

FINAL REPORT

Biogeochemical Processes that Control Natural Attenuation of Trichloroethylene in Low Permeability Zones

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Charles Werth, Ph.D.
University of Texas at Austin

Professor Albert Valocchi
Professor Robert Sanford
University of Illinois at Urbana-Champaign

Professor Timothy Strathmann.
Colorado School of Mines

Charles Schaefer, Ph.D.
CDM Smith

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LIST OF ACRONYMS

AFB	Air Force Base
Ag	Silver
AgCl	Silver chloride
APF	Aminophenyl fluorescein
CE	Coulombic efficiency
CSM	Colorado School of Mines
DCC	Dechlorinating culture
DCE	<i>cis</i> -1,2-dichloroethene
DHC	<i>Dehalococcoides</i> sp.
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
DNAPL	Dense non-aqueous phase liquid
DNPH	2,4-dinitrophenylhydrazine
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DoD	Department of Defense
<i>D vulgaris</i>	<i>Desulfovibrio vulgaris</i>
EPA	Environmental Protection Agency
ERSON	Environmental Restoration Statement of Need
ETM	Electron transfer mediator
Fe	Iron
FID	Flame ionization detector
ft bgs	Feet below ground surface
GC	Gas chromatograph
GC-FID	Gas chromatography with flame ionization detection
HCHO	Formate
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HPZ	High permeability zone
HS ⁻	Hydrogen sulfide
H ₂	Hydrogen
IAP	Ion activity product
IC	Ion chromatography
ICP-MS	Inductively coupled plasma mass spectrometer
IL	Illinois
IMZ	Immobile zone
IPR	In-Progress Review
K _{sp}	Solubility product
LPZ	Low permeability zone
MNA	Monitored natural attenuation
NO ₃ ⁻	Nitrate

OA	Organic acid
PCE	Tetrachloroethene
PDA	Photodiode array
RPM	Remedial Project Manager or Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RT3D	Reactive transport over three dimensions model
S	Sulfur
SEM-EDS	Scanning electron microscopy with energy dispersive spectroscopy
SERDP	Strategic Environmental Research and Development Program
SHE	Standard hydrogen electrode
SON	Statement of Need
SO ₄ ²⁻	Sulfate
SRB	Sulfate reducing bacteria
<i>S. oneidensis</i>	<i>Shewanella oneidensis</i>
TCD	Thermal conductivity detector
TCE	Trichloroethene
TOC	Total organic carbon
UV	Ultraviolet
VC	Vinyl chloride
VOC	Volatile organic compound
v/v	Volume per volume
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
1D	One dimensional
·OH	Hydroxyl radical
°C	Degrees Celsius

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Abstract

Introduction and Objectives

The overall goal of this research was to quantify the biotic and abiotic attenuation mechanisms that impact the fate and transport of trichloroethylene (TCE) within and at the boundaries of low permeability zones (LPZs) comprised of clays and silts, and to incorporate these processes into a computationally efficient model that can be used to directly address key questions regarding natural attenuation time scales and cleanup at TCE-impacted sites.

Technical Approach

Batch studies were performed to evaluate the biogeochemical conditions that promote abiotic TCE reaction under anaerobic and aerobic conditions in natural soils. These included evaluation of TCE reduction and oxidation using pure minerals alone in solution, and in the presence of iron or sulfur reducing bacteria, and evaluation of TCE reduction and oxidation using natural clayey soils known to contain Fe(II) minerals. TCE reaction rates in batch solutions were determined by monitoring TCE decay or reaction products. These were complemented with batch solution and mineral characterization, which involved measurement of dissolved and sorbed Fe(II), mineralogy using X-ray diffraction, mineral surface oxidation state using X-ray photoelectron spectroscopy (XPS), and oxidation reduction potential.

Flow cell studies were performed to determine the contribution of abiotic TCE reactions in the presence of an added electron donor and dechlorinating bacteria. The results were simulated using a newly developed and process-based numerical model. The model was used to assess the relevance of abiotic reaction in the flow cell and in larger systems using abiotic reaction rate constants from the flow cell, as well as from the literature.

Results

Batch study results show that a variety of minerals promote abiotic TCE reduction and oxidation, including Fe(II) carbonates, Fe(II) sulfides, and Fe(II)-containing illite. They also show that TCE reduction rates with iron sulfides are enhanced in the presence of either sulfate or iron-reducing bacteria. TCE reduction rate constants for pure minerals and clayey-soils generally increased with the amount of sorbed Fe(II) determined using a weak-acid extraction, but they were not related to mineral or clay surface areas or magnetic susceptibility. TCE reduction rate constants for pure minerals generally increases with decreasing redox potential of soil-solution slurries. However, this relationship was different for different minerals, indicating both reduction potential and mineralogy are important. Flow cell results show that biological reduction dominated TCE reduction, and abiotic TCE reduction was sustained concurrently at a lower level. Flow cell and larger scale modeling results indicate that in the absence of an added electron donor, abiotic TCE reduction can reduce the TCE back diffusion flux from LPZs by as much as 55%. A simplified method for incorporating reaction and back diffusion from clays was incorporated into RT3D.

Benefits

Results from these studies indicate that abiotic TCE reduction and oxidation are both important natural attenuation pathways for TCE in low permeability clays, and that more reducing conditions favor formation of a suite of Fe(II) minerals that contribute to these reaction pathways.

Executive Summary

Introduction

The Back Diffusion Challenge

It has long been recognized that the most challenging hazardous waste sites for groundwater remediation are those with DNAPL sources in highly heterogeneous and fractured aquifers (NRC, 2003, 2005, 2012). At large sites with a long history of contamination, DNAPL has slowly dissolved from trapped ganglia or pools resulting in large plumes with high levels of contamination (NRC, 2005, 2012). Dissolved contaminants in high permeability zones (HPZs) within these plumes can diffuse over long time periods into LPZs such as rock matrices, clay aquitards and lenses, a process commonly known as “matrix diffusion.” When contaminant mass in the high permeability source zone is depleted through natural attenuation or active remediation, back-diffusion of contaminants from LPZs into groundwater flowing through HPZs can occur for many decades (Chapman and Parker, 2005; NRC, 2005; Hadley and Newell, 2013). A prime example is the TCE DNAPL impacted site in Cocoa, FL illustrated in **Figure 1**, where TCE was released to the subsurface over approximately 10 years and back diffusion now appears to be limiting site cleanup. A critical question is how do reactive processes within LPZs attenuate the diffusive flux of TCE and other pollutants into adjacent high permeability groundwater zones, and what are the implications for managing dilute groundwater plumes at many DoD facilities.

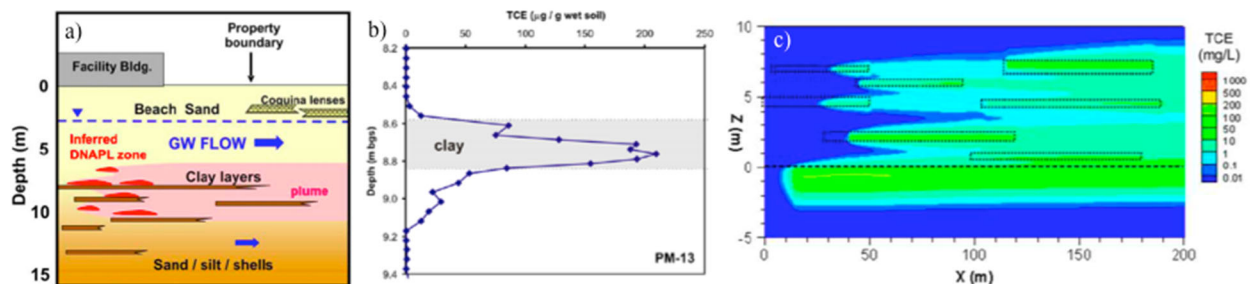


Figure 1. a) Schematic cross section of DNAPL source zone with LPZs at a metal fabricating and precision cleaning facility in Cocoa, FL where TCE was released from the mid- to late 1960s through 1977. b) Detailed TCE concentration profile from a core taken at the site. c) Simulation results showing TCE concentration profiles in water 50 years after the source zone is removed.

From Parker et al., 2008.

We hypothesize that a suite of naturally occurring reaction mechanisms may serve to significantly attenuate diffusive flux from LPZs, resulting in decreased impacts (both in magnitude and timeframe) of back-diffusion on groundwater quality. These mechanisms may occur within the LPZ itself, or at the interface between LPZs and HPZs. *Furthermore, we hypothesize that biogeochemical conditions (e.g., reducing, aerobic, organic carbon) will impact these naturally occurring reaction mechanisms in LPZs.* By attaining improved insight into the naturally occurring reaction mechanisms in LPZs, improved management (with respect to implementation of treatment and monitoring) and improved predictions of plume longevity will be attained.

Overview of TCE Reaction Pathways

Possible TCE reaction pathways are illustrated in **Figure 2**. Three chemical reaction pathways dominate the known biotic and abiotic anaerobic dechlorination reactions. First, hydrogenolysis involves replacing a chlorine with a proton through a two-electron transfer such as TCE dechlorination to dichloroethene (DCE) or chloroacetylene reacting to form acetylene. TCE dechlorination is typically promoted biologically in the subsurface, and in many cases involves the addition of an electron donor to stimulate dechlorinating bacteria. Among the dechlorinating bacteria, only *Dehalococcoides* are known to dechlorinate TCE all the way to ethene. Second, β -elimination, or dichloroelimination, is also a two-electron transfer reaction that results in a less saturated product; for example, during dichloroelimination of TCE, the double bond in TCE becomes a triple bond, forming chloroacetylene. This is generally considered an abiotic reaction, and mounting evidence shows ferrous iron associated minerals drive this process. Finally, hydrogenation reactions involve adding H_2 to a compound and further reducing it to a more saturated product, such as acetylene to ethene, or ethene to ethane.

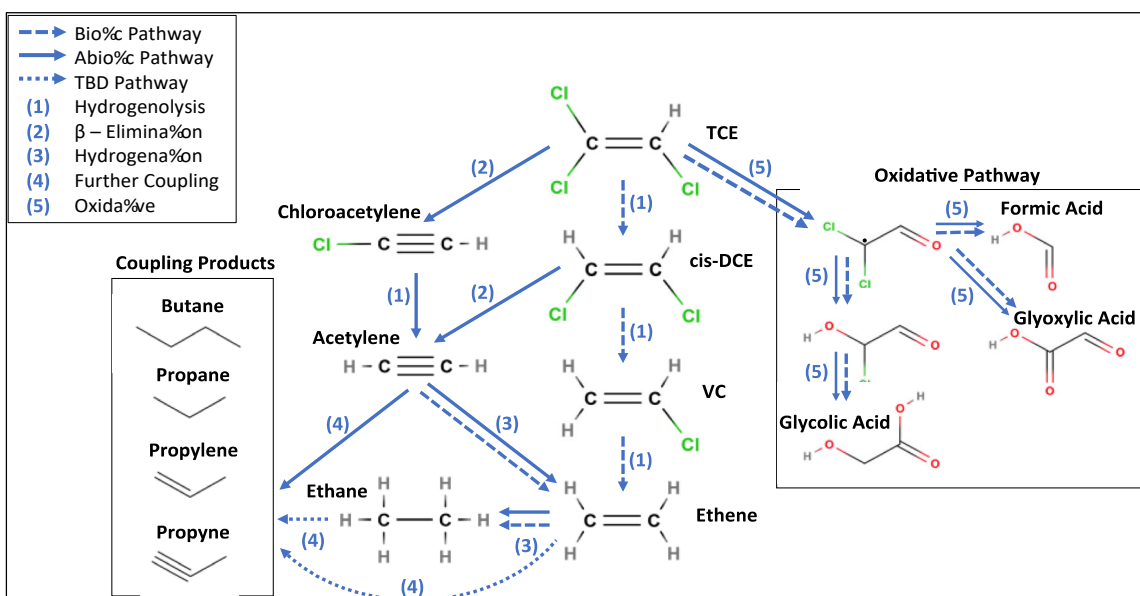


Figure 2. TCE Reaction Pathways in Groundwater.

Oxidative reactions are also possible for TCE, and these can be biotic or abiotic. The former is driven by co-metabolism, where an oxygenase enzyme produced for the oxidation of an energy yielding substrate (e.g., methane, toluene) fortuitously oxidizes TCE without benefit to the bacteria. There is growing evidence that the latter is promoted by hydroxyl radicals (Schaefer et al., 2018), that are produced when ferrous iron associated minerals react with oxygen to produce in sequence superoxide, hydrogen peroxide, and then hydroxyl radicals.

Objectives

The overall objectives of our SERDP proposal are to quantify the biotic and abiotic attenuation mechanisms that impact the fate and transport of trichloroethylene (TCE) within and at the boundaries of LPZs comprised of clays and silts, and to incorporate these processes into a computationally efficient model that can be used to directly address key questions regarding natural attenuation time scales and cleanup at TCE-impacted sites. Low permeability sediments

can limit microbial transport and affect the location and diversity of microbial activity (Bradford and Bettahar, 2005; Musslewhite et al., 2007; Olson et al., 2005; Wang et al., 2008). Reactive iron species can enhance overall transformation rates due to synergistic effects of such minerals on biological transformation (Weerasooriya and Dharmasena, 2001; Elsner, 2002), and due to the contribution from abiotic transformations (Lee and Batchelor, 2002a, 2002b; Ferrey et al., 2004; O'Loughlin and Burris, 2004; Jeong et al., 2011; Schaefer et al., 2013). Even if the rates of these processes within LPZs are low, they can substantially reduce the flux of TCE to HPZs, and ultimately provide a significant reduction in the longevity of TCE plumes that are sustained via back-diffusion. To attain the overall project goal, the following objectives were addressed:

- Obtain and characterize site material from a low permeability zone;
- Determine the abiotic reactivity of LPZ site materials for TCE, and the controlling biogeochemical conditions;
- Determine the abiotic reactivity of iron-enriched materials present at the site for TCE;
- Determine the contribution of biotic and abiotic TCE reaction rates on LPZ back diffusion;
- Develop a numerical model to uncouple the effect of biotic and abiotic reactions on TCE back diffusion, and to evaluate their relevance in larger systems;

Technical Approach

Collection and Characterization of Materials

Soil and groundwater were obtained from Site 34 at Pease Air Force Base in Portsmouth, New Hampshire (**Figure 3**). An upper sand and the underlying marine clay and silt were sampled. Geoprobe coring was used to obtain the sand and clay samples, and an existing well screened in the upper sand was used to obtain groundwater. The sand and clay materials were put on ice and shipped overnight to the laboratory for storage. CDM Smith had on hand sediment material from four other Department of Defense (DoD) sites due to prior SERDP work. This was used for analysis and comparison of abiotic reaction rates as a function of soil mineralogy and other measured properties. All solids were characterized for mineralogy using X-ray diffraction, size fractions using sieving, sorbed ferrous iron content using a weak acid extraction followed by analysis using the 1,10-phenanthroline method, and total organic carbon (Berns, 2020).

Three different microbial inocula were used in this work. A mixed dechlorinating community (DCC), originally obtained from river sediments on the Sangamon River (IL) in 2001 and maintained on PCE and lactate, was used in flow cell experiments. This community was characterized to determine whether the dehalogenase enzymes *tceA*, *vcrA*, and *bvcA*, as well as *Dehalococcoides*, were present by amplifying 16S rRNA genes in DNA. *Desulfovibrio vulgaris* was used as a model sulfate-reducing bacteria. *Shewanella oneidensis* was used as a model iron reducing bacteria. The DCC and *Desulfovibrio vulgaris* were both provided by Robert Sanford (co-investigator) at the University of Illinois. The *Shewanella oneidensis* was obtained from Kyle Michelson at the University of Texas at Austin.

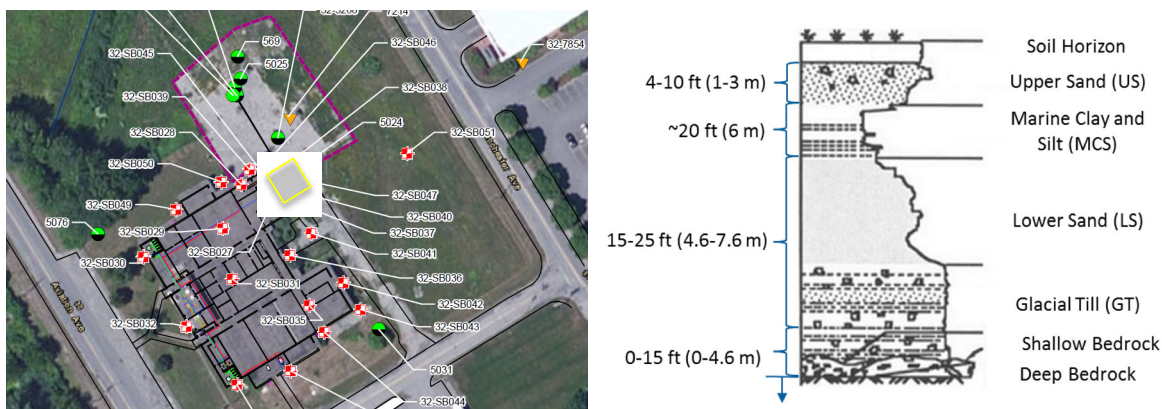


Figure 3. Pease AFB plan view and stratigraphic depth profile.

Batch Experimental Setup

Batch experiments were performed in either 40 mL glass vials, or 160 mL glass bottles, both sealed with Teflon-lined septa (**Figure 4**). The 40 mL vials had caps with Mininert® valves and the threads were sealed with epoxy. In some cases these vials were stored in secondary containers continuously sparged with nitrogen to ensure anaerobic conditions. The 160 mL bottles had crimp caps, and were stored upside down in a water to prevent TCE volatilization losses, and to minimize oxygen diffusion into anaerobic experiments.



Figure 4. Photos of 40 mL glass vials & 160 mL glass bottles used in batch studies, with double containment approaches to maintain anaerobic conditions and/or minimize volatilization losses.

Both vials and bottles were amended with groundwater or synthetic media, leaving room for headspace. In most experiments, natural solids or minerals were added directly to the batch experiments under anaerobic conditions before sealing, and then directing amending with either TCE alone or TCE plus oxygen. Natural solids were gamma-irradiated prior to abiotic experiments to prevent biological activity. In some experiments iron sulfide minerals were precipitated in the bottles under anaerobic conditions using either ferrous iron and sulfide salts only, ferrous iron salt and sulfate plus the sulfate reducing bacterium, or ferrihydrite and a sulfide salt plus the iron reducing bacterium. The iron sulfide minerals were allowed to age for three weeks before the bottles were amended with TCE.

Batch reactors were either mixed by hand or on a shaker platform. Periodically, head space samples from anaerobic bottles were taken and analyzed for TCE and TCE reduction products, including DCE, VC, ethene, and acetylene. This was done using gas chromatography coupled with flame ionization detection. Aqueous samples were taken from bottles amended with oxygen to measure TCE oxidation products, i.e., organic acids, using liquid chromatography. Mass balances on control bottles containing only TCE and water had losses of < 5% over six weeks. At the conclusion of selected batch experiments, solution reduction potentials were measured using electron transfer mediators (Gorski et al., 2016; Kocur et al., 2020). Also, solids were separated from water in an anaerobic chamber, and solids were analyzed for total and sorbed ferrous iron, mineralogy, magnetic susceptibility, and surface area.

Flow Cell Experimental Setup

A stainless steel and glass flow cell was constructed from two 0.64 cm-thick sheets of glass placed on the front and back faces of a stainless steel frame, separated with Viton gaskets to prevent leaks. One-eighth inch fittings tapped in the frame on the edges were used to introduce groundwater, and a thin, rectangular opening in the top of the frame was used to pack sand and clay. A stainless steel plate was used to seal the top of the flow cell. The inside dimensions of the flow cell were 16.5 cm x 2 cm x 16.5 cm (LxWxH). The flow cell setup with inlet reservoir, pump, sampling loop, and effluent reservoir is shown in **Figure 5**. At the bottom of the flow cell was a thin layer (~0.25cm) of Tenax beads, pre-equilibrated with TCE in a serum bottle for a week. Pease clay was mixed with 20 mL of the dechlorinating culture (DCC) inoculum, and then transferred out of the chamber and placed in the bottom of the flow cell. The top of the clay was then smoothed, and Pease sand was packed on top of the clay with stainless steel mesh on the left and right edges to distribute flow. During packing, the groundwater level in the flow cell was always maintained above the solids. After packing, a top plate was used to seal the flow cell and the experiment was performed in the fume hood. Flow only occurs in the sand, and not in the low permeability clay. As a result, any mass transport in the clay is controlled by diffusion.

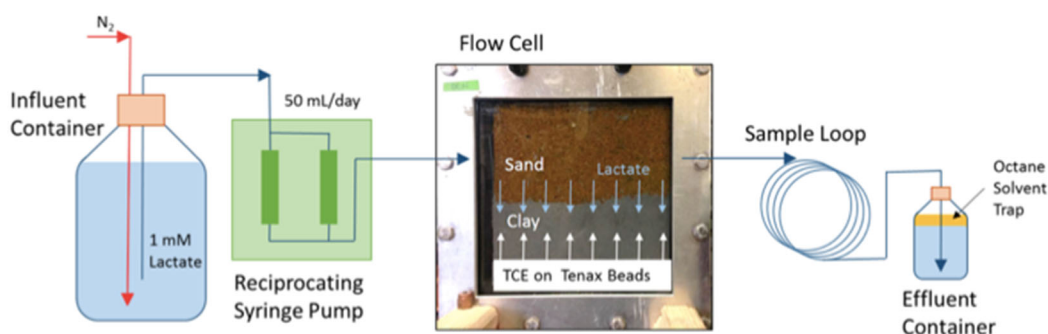


Figure 5. Flow cell setup.

Anaerobic groundwater was amended with 1 mM lactate as the electron donor and delivered to the flow cell. Its fermentation products diffused into the LPZ at the HPZ-LPZ interface. On the bottom of the flow cell, TCE on the Tenax dissolved into solution and diffused upward through the LPZ. The electron donors and TCE mixed via diffusion and promoted biological reaction. Reaction products that diffused out of the LPZ were quickly removed from the flow cell via

advection in the HPZ, and these were captured for analysis of TCE and reaction products. A second flow cell experiment was conducted in the same way for the first 70 days, and then groundwater containing oxygen but no lactate was used to promote abiotic TCE oxidation. Flow cell experiments were terminated after 180 days and frozen at -80°C. The frozen clay was then cut into 1 cm-wide by 0.5 cm tall sections, placed into separate head space vials, thawed, and analyzed for volatile products.

1D Diffusion Reaction Model

The model was developed and coded in MATLAB to simulate flow cell effluent concentrations, and the corresponding depth profiles in the LPZ. Biological dechlorination of TCE to cis-DCE, to VC, and to ethene was considered, and reactions were modeled using Dual Monod kinetics. Abiotic reactions of TCE and cis-DCE with reactive minerals were considered and modeled using 1st order kinetics (with respect to TCE or cis-DCE). Reactive mineral concentrations were assumed to remain constant because rates of abiotic reduction in batch experiments appeared to be constant for over 3 months. It was assumed that lactate completely fermented and oxidized to acetate, H₂, and propionate in the HPZ, and the fermentation products diffuse into the LPZ. Propionate is not a direct electron donor for dechlorination, and hydrogen produced from propionate fermentation was determined to be insignificant. Therefore, it is assumed that one microbial community uses either hydrogen or acetate for the first dechlorination step, and a second community uses only hydrogen, but can facilitate all three dechlorination steps.

Computationally Efficient Numerical Model Simulation Using RT3D

A new Immobile Zone (IMZ) Model was implemented within the commercial software RT3D to more provide a tool for practitioners to more computationally simulate diffusion and reaction in low permeability zones. The approach treats the LPZ as an impermeable region in MODFLOW, while in RT3D the LPZ is conceptualized as a series of immobile zones coupled with a mobile zone at the HPZ interface. Finite volume discretization of diffusion and reaction within the LPZ is then modeled as mass transfer and reaction among several immobile species. This results in a simulation domain with significantly fewer grid cells compared to that required if all LPZs are discretized, providing potential for improved computational efficiency. Cases evaluated included a layer of HPZ over an LPZ, a thin/thick lens of LPZ embedded in HPZ, and multiple lens of LPZs embedded in HPZ.

Results

Anaerobic and Aerobic Batch Experiments with Natural Sediments

In the absence of oxygen (<26 µM), TCE dechlorination proceeded via a reductive pathway generating acetylene and/or ethene. Reductive dechlorination rate constants up to $3.1 \times 10^{-5} \text{ d}^{-1}$ were measured, after scaling to in situ solid water ratios. In the presence of oxygen greater than 120 µM, TCE dechlorination proceeded via an oxidative pathway generating formic/glyoxylic and glycolic/acetic acids, and oxidative dechlorination rate constants (again scaled to in situ conditions) up to $7.4 \times 10^{-3} \text{ d}^{-1}$ were measured. These rates correspond to half-lives of 60 and 0.25 years for abiotic TCE dechlorination under anaerobic and aerobic conditions, respectively,

indicating the potentially large impact of aerobic TCE oxidation in the field. For both reductive and oxidative TCE dechlorination pathways, measured first-order rate constants increased with increasing ferrous iron content, suggesting the role of iron oxidation. Hydroxyl radical formation was measured and increased with increasing oxygen and ferrous iron content. Rate constants associated with TCE oxidation products increased with increasing hydroxyl radical generation rates, and are zero in the presence of a hydroxyl radical scavenger, suggesting that oxidative TCE dechlorination is a hydroxyl radical driven process.

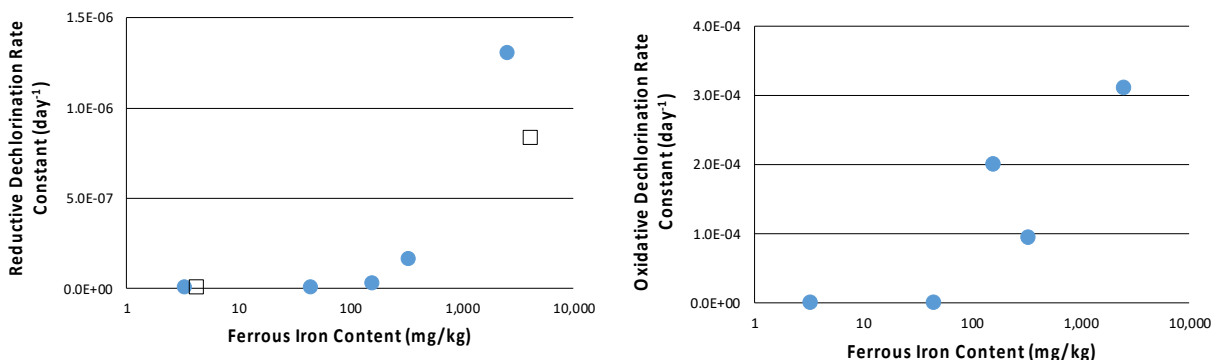


Figure 6. Left - Reductive first order dechlorination rate constants (k_{an}) plotted as a function of sediment ferrous iron content in the anaerobic experiments. ●=TCE data, □=PCE data from Schaefer et al., 2017. Right - Oxidative TCE first order dechlorination rate constants (k_{ox}) plotted as a function of sediment ferrous iron content in the aerobic experiments.

Anaerobic and Aerobic Batch Experiments with Pure Minerals Added

Measurable TCE reduction under anoxic conditions was observed for ankerite, siderite, and illite, but not for biotite; acetylene and trace levels of ethene were the identified dechlorination products. With the exception of biotite, first-order dechlorination rate constants increased with increasing ferrous content. Measurement of reduction potentials among the four minerals examined showed that biotite had the least reducing value (+84 mV vs. SHE), and thus may have lacked the reductive capacity to dechlorinate TCE. When normalizing measured first-order dechlorination rate constants to the estimated ferrous iron specific area, reduction potentials provided a reasonable prediction of TCE reductive dechlorination. Under oxic conditions, hydroxyl radicals were generated for each of the four minerals. However, the order of mineral activity differed from that observed under anoxic conditions, with no readily apparent correlation to mineral properties. In terms of TCE and oxygen reduced per mole of ferrous iron initially present in each mineral, illite was the most reactive of the four minerals. Together, the results suggest that several ferrous minerals contribute to abiotic dechlorination in the natural environment, and (at least for TCE reduction under anoxic conditions) measurement of ferrous mineral content and reduction potential of field samples may serve as useful tools for estimating TCE dechlorination rate constants.

Batch Experiments with Iron Sulfide Minerals in the Presence of a Sulfate Reducing Bacterium

Pseudo first order rate constants for TCE transformation to acetylene by iron sulfide minerals in systems with biological sulfate reduction increase with increasing ferrous iron loading from 2 mM (3.63×10^{-5} 1/day) to 50 mM (1.13×10^{-4} 1/day), and then decrease sharply at 100 mM (3.13×10^{-6} 1/day). By comparison, the maximum rate constant for TCE transformation by iron sulfide minerals under abiotic conditions peaks at 2 mM, and is only 3.17×10^{-5} 1/day. TCE transformation rate constants for experiments performed with cell exudates were similar to those with live cells, suggesting redox buffering by cell-impacted media and not the cells themselves imparted greater activity. Geochemical modeling results indicate greater TCE transformation rates are associated with the presence of mackinowite, but not ferrihydrite (Figure 7). TCE transformation rates do not correlate to the concentration of aqueous ferrous iron, but do correlate to measured reduction potentials.

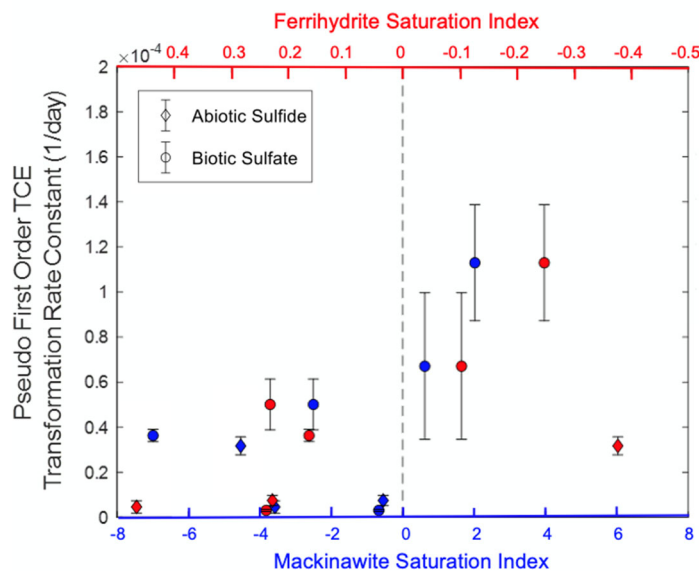


Figure 7. TCE transformation rate constants versus mineral saturation index.

Batch Experiments with Iron Sulfide Minerals in the Presence of an Iron Reducing Bacterium

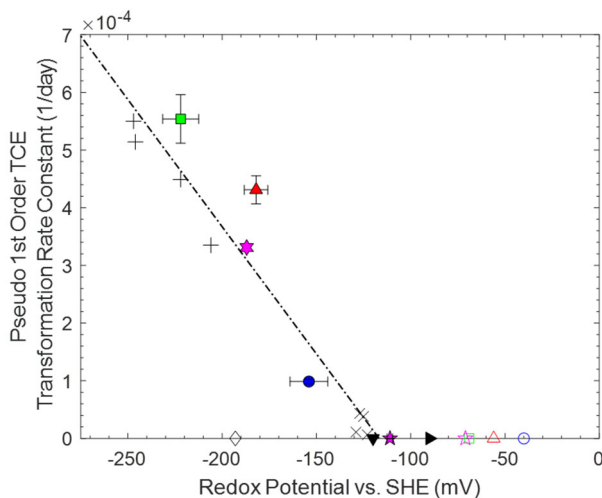


Figure 8. TCE reduction rate constant by iron sulfide minerals vs. reduction potential.

transformation was observed. It is proposed that iron-reducing bacteria “re-reduce” oxidized mineral surfaces, promoting faster TCE transformation, and that faster TCE transformation is favored when FeS formation is favored over ferrihydrite and FeCO_3 .

In the presence of *Shewanella oneidensis*, TCE transformation rates are approximately an order of magnitude higher than under abiotic conditions, regardless of whether the source of iron is ferrihydrite or aqueous Fe^{2+} . Data from XRD and XPS paired with MINTEQA calculations indicate that conditions promoting the formation of mackinawite (FeS) had the highest TCE transformation rates. Also, conditions that favored siderite (FeCO_3) or ferrihydrite formation limited TCE transformation. Higher TCE transformation rates were not related to aqueous ferrous iron concentrations, but they were associated with lower redox potentials. Also, above -125 mV (vs. SHE) no TCE

Flow Cell and 1D Dimensional Modeling

Flow cell effluent measurements indicate vinyl chloride (VC) is the dominant TCE transformation product, and that small but sustained concentrations of acetylene were sustained over the entire 180 time period. Depth profiles through the LPZ in the flow cell (**Figure 9**) showed that cis-DCE and VC production were greater near the interface of the flow cell, indicating that the biotic processes are more important near the electron donor source. The depth profiles also showed higher concentrations of acetylene near the base of the depth profile, where the TCE was highest, indicating that abiotic degradation of TCE to acetylene was occurring simultaneously.

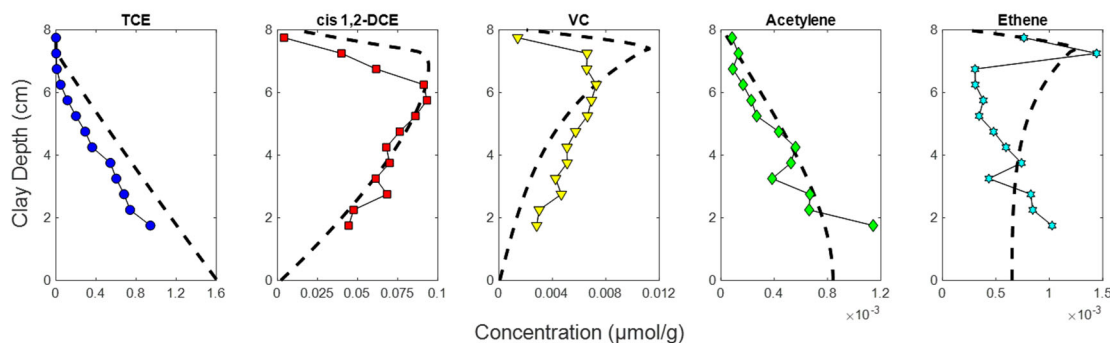


Figure 9. Flow cell depth profiles through clay (LPZ), with a comparison to the numerical model (dashes). All concentrations are reported as mass of analyte in headspace per mass of clay analyzed at each depth, and each data point is an average of three measurements at depth.

The 1D diffusion reaction model accurately simulated depth profiles in the flow cell, as well as time scales of dominant products and reactants in the effluent. However, it failed to capture the magnitude of some product flux values. Additional complexities could be added to the model to improve its capabilities, albeit with more model parameters. Biotic and abiotic flow cell parameters were used to simulate a field site with a 300 cm thick LPZ. Abiotic processes contributed at most a 2.0% reduction in TCE flux after 730 days. When abiotic rate constants were changed to that observed in batch, or to rate constants previously reported for a pyrite rich mudstone, the TCE flux reduction was 21% and 53%, respectively, after 730 days. Though biotic processes dominated TCE transformation in the flow cell experiment, the simulations indicate that abiotic processes have potential to significantly contribute to TCE attenuation in electron donor limited environments provided suitable reactive minerals are present.

Numerical Model Development and Computationally Efficient Incorporation into RT3D

The IMZ-RT3D model results show that as long as each LPZ is divided into a sufficient number of immobile species, the results are in good agreement with the full-RT3D model with respect to predicting mass of solute remaining in the model domain and average outflow concentration for all cases. However, one obvious discrepancy is the consistent and small overestimation of total mass of solutes stored in the LPZ by the IMZ-RT3D compared to the full-RT3D model. Another discrepancy is larger errors between the IMZ and full model for the thin lens compared to the thick lens. This could be attributed to larger gradients of concentration in the thin relative to the thick

lens, which can cause higher mass transfer rates and consequently larger errors in numerical approximation. However, considering that IMZ-RT3D model is estimating the full model, the discrepancies are slight as long as enough immobile zones are used for IMZ-RT3D model. The results indicate that the IMZ-RT3D model is capable of modeling a flow field with complex heterogeneity LPZ structures, as well as arbitrary reaction kinetics like dual-Monod.

Implications for Future Research and Benefits

The conceptual model that emerges from this work and others for abiotic reactions is illustrated in Figure 10. Under anaerobic conditions, structural or sorbed ferrous iron donates an electron directly to TCE, promoting its reduction. The ferrous iron is oxidized, possibly coating the ferrous iron mineral. When iron or sulfur reducing bacteria are present, the oxidized iron can be reduced back to ferrous iron and possibly regain activity. A similar scenario exists for aerobic conditions, except that now ferrous iron donates an electron to oxygen, which by way of superoxide and hydrogen peroxide produces hydroxyl radicals. These radicals then oxidize TCE. In the absence of reducing conditions, the ferrous iron is depleted under oxidizing conditions.

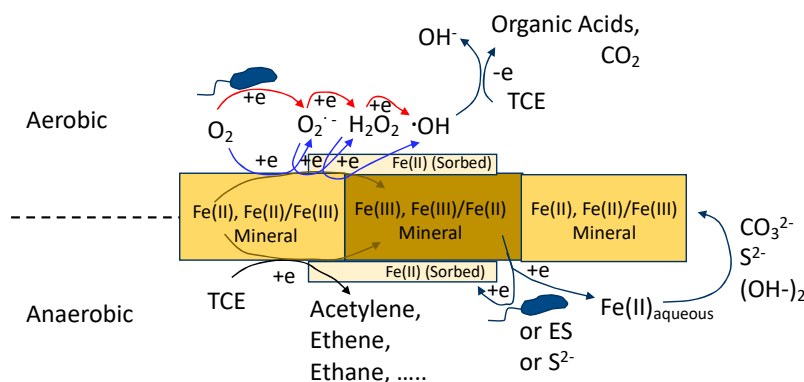


Figure 10. Conceptual model of TCE abiotic reactions under aerobic and anaerobic conditions.

Key questions that were not addressed in this work are the following:

- Are there transient reactive mineral intermediates that form and promote TCE reduction and oxidation that we did not detect?
- Do both sorbed and structural iron contribute to TCE reduction?
- Do both sorbed and structural iron contribute to TCE oxidation?
- Can the mineral capacity for TCE oxidation effectively be regenerated under reducing conditions?
- Do mineral surfaces enhance TCE oxidation with hydroxyl radicals?
- Can the reduction potential be used to indicate abiotic TCE reactivity of field samples?

Further work should address these open questions to gain a better understanding of when abiotic TCE transformation is likely to occur, and when it will be a dominant process in low permeability zones.

1.0 Objectives

This SERDP Proposal was submitted in response to the SERDP Statement of Need ERSON-15-01 that addressed the need to improve understanding of long-term natural attenuation processes on contaminants in groundwater. Tetrachloroethene (PCE) and trichloroethene (TCE) were noted as compounds of particular interest. The overall objective of this research was to quantify the biotic and abiotic attenuation mechanisms that impact the fate and transport of trichloroethylene (TCE) within and at the boundaries of LPZs comprised of clays and silts, and to incorporate these processes into a computationally efficient model that can be used to directly address key questions regarding natural attenuation time scales and cleanup at TCE-impacted sites. More specifically, our research was directed to address the following key questions:

- To what extent is microbially enhanced reductive dechlorination occurring within LPZs relative to adjacent HPZs (both under ambient conditions, and in response to active bioremediation efforts), and what factors control this difference?
- What is the role of ferrous mineral-induced abiotic reactions (reductive and oxidative) on TCE fate and diffusive flux from LPZs?
- What are the overall impacts of coupled biotic/abiotic processes (e.g., biologically facilitated iron-cycling between ferric and ferrous iron), along with diffusion, on TCE fate and flux from LPZs?
- What are the impacts of electron-shuttling compounds (e.g., extracellular quinones, flavins) that diffuse from HPZs into LPZs on biotic/abiotic reactions within the LPZs?
- How do biogeochemical conditions (e.g., dissolved oxygen, organic carbon) in LPZs and adjacent HPZs impact the above processes?
- How can the complex and interdependent processes discussed above be integrated into a computationally efficient modeling framework that can be used to help manage TCE-impacted sites, including remedial action selection and implementation, long term monitoring, and expectations of monitored natural attenuation (MNA) timeframes?

To address these questions, our research involved evaluation of biotic and abiotic TCE reaction in natural and iron-enriched fractions of LPZ materials using batch reactors, a flow cell, and numerical modeling. Batch studies were performed under well-mixed conditions and allowed us to determine how properties of groundwater, LPZ materials, or pure minerals with LPZ materials affect biotic and abiotic TCE transformation rates. Of particular interest was the role of LPZ mineral components in promoting abiotic TCE reduction under anoxic conditions, and abiotic TCE oxidation under aerobic conditions. Flow cell studies were designed to evaluate how mass transfer limitations of reactants affects the extent and pathways of TCE transformation, and the impact of these transformation reactions on TCE flux from LPZs. Controlling mechanisms in the flow cell were evaluated using a newly developed numerical model, and this model was used to assess the

relevance of abiotic TCE reactions at larger scales using TCE concentration profiles from field sediments and abiotic TCE reduction rates from a number of different studies in the literature. Last, the reactions identified in the numerical model were incorporated into RT3D using a custom reaction module, and RT3D was modified to avoid fine-scale discretization in LPZs.

2.0 Background

2.1 Chlorinated Ethenes

Groundwater is an essential source of water in the United States. In 2015, 84.7 billion gallons of groundwater were withdrawn per day (Dieter et al., 2018). Of this total groundwater use, 68% was used for irrigation and 18% was used for public supply (Dieter et al., 2018). Chlorinated solvents are a class of persistent groundwater contaminants that are regularly detected in shallow groundwater sources (Desimone et al., 2014), with the most problematic being chlorinated ethenes. A study of volatile organic compounds (VOCs) in United States groundwater showed detections of two chlorinated ethenes, i.e., tetrachloroethene (PCE) and trichloroethene (TCE), exceeding 0.2 $\mu\text{g/L}$ in 2% of approximately 3,500 aquifer samples, and exceeding 0.02 $\mu\text{g/L}$ in 6% of these samples (Zogorski, 2006). Chlorinated ethenes in shallow groundwater also present a vapor intrusion risk, where volatile compounds partition into the gas phase and migrate through the vadose zone and base slab, allowing them to be inhaled indoors (Burk and Zarus, 2013). Chlorinated ethenes are known or suspected carcinogens and have been shown to adversely affect the human liver, kidneys, central nervous system, and reproductive system. Because of these health concerns, the Environmental Protection Agency (EPA) set a maximum contaminant level (MCL) of 5 $\mu\text{g/L}$ for both PCE and TCE per the requirements detailed in the Safe Drinking Water Act. MCLs are enforceable and have led to groundwater remediation efforts at many contaminated sites.

In the last 30 years, studies have shown that low permeability aquifer materials such as silts and clays, can cause chlorinated ethenes to be especially persistent in the subsurface (Chapman and Parker, 2005; Liu and Ball, 2002; Parker et al., 2008). Because chlorinated ethenes are denser than water, they will move downward through the subsurface until a low permeability zone (LPZ) limits their migration. Over time, chlorinated ethenes will diffuse into the LPZ matrix. As illustrated in **Figure 2.1**, following remediation efforts that preferentially target high permeability zones (HPZs), chlorinated ethenes will back diffuse from LPZs, re-contaminating HPZ aquifers (Parker et al., 2004). At some sites, LPZs make it especially challenging to maintain groundwater concentrations below the MCLs. Our research effort focused on understanding the relative contributions of biotic and abiotic processes that control the release of TCE from LPZs. TCE was chosen because 1) chlorinated ethenes are a subclass of chlorinated solvents that are particularly resistant to transformation, 2.) PCE, which is often a precursor to TCE, yields more energy during reduction reactions and is more readily dechlorinated, and 3.) TCE is ranked sixteen on the Agency for Toxic Substances and Disease Registry Substance Priority List (ATSDR, 2019), and is a precursor to vinyl chloride which is ranked number four.

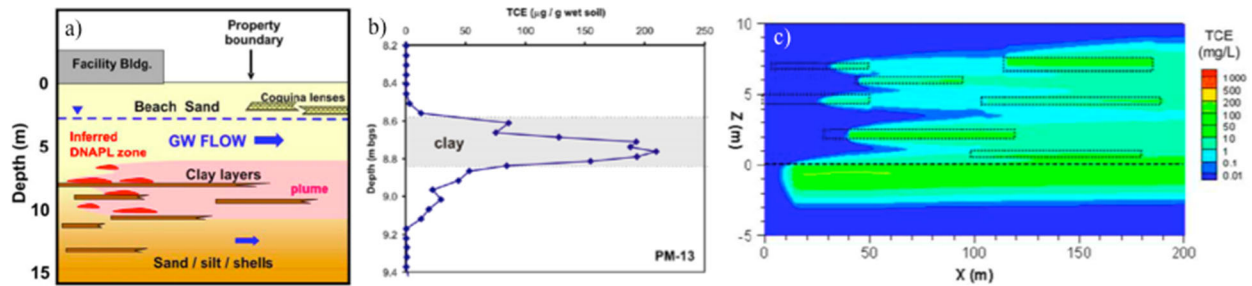


Figure 2.1. a) Schematic cross section of DNAPL source zone with LPZs at a metal fabricating and precision cleaning facility in Cocoa, FL where TCE was released from the mid- to late 1960s through 1977. b) Detailed TCE concentration profile from a core taken at the site. c) Simulation results showing TCE concentration profiles in water 50 years after the source zone is removed. From Parker et al., 2008.

Biogeochemical mechanisms are known to control the fate and transport of TCE in the environment. Both biotic and abiotic processes can degrade TCE, with some daughter products being more toxic than TCE itself and some products being innocuous. Groundwater remediation efforts often focus on stimulating biotic or abiotic processes *in situ* (in the aquifer), but it can be challenging and expensive to completely degrade TCE to its nontoxic daughter products. Research in the areas of biological reductive dehalogenation, also referred to as chlororespiration, have focused on a group of microorganisms that gain energy from transforming TCE to less chlorinated products (Löffler and Edwards, 2006; Maymó-Gatell et al., 1997; McCarty et al., 2020). Other research has shown that a range of microorganisms can promote cometabolic TCE transformation, either under aerobic or anaerobic conditions, whereby the organisms do not derive energy from reactions with TCE, but instead biological metabolites fortuitously react with TCE (Alvarez-Cohen and McCarty, 1991; Fantroussi et al., 1998; Hazen, 2010; Holliger et al., 2003; Wilson and Wilson, 1985). Microcosms with methanogenic, sulfate-reducing, and iron-reducing bacteria have all shown potential to contribute to TCE transformation. Finally, abiotic TCE transformation can be promoted by many natural and anthropogenic materials, with reduced iron and sulfur minerals showing promise for promoting efficient natural attenuation (Butler and Hayes, 1999, 1998; Culpepper et al., 2018; Ferrey et al., 2004; He et al., 2010; Jeong and Hayes, 2007; Lee and Batchelor, 2002a, 2002b).

Reactive iron minerals have potential to facilitate efficient natural attenuation of TCE *in situ*. Because the formation of these minerals is strongly dependent on metal reducing bacteria active in groundwater environments, elevated TCE transformation has been regularly documented under iron and sulfate reducing conditions (AFCEE, 2008; Henry, 2010; Kennedy et al., 2006b, 2006a; Scheutz et al., 2008), either in groundwater or within engineered biowalls. Previous studies have shown enhanced transformation kinetics of chlorinated solvents by reduced iron minerals in the presence of active microbial communities (Butler et al., 2013; Gander et al., 2002; Huo et al., 2016; Nie et al., 2020), but further mechanistic interpretation of these observations is needed. Biotic

processes might be either causative, creating reduced minerals, or synergistic, enhancing abiotic reactions with TCE-reactive minerals, or both (Brown et al., 2009).

2.2 Anaerobic Transformation of Chlorinated Ethenes

Three chemical reaction pathways dominate the known biotic and abiotic anaerobic dechlorination reactions. First, hydrogenolysis involves replacing a chlorine with a proton through a two-electron transfer such as TCE dechlorination to dichloroethene (DCE) (Equation 2.1) or chloroacetylene reacting to form acetylene. β -elimination, or dichloroelimination, is also a two-electron transfer reaction that results in a less saturated product; for example, during dichloroelimination of TCE, the double bond in TCE becomes a triple bond, forming chloroacetylene (Equation 2.2). Finally, hydrogenation reactions involve adding H_2 to a compound and further reducing it to a more saturated product, such as acetylene to ethene (Equation 2.3), or ethene to ethane.

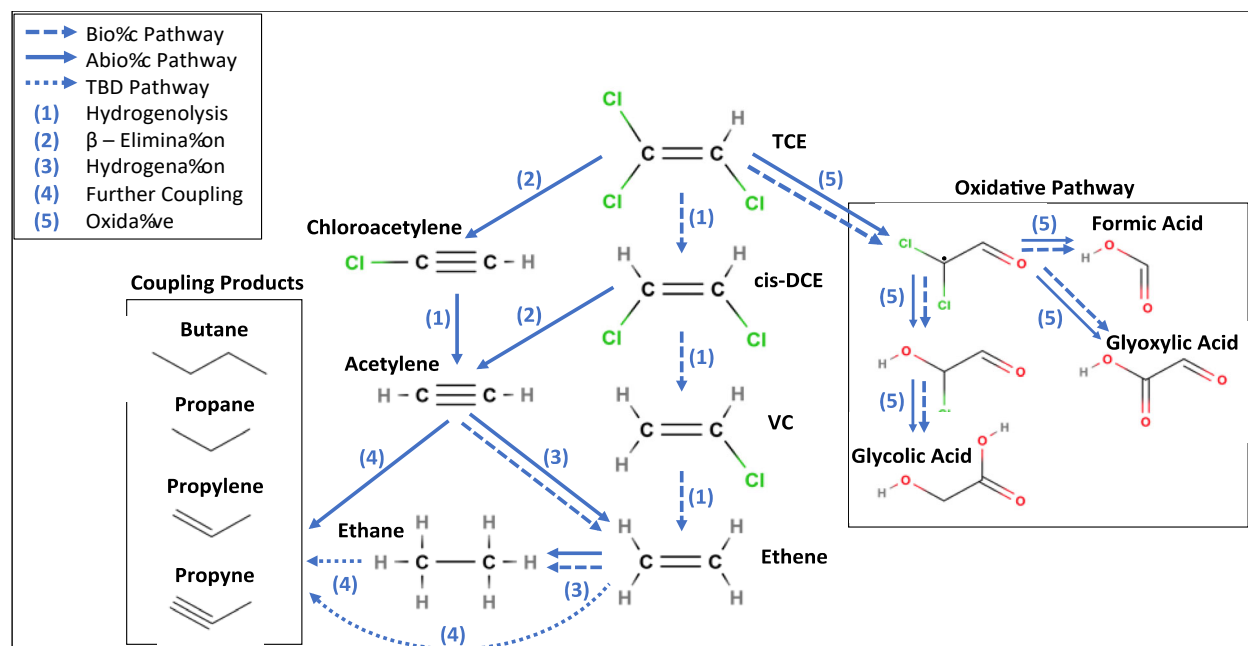
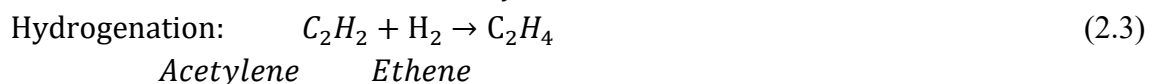
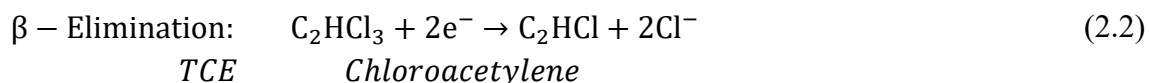
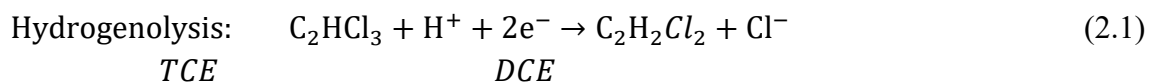


Figure 2.2 TCE Reaction Pathways in Groundwater.

Biological reductive dechlorination involves sequential removal of chlorine via hydrogenolysis reactions promoted by dehalogenase enzymes. Several different genera of bacteria are capable of yielding energy from biological reductive dehalogenation of TCE, including *Desulfomonas*, *Dehalobacter*, *Desulfitobacterium* and others (Bradley, 2000; Dolinová et al., 2017; McCarty et al., 2020), but only bacteria of the *Dehalococcoides* genus are known to be capable of gaining energy from each hydrogenolysis reaction from TCE to *cis*-dichloroethene (*cis*-DCE), to vinyl chloride (VC), and finally to ethene. This reaction pathway is shown in the center of **Figure 2.2**. Hydrogenolysis can also be promoted by abiotic processes under certain conditions (Arnold and

Roberts, 2000; Butler and Hayes, 2001). The ability of chlororespiring bacteria to obtain energy by dechlorinating TCE depends on many environmental factors including temperature, pH, reducing conditions, hydrogen thresholds (Löffler et al., 1999; Yang and McCarty, 1998), and the ability to compete with other bacteria for electron donor (David et al., 2015; Wei and Finneran, 2011).



Biologically mediated TCE transformation can be promoted by a variety of mechanisms other than biological reductive dechlorination. During cometabolic TCE transformation, microorganisms do not directly obtain energy from transforming TCE. Under anaerobic conditions, cometabolic processes might promote any of the three reactions summarized in Equations 2.1-2.3. Bacteria can biosynthesize enzymes, cofactors, and other metabolites that might contribute to anaerobic dechlorination of chlorinated solvents. When these compounds catalyze the reaction of TCE to less reduced products, it is considered anaerobic cometabolism, but these compounds might also simply mediate electron transfer, which is not strictly considered cometabolism. Biogenic enzymes and cofactors can act as electron transfer mediators, or metal reducing bacteria can promote cycling of iron or sulfur compounds that can act as electron transfer mediators. Some biologically associated compounds that have been shown to promote or enhance dechlorination of chlorinated ethenes via catalysis or electron transfer are listed below. (Note: some compounds identified have been observed to dechlorinate chlorinated alkanes and other chlorinated compounds, but not chlorinated alkenes like TCE, which are considerably more persistent. Other compounds are known electron transfer mediators in iron-sulfur systems). Compounds listed here may be relevant for TCE transformations via cometabolism or electron transfer in iron and sulfate reducing systems.

Finally, TCE may be transformed by abiotic reactions with reactive mineral species. Reduced iron minerals have shown great potential for TCE transformation, especially iron sulfide minerals, such as mackinawite, that have reported optimal TCE half lives of ~25 days via surface catalyzed reactions (Butler and Hayes, 2001, 1999). While abiotic TCE transformation rates are typically slower than biological reductive dechlorination, abiotic reactions can sometimes be more desirable because they often select for acetylene as a product (left side of **Figure 2.2**), and do not involve DCE and VC intermediates, which can be more mobile and toxic than TCE. A comparison of TCE transformation rates by iron sulfides, oxides, green rusts, and clay is presented in **Figure 2.3**. Abiotic reactions with these mineral species have been shown to promote hydrogenolysis and β -

elimination reactions, with β -elimination often accounting for most of the TCE mass reacted (Equation 2.2). Abiotic reductive transformation of TCE has been reported for pure mineral species as well as sterilized heterogeneous soils and sediments (Schaefer et al., 2018, 2017), indicating potential for natural mineral species to contribute to natural attenuation of TCE in contaminated groundwaters. (Berns et al., 2019; Culpepper et al., 2018; He et al., 2015, 2010; Lee and Batchelor, 2002a, 2002b; Liang et al., 2009, 2007; Schaefer et al., 2017)

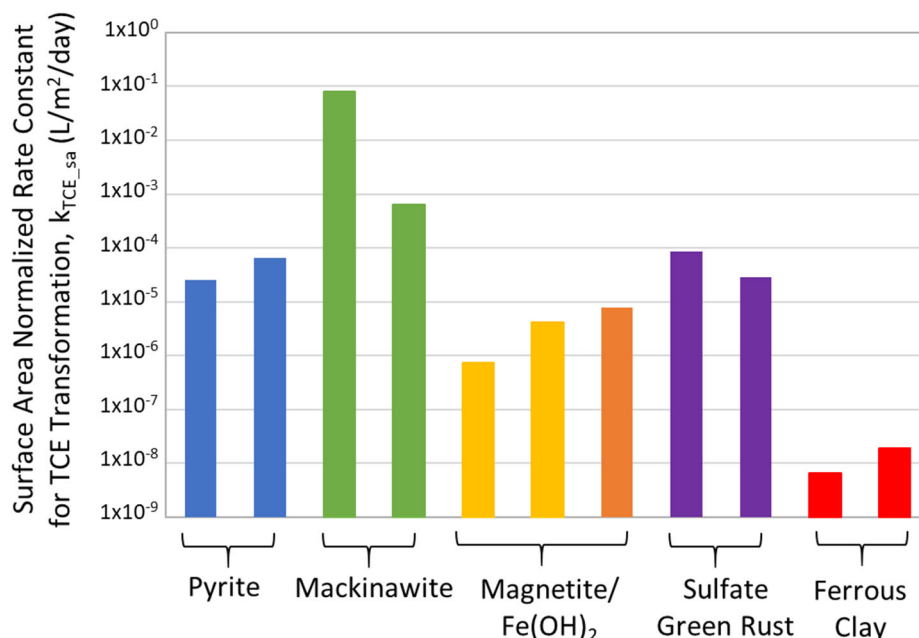


Figure 2.3. Abiotic TCE transformation rates promoted by different iron minerals in the absence of oxygen. Data from He et al. (2015), Lee & Batchelor (2002a), Liang et al. (2009), He et al. (2010), Liang et al. (2007), Lee & Batchelor (2002b), Culpepper et al. (2018), Schaefer et al. (2017), Berns et al. (2019).

2.3 Aerobic Transformation of Chlorinated Ethenes

TCE may be also transformed abiotically with reactive mineral species under aerobic conditions (Pham et al., 2008; 2009). As with anaerobic reactions, reduced iron minerals have shown great potential to promote this reaction, especially iron sulfide minerals, such as mackinawite. The mechanism has not been confirmed, but data from aqueous ferrous iron studies suggests that structural or sorbed phase ferrous iron react with oxygen to form superoxide (Li et al., 2007). The ferrous iron then reacts with superoxide to form hydrogen peroxide, which then reacts again with ferrous iron to form the hydroxyl radical. These radicals then oxidize TCE to form organic acids.

While abiotic TCE transformation rates under aerobic conditions are typically slower than biological reductive dechlorination, they can also be more desirable because they typically select for the aforementioned organic acids (right side of **Figure 2.2**). This avoids the intermediates

DCE and VC, which can be more mobile and toxic than TCE. A comparison of TCE transformation rates by nontronite, iron sulfides, and ferrous clays are presented in **Figure 2.4**.

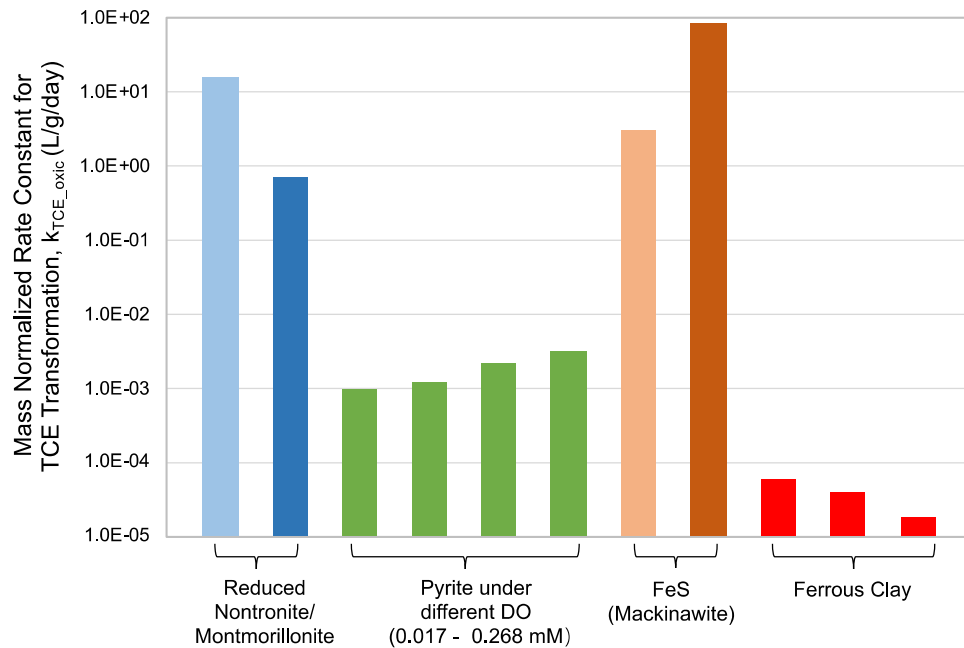


Figure 2.4. Abiotic TCE transformation rates promoted by different iron minerals in the presence of oxygen. Data from Liu et al. (2017), Pham et al. (2009), Pham et al. (2008), Cheng et al. (2020), Schaefer et al. (2018).

3.0 Materials and Methods

3.1 Overall Approach and Rationale

The overall experimental approach to address the project objectives are addressed in five research tasks, and several associated subtasks. These are illustrated in **Figure 3.1**. Task 1 involved obtaining and characterizing site material, and we collected and characterized groundwater sediments and groundwater from Pease Air Force Base (AFB). Both low (i.e., clay/silt) and high (sand) permeability sediments were collected, including groundwater from the high permeability zone. Pease AFB was chosen because at this site back diffusion of TCE is occurred from the low to high permeability sediments. Sediment cores were characterized for mineralogy to identify potentially reactive iron-bearing minerals, total organic carbon to determine potential electron donating capacity, and magnetic susceptibility as an indicator of the mineral magnetite. Groundwater was analyzed for pH, alkalinity, dissolved oxygen, dissolved organic carbon (DOC), TCE, TCE reaction products, and relevant ions (e.g., Fe^{2+} , NO_3^- , SO_4^{2-} , HS^-). Efforts were also taken to characterize the dechlorinating microbial community naturally present in the low permeability clay, but we were not successful in extracting sufficient DNA for analysis.

Task 2 involved batch studies to evaluate the biological (biotic) and non-biological (abiotic) TCE reactivity of Pease AFB materials, as well as several low permeability clays from other sites, and pure iron minerals that were identified in these clays. These minerals included iron carbonates, iron sulfides, illite, and biotite. *This task allowed us to test the hypothesis that both biotic and abiotic transformation processes are occurring in LPZ material, and are potentially enhancing natural attenuation of TCE.* TCE reactivity was evaluated by measuring either the disappearance of TCE, or the appearance of TCE reaction products. No biotic TCE reactivity was observed in the LPZ materials unless they were amended with a dechlorinating culture, so anaerobic biotic reactivity was then evaluated by adding a laboratory grown dechlorinating culture (DCC). Abiotic TCE reactivity was evaluated under both anaerobic and aerobic conditions. The former assessed abiotic TCE reduction, whereas the latter evaluated abiotic TCE oxidation. In several experiments performed with iron sulfide minerals, both iron and sulfate reducing cultures were added to the bottles to promote more reducing conditions. TCE reactivity in all experiments was quantified by pseudo first order rate constants. These rate constants were compared to different material and material-solution properties. These include total and ferrous iron in solution, sorbed ferrous iron, total and structural ferrous iron of the solids, and magnetic susceptibility.

Task 3 required constructing a two-dimensional flow cell, filling the flow cell with low permeability clay overlain by high permeability sand, and then evaluating the impact of biotic and abiotic TCE reactions in the clay on back diffusion to the sand. *This task allowed us to test the hypotheses that contributions of biotic and abiotic reaction mechanisms depend on permeability, reactive minerals, dissolved oxygen, and associated mass transfer limitations, and these reactions*

control the flux of TCE and transformation products out of the LPZ. Pease AFB materials were used in these experiments, and a source of TCE was created at the bottom of the clay using Tenax beads equilibrated with this chemical. Also, the clay was premixed with the aforementioned DCC prior to placing in the flow cell. Two flow cell experiments were conducted. In one experiment, anaerobic groundwater containing the electron donor lactate were continually purged through the high permeability sand to create an electron donor source at the sand-clay interface, that diffused into the clay and promoted biological reaction of TCE. In the other experiment, this same protocol was followed for 70 days, at which point aerobic groundwater without an electron donor was continuously purged through the high permeability sand to promote oxygen diffusion into the clay and abiotic TCE oxidation. All flow cell effluent was collected in a bypass loop, which was injected into bottles with headspace and analyzed for TCE and reaction products.

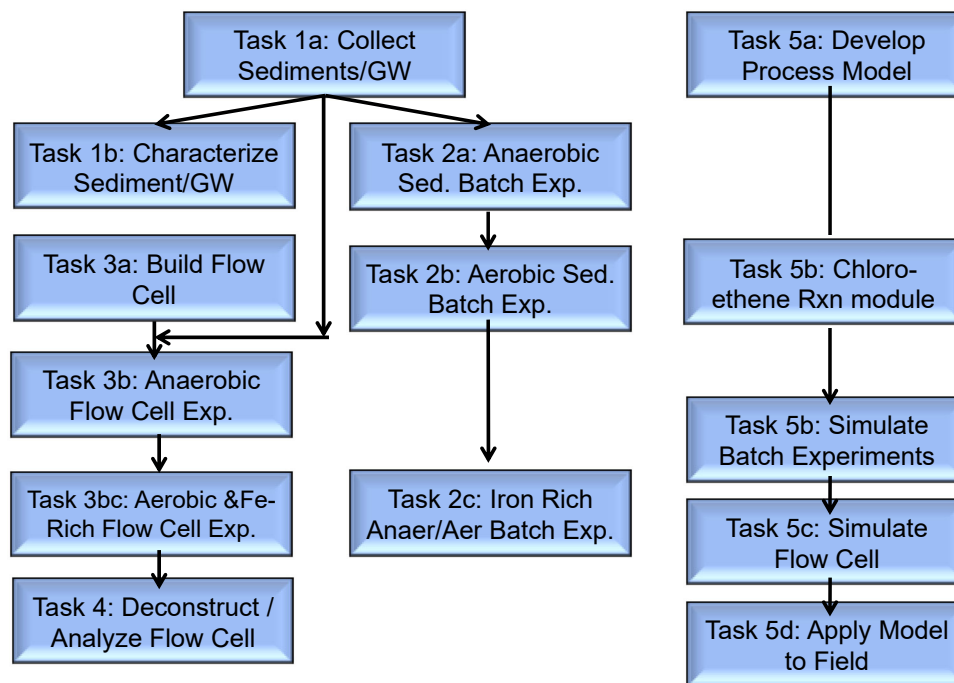


Figure 3.1. Experimental approach, segregated into Tasks.

For Task 4, the flow cells were frozen at the end of each experiment. The external frames were then removed, and the clay and sand were cut along vertical and horizontal directions to create small frozen cubes. These cubes were then placed into headspace vials, allowed to thaw, and then the headspace was analyzed for TCE and reaction products. *This approach allowed us to test the hypothesis that microbial ecology and mineral oxidation state will vary with depth into the LPZ due to mass transfer of electron acceptors and donors from the HPZ.* Unfortunately, we could not extract sufficient DNA from the clay to assess changes in microbial ecology, and mineralogical analyses showed no changes with depth. However, we were able to measure changes in TCE and

reaction products with depth, and this allowed us to distinguish biotic and abiotic reactions occurring in the flow cell.

Task 5 involved development a one-dimensional (1D) numerical model to simulate flow cell experiments, extension of this model to a larger scale, and then incorporation of key components of this model into the commercial software RT3D in a computationally efficient manner. *This task allowed us to test the hypothesis that natural attenuation can be accurately captured using RT3D with new modules for relevant reactions and diffusive mass transfer processes, and that these modules will allow us to quantitatively uncouple and predict the contribution of different natural attenuation mechanisms in LPZ experiments.* For all models, operator splitting was used to separate development of the transport and reaction modules.

3.2 Materials

Unless specified, all chemicals were obtained from Sigma-Aldrich (ACS grade). Trichloroethene (TCE) was obtained as a pure solvent. Gas standards (methane, ethane, ethene, acetylene, propane, propylene, methyl acetylene, butane in a nitrogen balance) were purchased from Sigma Aldrich (St. Louis, MO). Acetylene was also obtained as a standard dissolved in methanol (Matheson Tri-Gas, stabilized with acetone; SPEX CertiPrep, 1000 µg/mL in methanol). Iron and sulfur salts were used to make stock solutions: Fe(II)Cl₂·4H₂O (Sigma-Aldrich, puriss) for ferrous iron, Na₂SO₄ for sulfate, and Na₂S·9H₂O for sulfide. All solutions were prepared with Milli-Q water that was degassed for one hour with N₂. Ferrous iron solutions were prepared with degassed Milli-Q water that was then stirred in the anaerobic chamber (Coy Laboratory Products, N₂/H₂ 97%/3%) overnight to remove trace oxygen before adding Fe(II)Cl₂·4H₂O. Lactate solutions were made by mixing 60% (w/w) sodium DL-lactate (Sigma-Aldrich) with Milli-Q water. Ferrihydrite slurries were prepared using the standard method of Cornell and Schwertmann (2003), and final concentrations were determined by ICP-MS after digestion in HCl.

In selected batch experiments using Pease AFB sediments, and in flow cell experiments, groundwater from Pease AFB was used as the growth medium. In selected batch experiments using different clay materials, Milli-Q water was used after amendment with CaCl₂ to serve as background electrolyte. In selected batch experiments using iron sulfide minerals, a tris-buffered medium was used. It was prepared at 100 mM using Trizma base and Trizma hydrochloride (pH 7.5) with the following per liter of Milli-Q water: 0.027 g NH₄Cl, 0.068 g KH₂PO₄, 0.2 g MgCl₂·6H₂O, 0.15 g CaCl₂·2H₂O, 0.25 g NaCl (Fisher Scientific), 0.25 g yeast extract (Fisher Scientific), 5 mL ATCC mineral solution (MD-TMS), 2.5mL ATCC vitamin solution (MD-VS). In selected batch experiments using iron sulfide, a bicarbonate-buffered medium was used. It was prepared by degassing a 100mM bicarbonate solution for one hour with N₂/CO₂ 80%/20% (pH 6.9). The medium contained the following per liter of Milli-Q water: 0.027 g NH₄Cl, 0.068 g KH₂PO₄, 0.2 g MgCl₂·6H₂O, 0.15 g CaCl₂·2H₂O, 0.25 g NaCl (Fisher Scientific), 0.25 g yeast

extract (Fisher Scientific), 5 mL ATCC mineral solution (MD-TMS), 2.5mL ATCC vitamin solution (MD-VS).

3.3 Pease AFB Material Collection

Soil and groundwater were obtained from Site 34 at Pease Air Force Base in Portsmouth, New Hampshire (**Figure 3.2**). An upper sand and the underlying marine clay and silt were targeted for sampling (Soloyanis et al., 1996). Soil cores were pushed using a Geoprobe 7822DT direct push rig equipped with MC5 tooling (**Figure 3.3**). Cores were sealed with endcaps, placed in a cooler on ice and shipped overnight to the laboratory, where they were then stored in a 4°C room in an argon-filled container to minimize oxygen exposure. Site material was also obtained from four other clayey formations at Department of Defense (DoD) sites. This was not used in flow cell work, but in batch studies for comparison to the Pease AFB sediments. Prior to use in abiotic batch experiments, sediments were sterilized via gamma irradiation (2.5 M-rads) to limit microbial activity. Sediments were homogenized by hand in a glass bowl within the anaerobic chamber; any large solids (> 5 mm) present in the sediments were discarded.

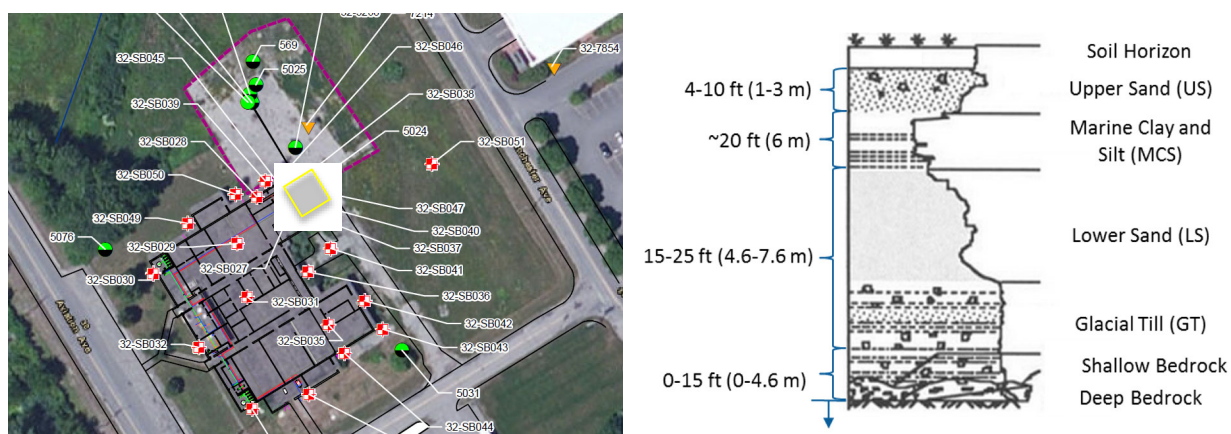


Figure 3.2. Pease AFB plan view and stratigraphic depth profile.

Groundwater was pumped from a nearby well directly into Cornelius kegs that had been prewashed and filled with nitrogen to keep the water anaerobic. It was also shipped to the laboratory and stored at 4°C until use.



Figure 3.3. Sediment core and groundwater collection at Pease AFB.

3.4 Iron-Enriched Material

The iron-enriched mineral fractions identified in the Pease AFB clay and other low permeability materials were either purchased from commercial vendors or made synthetically in the laboratory. Siderite was purchased from SIDCO Minerals Inc. (Texarkana, TX), illite was purchased from greenclays.com (Las Vegas, NV), biotite was purchased from Ontario, CA, and ankerite was acquired from Austria. Iron sulfides were directly precipitated from solution as described in the methods section below for batch experiments

3.5 Microbial Community

A mixed dechlorinating community (DCC), originally obtained from river sediments on the Sangamon River (IL) in 2001 and maintained on PCE and lactate, was used in this study. Acetate and hydrogen (H_2), resulting from the fermentation (and oxidation) of lactate, promoted biological reductive dechlorination. We evaluated whether the dehalogenase enzymes *tceA*, *vcrA*, and *bvcA*, as well as *Dehalococcoides*, were present by amplifying 16S rRNA genes in DNA extracts using PCR primers from Behrens et al. (Behrens et al., 2008). Cultures for this study were maintained on nitrogen-sparged groundwater from Pease AFB containing 1 mM lactate and 100 μ M TCE.

Desulfovibrio vulgaris was used as a model sulfate-reducing bacteria. It was grown anaerobically on both the tris-buffered and groundwater media. Media was degassed for 60 minutes with N_2 , sealed with stoppers and crimp caps, and autoclaved. The *D. vulgaris* was maintained by transferring to fresh medium every two weeks; 3 mL of the previous generation was added to new medium (total volume 100 mL) with sulfate and lactate and then incubated at 30°C. Cultures used for experiments were washed with buffer and adjusted to an OD 600 of 0.1, before adding 3 mL to each biotic batch serum bottle.

Shewanella oneidensis was used as a model iron reducing bacteria. Cells were streaked on LB agar plates (Fisher Scientific) from a stock stored at -80°C and incubated overnight at 30 °C. Colonies

from the plates were selected and rinsed in buffer before inoculating experiments. Lactate was supplied as the carbon source and electron donor and ferrihydrite was the electron acceptor.

3.6 Batch Experimental Setup

Batch experiments were performed in either 40 mL glass vials, or 160 mL glass bottles, both sealed with Teflon-lined septa (**Figure 3.4**). The 40 mL vials had caps with Mininert® valves and the threads were sealed with epoxy. In some cases these vials were stored in secondary containers continuously sparged with nitrogen to ensure anaerobic conditions. The 160 mL bottles had crimp caps, and were stored upside down in a water to prevent TCE volatilization losses, and to minimize oxygen diffusion into anaerobic experiments. Both vials and bottles were amended with groundwater or synthetic media, leaving room for headspace. In most experiments natural solids or minerals were added to the batch experiments before sealing, and then they were amended with TCE. Natural solids were typically gamma-irradiated prior to abiotic experiments to prevent biological activity. Batch reactors were either mixed by hand or on a shaker platform. Mass balances on control bottles containing only TCE and water demonstrated losses of less than 5% over six weeks in both vial/bottle setups. Details of the different batch experiments follow.



Figure 3.4. Photos showing 40 mL glass vials and 160 mL glass bottles used in batch studies, along with double containment approaches to maintain anaerobic conditions and/or minimize volatilization losses.

3.6.1 Anaerobic Batch Experiments with Natural Sediments

These experiments were performed in both 40 mL vials and 160 mL bottles with varying conditions. For batch experiments in 40 mL bottles, eight different aquifer solids were evaluated and these were obtained from various DOD sites including Pease AFB. Three of these are called soils, and five sediments. However, this distinction is arbitrary and the names are used because they match terminology in the publications in which they were presented. All preparations were performed in an anaerobic chamber. Briefly, soil (6 g) was placed in 40 mL glass vials. Approximately 15 mL of 5 mM CaCl_2 electrolyte solution sparged with nitrogen gas was then

added. A contaminant spiking solution, which contained neat PCE or TCE in the electrolyte, was prepared using the same nitrogen-sparged electrolyte. Either the spiking solution (15 mL) or the contaminant-free electrolyte was added to each vial to bring the final aqueous volume to 30 mL, and TCE or PCE concentrations to 3 mM. Use of both contaminant-spiked and non contaminant-spiked conditions served as a means to distinguish generation of PCE/TCE dechlorination products (e.g., ethene, ethane, acetylene, propane) due to current abiotic/biotic reaction from that of gas generation due to any other carbon sources and/or any accumulation of these gases in the soil samples from dechlorination that may have occurred *in situ* prior to sample collection. The vials were capped with the aforementioned Mininert® valves. The vials were then removed from the anaerobic chamber and placed in the secondary containers under a stream of nitrogen. The vials were gently inverted several times to enhance mixing, then returned to the anaerobic chamber and placed on shakers at 50 RPM. Elevated temperature conditions were also evaluated, and these vials were placed in an Innova 44R incubator and shaker table (New Brunswick Scientific Co., Edison, NJ). Vial headspace was sampled as a function of time for chlorinated ethenes and reduced gases.

For batch experiments in 160 mL bottles, abiotic reactivity of the Pease clay with both TCE and cis-DCE was evaluated. The glass bottles (160 mL) were first filled with 100 mL of Milli-Q water and degassed with nitrogen for 30 min. The bottles were then crimp-sealed with Teflon-lined butyl stoppers and autoclaved. After returning to room temperature, the bottles were placed in an anaerobic chamber, opened, and amended with 10 g of Pease clay. After re-capping, TCE or cis-DCE was added neat to obtain an initial aqueous concentration of 400 μ M after partitioning between phases. Concentrations of volatile compounds were then measured over time in the headspace of the batch reactors, and the total mass of reactants and products in the batch bottles was determined assuming equilibrium partitioning. All batch experiments were performed in duplicate or triplicate.

3.6.2 Aerobic Batch Experiments with Natural Sediments

Aerobic batch experiments were performed in 40 mL vials and monitored similarly to the anaerobic experiments. The difference is that aerobic experiments had ultra-high purity oxygen injected into the vial headspace just prior to amending with TCE. Equal amounts of vial headspace were removed and replaced with oxygen to maintain atmospheric pressure. Vial headspace was periodically monitored and maintained above 8% O₂, which (based on Henry's Law) resulted in aqueous dissolved oxygen concentrations being maintained above 120 μ M. Controls were also performed that contained no added TCE. This allowed monitoring of hydroxyl radicals produced over time from reaction of oxygen with ferrous iron.

3.6.3 Anaerobic and Aerobic Batch Experiments with Pure Minerals Added

Anaerobic batch experiments with pure minerals were performed in 40 mL vials using siderite, illite, biotite, and ankerite following the same methods described for anaerobic batch experiments with natural sediments. However, instead of adding 6 g of natural sediment, 6 g of a pure mineral was added. Otherwise, the procedures for the anaerobic batch experiments were the same. There is an exception for two experimental conditions. This involved amending experiments with pure illite and siderite with 1.8 mM of ferrous iron (from $\text{FeCl}_2\text{-H}_2\text{O}$). This allowed evaluation of the effect of sorbed iron on TCE reactivity. At the conclusion of anaerobic batch experiments, the reduction potential and pH were measured.

Aerobic batch experiments with pure minerals were also performed in 40 mL vials using siderite, illite, biotite, and ankerite, following the similar methods described for aerobic batch experiments with natural sediments. However, no TCE was added to the bottles. Instead, only the generation of hydroxyl radicals was monitored, as these have been shown to oxidatively dechlorinate TCE (Pham et al., 2008; Liu et al., 2017; Schaefer et al., 2018). Bottle headspace was flushed with air at time zero and daily thereafter; periodic aqueous samples were collected during the 6-day duration of the experiments. Bottles were placed on a shaker (50 rpm) during incubation. At the conclusion of aerobic batch experiments, the pH was measured.

3.6.4 Anaerobic Batch Experiments with *In Situ* Generated Iron Sulfide Minerals Under Sulfate Reducing Conditions

Five sets of batch experiments were conducted in this study as shown in **Table 3.1**. They are classified as 1) abiotic sulfide, 2) biotic sulfate, 3) abiotic sulfate/sulfide, 4) microbial product and 5) natural groundwater experiments. The first four sets of experiments were prepared with a tris-buffered medium to avoid large pH shifts and independently probe the influence of sulfur source, iron concentration, and presence of bacteria. The last set used Pease AFB groundwater instead of tris-buffered medium to evaluate whether the primary reactivity trend observed with tris-buffered media extends to natural systems. All batch experiments were run in triplicate, except for control bottles with no sulfur or no iron. The batch experiments were performed in 160 mL glass serum bottles filled with 100 mL of medium (either tris-buffered or groundwater) that was degassed with N_2 for one hour and sealed with Teflon-lined butyl stoppers and crimp caps. The medium was autoclaved and allowed to cool to room temperature before adding ferrous iron (Fe^{2+}), sulfate (SO_4^{2-}), sulfide (HS^-), lactate, and/or *D. vulgaris*.

In the abiotic sulfide experiments, serum bottles were amended to contain 1 mM HS^- , 10 mM lactate and varying amounts of ferrous iron for Fe:S ratios 0:1, 2:1, 20:1, 50:1. The lactate was added to replicate media conditions in the biotic sulfate experiments minus bacteria. In the biotic sulfate experiments, 3% *D. vulgaris* (v/v) was added to serum bottles with 1 mM SO_4^{2-} as the sulfur source and electron acceptor and 10 mM lactate as the carbon source and electron donor. Bacteria were excluded in abiotic sulfide experiments; sulfur was added as 1mM HS^- , and 10 mM

lactate was included to allow for comparison with biotic experiments. Both abiotic and biotic experiments were conducted at iron:sulfur ($\text{Fe}^{2+}:\text{S}$) molar ratios of 0:1, 2:1, 20:1, and 50:1, which were chosen based on results from screening experiments. A ratio of 0:1 is sulfur only. Abiotic sulfide experiments (no *D. vulgaris*) were conducted at a ratio of 20:0 to evaluate ferrous iron alone. Biotic sulfate experiments were also conducted at $\text{Fe}^{2+}:\text{S}$ ratios of 35:1 and 100:1 to explore transitions and limits in TCE reactivity.

Abiotic sulfate/sulfide control experiments were conducted to probe the potential TCE reactivity of a system with Fe^{2+} and either SO_4^{2-} or mixed $\text{SO}_4^{2-}/\text{HS}^-$ in the absence of *D. vulgaris*. These experiments consisted of 10 mM lactate and no bacteria with 50 mM Fe^{2+} . Either 1mM SO_4^{2-} or both 0.5 mM SO_4^{2-} and 0.5 mM HS^- were added as sulfur sources.

Table 3.1. Experimental conditions used to evaluate iron sulfide reactivity for TCE in the presence and absence of sulfate reducing bacteria.

Experiment Set	Sample Name	Sulfate (mM)	Sulfide (mM)	Ferrous Iron (mM)	Lactate (mM)	<i>D. Vulgaris</i>
Abiotic Sulfide	X:HS- 0:1		1		10	no
	Fe2+:HS- 2:1		1	2	10	no
	Fe2+:HS- 20:1		1	20	10	no
	Fe2+:HS- 50:1		1	50	10	no
Biotic Sulfate	X:SO42- 0:1	1			10	yes
	Fe2+:SO42- 2:1	1		2	10	yes
	Fe2+:SO42- 20:1	1		20	10	yes
	Fe2+:SO42- 35:1	1		35	10	yes
	Fe2+:SO42- 50:1	1		50	10	yes
	Fe2+:SO42- 100:1	1		100	10	yes
Abiotic Sulfate/Sulfide	Fe2+:X 20:0			20	10	no
	Fe2+:SO42- 50:1	1		50	10	no
	Fe2+:HS-:SO42- 50:0.5:0.5	0.5	0.5	50	10	no
Microbial Product	Fe2+:HS- 50:1 Autoclaved		1	50	10	autoclaved cells
	Fe2+:HS- 50:1 Filtered		1	50	10	filtered (no cells)
	Fe2+:X 50:X Filtered			50	10	filtered (no cells)
	X:X 0:0 Filtered Culture				10	filtered (no cells)
Groundwater Biotic	Fe2+:SO42- 1:2	2		1	10	yes
	Fe2+:SO42- 1:1	1		1	10	yes
	Fe2+:SO42- 2:1	1		2	10	yes
	Fe2+:SO42- 4:1	1		4	10	yes
	Fe2+:SO42- 6:1	1		6	10	yes
	Fe2+:SO42- 8:1	1		8	10	yes
	Fe2+:SO42- 10:1	1		10	10	yes
	Fe2+:SO42- 20:1	1		20	10	yes

The final experimental set used Pease Air Force Base groundwater and was conducted with 1 mM SO_4^{2-} , 10 mM lactate, and 3% *D. vulgaris* (v/v). Ferrous iron was added to obtain a range of $\text{Fe}^{2+}:\text{SO}_4^{2-}$ molar ratios (1:2, 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, and 20:1).

Microbial product experiments aimed to distinguish between the role of active microbial metabolism and the presence of inactive microbial material on TCE transformation. Autoclaved and filtrate from *D. vulgaris* cultures were used to explore the contributions of dead cellular material and soluble microbial species, respectively. Autoclaved cultures were cooled to room temperature before adding 50 mM Fe^{2+} , 1 mM HS^- , and 10 mM lactate. *D. vulgaris* cultures were passed through 0.2 μM filters anaerobically to separate cells from smaller soluble species. The filtrate was then resealed in new, sterile serum bottles before the same iron, sulfur, and lactate additions. Additional microbial product experiments were conducted without a sulfur source and without both iron and sulfur to evaluate potential TCE reactivity in the absence of iron sulfide precipitates. For these control experiments, the cultures were grown on 10 mM lactate and 1 mM sulfate, and then the culture was bubbled with nitrogen to remove sulfide, before adding iron and TCE.

After adding all aforementioned constituents to batch experiments, they were aged for 3 weeks at 30°C to promote growth and activity of *D. vulgaris*, as well as iron sulfide precipitation. Dark black mineral precipitates usually appeared within seconds of adding all constituents in the abiotic experiments, and biotic experiments were initially grey and turned dark black in color within a week and did not change color for the remainder of the aging time. After aging, the batch experiments were sampled for pH and total aqueous iron; the latter was determined by removing an aliquot, filtering through a 0.2 μm filter, and immediately acidifying with nitric acid. Neat TCE was then added to each batch experiment to obtain an initial aqueous concentration of 400 μM after partitioning between media and headspace, and experiments were stored at 25°C after spiking with TCE. Concentrations of TCE and acetylene were monitored over time.

At the conclusion of each experiment, the reduction potential was measured, filtered aqueous samples were taken for total and ferrous iron measurement, and solids and solution were separated using vacuum filtration with a hydrophilic, PVDF Durapore membrane filter. The solids were frozen for later analysis, which included analysis of total and sorbed ferrous iron, mineralogy, surface area, and mineral oxidation state.

3.6.5 Anaerobic Batch Experiments with *In Situ* Generated Iron Sulfide Minerals Under Iron Reducing Conditions

Biotic and abiotic batch experiments with either ferrihydrite or ferrous iron were conducted under anaerobic conditions with 100 mL of solution in 160 mL serum bottles. Buffered media was degassed with N_2/CO_2 80%/20% for one hour and the serum bottles were sealed with Teflon-lined butyl stoppers and crimp seals. The medium was then autoclaved and cooled to room temperature. Ferrihydrite ($\text{Fe}(\text{OH})_3$), ferrous iron (Fe^{2+}), sulfide (HS^-), lactate, and *S. oneidensis* were added to the bottles and incubated at 30°C for three weeks to achieve a pseudo equilibrium in the solid precipitates.

Five sets of batch experiments were conducted and are classified as 1.) biotic ferrihydrite, 2.) abiotic ferrihydrite, 3.) biotic ferrous iron, 4.) abiotic ferrous iron, and 5.) no sulfur biotic ferrous iron (**Table 3.2**). The no sulfur biotic ferrous iron experiments were a control for the role of sulfur and have limited discussion throughout. Biotic experiments were inoculated with *S. oneidensis*. The first four experimental sets used iron (ferrihydrite or Fe^{2+}) to sulfide ratios of 1:1, 2:1, 5:1, and 10:1; the fifth set was conducted with the same iron concentrations but without sulfide. While it is known that ferrihydrite has a hydrous mineral structure, is denoted as $\text{Fe}(\text{OH})_3$ for simplicity in figures, tables, and text hereafter.

Table 3.2. Experimental conditions used to evaluate iron sulfide reactivity for TCE in the presence and absence of iron reducing bacteria.

Experiment Set	Sample Name	Sulfide (mM)	Ferrihydrite (mM)	Ferrous Iron (mM)	Lactate (mM)	<i>S. Oneidensis</i>
Biotic Ferrihydrite	Fe(OH)3:HS- 1:1	1	1		10	yes
	Fe(OH)3:HS- 2:1	1	2		10	yes
	Fe(OH)3:HS- 5:1	1	5		10	yes
	Fe(OH)3:HS- 10:1	1	10		10	yes
	Fe(OH)3:X 1:0		1		10	yes
	Fe(OH)3:X 10:0		10		10	yes
	X:HS- 0:1	1			10	yes
Abiotic Ferrihydrite	Fe(OH)3:HS- 1:1	1	1		10	no
	Fe(OH)3:HS- 2:1	1	2		10	no
	Fe(OH)3:HS- 5:1	1	5		10	no
	Fe(OH)3:HS- 10:1	1	10		10	no
Biotic Ferrous Iron	Fe2+:HS- 1:1	1		1	10	yes
	Fe2+:HS- 2:1	1		2	10	yes
	Fe2+:HS- 5:1	1		5	10	yes
	Fe2+:HS- 10:1	1		10	10	yes
Abiotic Ferrous Iron	Fe2+:HS- 1:1	1		1	10	no
	Fe2+:HS- 2:1	1		2	10	no
	Fe2+:HS- 5:1	1		5	10	no
	Fe2+:HS- 10:1	1		10	10	no
No Sulfide Biotic Ferrous Iron	Fe2+:X 1:0			1	10	yes
	Fe2+:X 2:0			2	10	yes
	Fe2+:X 5:0			5	10	yes
	Fe2+:X 10:0			10	10	yes

The biotic ferrihydrite experiments were conducted to evaluate how ferrihydrite loading influences the potential for TCE transformation during biological iron reduction by *S. oneidensis*. Three controls were included in this set: 1 mM ferrihydrite with no sulfide, 10 mM ferrihydrite with no

sulfide, and 1 mM sulfide with no ferrihydrite. Except for the controls, experiments in this set were conducted in triplicate. The abiotic ferrihydrite set was included to evaluate the extent to which secondary reactive minerals may precipitate in the presence of sulfide, but in the absence of biological iron reduction, and did not have replicates. The conditions are identical to the biotic ferrihydrite experiments, except lacking *S. oneidensis*.

Both abiotic and biotic ferrous iron experiments were conducted to evaluate potential differences in TCE transformation kinetics when reduced iron is initially available in solution as opposed to being initially present in the oxidized form as ferrihydrite. The biotic ferrous iron experiments further evaluated conditions in which *S. oneidensis* is able to “re-reduce” iron that became oxidized during the experiments. Finally, the no sulfide biotic ferrous iron set evaluated the potential for TCE transformation in the absence of sulfide, and targeted potential reactions with ferrous iron and soluble microbial species. These three experimental sets were conducted without replicates.

All experimental sets were aged for three weeks at 30 °C to either promote Fe(III) reduction by *S. oneidensis* coupled with sulfide precipitation, or to allow for abiotic iron sulfide precipitation. After three weeks, the batch experiments were sampled for total aqueous iron by filtering an aliquot through a 0.2 µm filter and acidifying with nitric acid. The pH was also measured after aging. TCE was then added to the experiments neat to achieve an initial aqueous phase concentration of 400 µM. After adding TCE, batch experiments were stored at 25°C. Five measurements were taken over five weeks for TCE and acetylene. Before terminating the batch experiments, reduction potential and pH were measured. Solid precipitates were then separated from the aqueous phase using vacuum filtration, and the solids were frozen and stored for later characterization.

3.7 Flow Cell

A stainless steel and glass flow cell was constructed from the components shown in **Figure 3.5**. Two 0.64 cm-thick sheets of glass were placed on the front and back faces of a stainless steel frame, separated with Viton gaskets to prevent leaks. Aluminum windows were bolted to the outside using holes bored through the windows and frame. One-eighth inch fittings tapped in the frame on the edges were used to introduce groundwater, and a thin, rectangular opening in the top of the frame was used to pack sand and clay. A stainless steel plate was used to seal the top of the flow cell. The inside dimensions of the flow cell were 16.5 cm x 2 cm x 16.5 cm (LxWxH).

The flow cell setup with inlet reservoir, pump, sampling loop, and effluent reservoir is shown in **Figure 3.6**. At the bottom of the flow cell is a thin layer (~0.25cm) of Tenax beads, pre-equilibrated with TCE in a serum bottle for a week. Pease clay was initially mixed with 20 mL of the dechlorinating culture (DCC) inoculum in an anaerobic chamber and aggregated into ~1cm balls. These were then transferred out of the chamber to a fume hood, placed in the bottom of the flow cell in approximately 1 cm lifts, and compacted together into a composite layer using a

stainless steel rod. The top of the clay was then smoothed with the rod. For the HPZ, Pease sand was packed on top of the clay with stainless steel mesh on the left and right edges to distribute flow through the sand uniformly. During packing, the groundwater level in the flow cell was always maintained above the solids. The groundwater was sparged with nitrogen before adding to the flow cell to maintain anoxic conditions. After packing, a top plate was used to seal the flow cell and the experiment was performed in the fume hood. The flow cell represents a pseudo two-dimensional system with LPZ/HPZ interface created between Pease AFB clay on the bottom, and Pease AFB sand on the top. Flow only occurs in the sand, and not in the low permeability clay. As a result, any mass transport in the clay is controlled by diffusion.

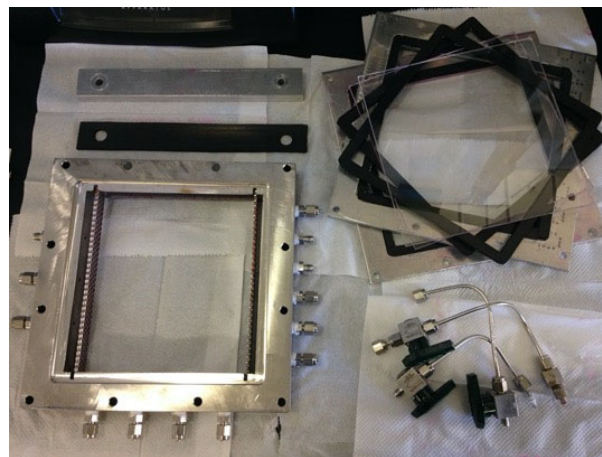


Figure 3.5. Flow cell components.

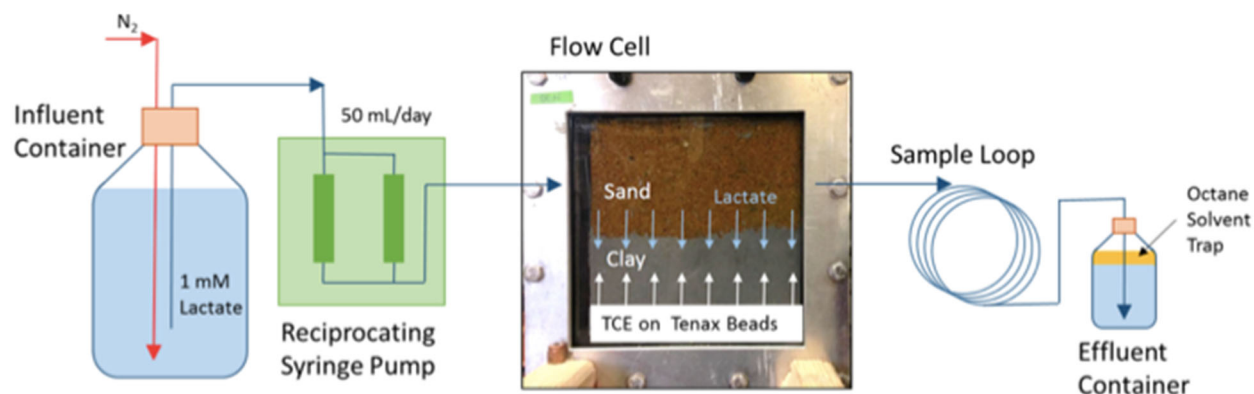


Figure 3.6. Flow cell setup.

Influent groundwater, amended with 1 mM lactate as the electron donor, was continuously sparged with nitrogen and introduced to the flow cell HPZ with a reciprocating (push/pull) syringe pump (Harvard Apparatus PhD Ultra) at 50 mL/day (retention time in sand is 1.5 days). Lactate in the HPZ fermented into acetate, propionate, and hydrogen, and these solutes diffused into the LPZ at the interface. On the bottom of the flow cell, TCE on the Tenax dissolved into solution and diffused upward through the LPZ. The electron donors and TCE mixed via diffusion and promoted biological reaction. Reaction products that diffused out of the LPZ were quickly removed from the flow cell via advection in the HPZ. Flow cell effluent was either collected in a 180 cm (4.5 mL volume) stainless steel sample loop, or a 200 mL effluent container (which was replaced with a clean container regularly). Effluent in the stainless steel sample loop was purged directly into a

bottle, and the headspace was analyzed for volatiles. Solution in the bottle was analyzed for inorganic anions to estimate reducing conditions, and organic acids to monitor utilization of electron donor. A second flow cell experiment identical to the first was run for 70 days. However, after this time, new groundwater saturated with air and containing no lactate was delivered to the flow cell HPZ to promote abiotic TCE oxidation.

The flow cell experiments were terminated after 180 days by closing the flow valves and freezing at -80°C . The flow cell was then unbolted, the glass was removed, and the frozen LPZ and HPZ material was removed from the frame. The frozen clay comprising the LPZ was cut into 1cm-wide

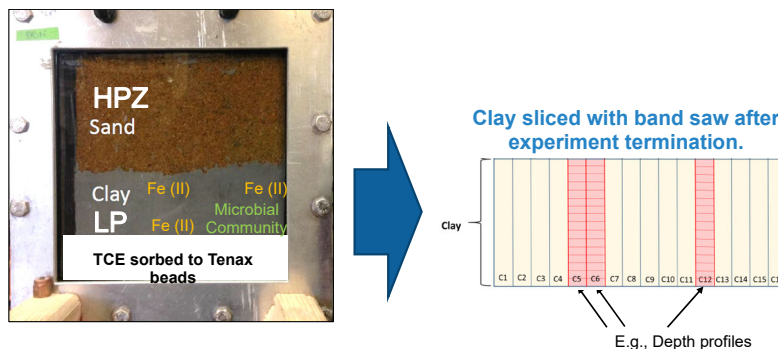


Figure 3.7. Illustration of how the clay was sliced into small segments for analyses.

vertical sections with a band saw (Ryobi 9 inch), and then further cut into 0.5cm depth increments (**Figure 3.7**). Each 1 cm x 2 cm x 0.5 cm frozen block was placed in an 8.5 mL vial, crimp-sealed with a Teflon-lined butyl stopper and allowed to thaw overnight at room temperature. Headspace in the vials was then analyzed for volatiles compounds. Each vertical profile was evaluated in triplicate, and the concentrations reported at each depth are an average of the three measurements. A fourth vertical profile was used to determine ferrous iron content; these profiles were also determined from frozen blocks (1 cm x 2 cm x 0.75 cm) cut from the flow cell material.

3.7.1 Tenax Equilibration

The amount of TCE needed to fully saturate Tenax beads was calculated using the Freundlich isotherm constants for TCE and Tenax determined by (Xing et al., 1996). A 160 mL bottle (crimp sealed using a Teflon-lined stopper) with 100 mL Milli-Q water, ~6 mL of TCE, and 2g of Tenax beads was shaken vigorously for ~5 minutes, and then placed on a bottle roller for one week to equilibrate. After removing from the bottle roller, the bottle was again shaken vigorously for ~5 minutes. In the hood, the crimp and stopper were removed, and the contents of the bottle were poured through a mesh to separate the Tenax beads and water. The TCE-saturated Tenax beads were immediately placed in the base of the flow cell, and covered with a thin layer of clay, before packing the remaining flow cell material.

3.8 Analytical Methods

3.8.1 GC Method for Volatiles

Volatiles headspace samples were quantified using either an Agilent 6890A gas chromatograph equipped with an FID and Agilent GS-Q column (dimensions 30m 0.32mm, Agilent 113-3432), or a Shimadzu 2010+ gas chromatograph equipped with a Flame Ionization Detector (FID) and an RT-QS-BOND fused silica PLOT column. Oven temperature programs varied slightly, and one approached used: start 40°C, ramp 10°C/min to 80°C, hold 2 minutes, ramp 50°C/min to 200°C, hold 5.5 minutes. Inlet and FID were held at 250°C, and the column flow rate was approximately 2mL/min. Aqueous concentrations were determined by applying Henry's Law. Aqueous quantification limits for TCE, DCE, and VC were approximately 9.7×10^{-5} , 2.4×10^{-4} , 3.9×10^{-5} mM, respectively; aqueous quantification limits for the reduced gases ranged from 1×10^{-6} to 1×10^{-7} mM.

When measured, O₂/CO₂ concentrations were determined using the aforementioned Shimadzu 2010+ gas chromatograph, but with a separate injector inlet connected to a Thermal Conductivity Detector (TCD) and a Sigma-Aldrich Carboxen-1010 PLOT capillary column. Standards were prepared daily from ultra-high purity oxygen 99.999% (Airgas, Cat# 001043) and carbon dioxide 99.8% (Sigma-Aldrich, Cat# 23402) balanced in nitrogen.

3.8.2 HPLC Method for Anions and Organic Acids

Inorganic anions and organic acids (OA) were analyzed using a Dionex ICS-2100 or DX-500 Ion Chromatograph system with a conductivity detector. For inorganic anions, diluted samples were analyzed using KOH as the eluent with a low gradient for separation of mono and divalent anions. For organic acids, diluted samples were treated with a ThermoScientific® OnGuard II Ba/Ag/H (cat#063955) pretreatment cartridge to remove sulfate, chloride, and alkaline earth and transition metals. The analytical column used to separate the OAs was a ThermoScientific® IonPac AS11-HC with an AG11-HC guard column. Standards were prepared daily from reagent grade chemicals acetic acid (cat# 695092-100ML), lactic acid (cat# 252476-100G), glycolic acid (cat# 124737-25G), formic acid (cat# F0507-100ML), propionic acid (cat# P1386-500ML), butyric acid (cat# B103500-100ML), and glyoxylic acid (cat# 260150-250G-A) from Sigma-Aldrich. Formic and glyoxylic acid peaks co-eluted, so these compounds could not be distinguished independently from one another; the same occurred for glycolic and acetic acids.

3.8.3 Solution Characterization Methods

The pH was determined by using an Orion 720A+ meter and Orion 9107BN pH probe. Total organic carbon was analyzed by Fremont Analytical (Seattle, WA) using EPA Method 9060. Aqueous ferrous iron was determined from samples taken and filtered with a 0.22 µm syringe filter, and then analyzed using the 1,10-phenanthroline method (Amonette and Templeton, 1998).

For batch experiments performed with natural soils, hydroxyl radicals were quantified using a chemistry that measures the formaldehyde (HCHO) generated from the reaction of hydroxyl

radicals ($\bullet\text{OH}$) and dimethyl sulfoxide (DMSO). The HCHO generated from the reaction is then derivatized with 2,4-dinitrophenylhydrazine (DNPH) to the stable hydrazone form (HCHO-DNPH) and measured using High-Performance Liquid Chromatography (HPLC) with ultraviolet (UV) detection. HPLC analysis was performed using a Shimadzu Nextera-i LC-2040C HPLC equipped with a Kinetex 2.6 μm 100A 100x4.6mm Reversed Phase C18 column and a Photo Diode Array (PDA) detector at 355nm wavelength. Sample volumes (2 mL) were collected from the experimental vials at designated time points, derivatized with DNPH for 30 minutes in phosphate buffer (pH 4), and then measured immediately on the HPLC. Analytical standards were prepared daily by derivatizing HCHO with DNPH, using the same procedures as the samples.

For experiments performed with pure minerals, hydroxyl radicals were also measured using the aminophenyl fluorescein (APF) method (Cohn et al., 2008; Cohn et al., 2009), which is based upon hydroxyl radical transformation of the APF into fluorescein. For each mineral, 1.7 g were placed in the 40 mL amber glass vials with 10 mL of 0.1 M phosphate buffer (pH=7.4) and APF at 25 μM . Periodic sampling was performed by removing a 400 μL aliquot that was transferred into a 15mL conical centrifuge tube and centrifuged at 10,000 g for 5 minutes. The supernatant was subsequently analyzed using a Promega Quantus Fluorometer, where a blue fluorescent channel with an excitation filter at 495nm and emission filter at 510-580nm was used to quantify fluorescein.

3.8.4 Solids Characterization Methods

All sediment mineralogy characterization was performed on homogenized samples prior to performing abiotic dechlorination experiments. All pure mineral characterization work was performed on samples obtained after abiotic dechlorination experiments. Sediment mineralogy was determined by MUD Geochemical, Inc. (Austin, TX) using x-ray diffraction (XRD) with a Bruker D2 Phaser. In most cases, duplicate samples were analyzed. Magnetic susceptibility was determined by Microbial Insights, Inc. (Knoxville, TN) using a Barrington MS2B meter (Ferry et al., 2004; USEPA, 2009). Ferrous mineral content was determined by McCampbell Analytical, Inc. using the 1,10-phenanthroline method following extraction using a weakly acidic 2.5% HCl solution (Amonette & Templeton, 1998). Aqueous total iron was measured using a Varian ICP-OES.

Scanning electron microscopy was conducted on selected solids using a Hitachi S5500 SEM/STEM equipped with energy dispersive spectroscopy (EDS). A Kratos Axis Ultra DLD equipped with an anaerobic transfer chamber was used for X-ray photoelectron spectroscopy (XPS). Samples were prepared for XPS in the anaerobic chamber and taken from the chamber to the XPS instrument in an anaerobic capsule. Fitting models for XPS data were developed after Lan and Butler (2016), Herbert et al. (1998), and Mullet et al. (2002). Iron spectra were fit with a

multiplet for Fe(III)-O as well as three separate peaks for Fe(III)-S, Fe(II)-O, and Fe(II)-S; sulfur spectra were fit with doublets for S²⁻, Sn²⁻, and SO₄²⁻.

3.8.5 Solid-Solution Characterization Methods

Reduction potentials were measured using a mediated approach with electron shuttles (Gorski et al., 2016; Kocur et al., 2020) under anaerobic conditions (Ag/AgCl (3M) Mettler Toldeo Redox Micro ORP Electrode). Between measurements, the electrode was rinsed in 0.1 N HCl for 30 minutes.

3.9 Geochemical Equilibrium Modeling

Geochemical equilibrium calculations were for batch iron sulfide experiments conducted using default values for most species in Visual MINTEQ version 3.1. Only chloride green rust, sulfate green rust, and three hydroxy green rusts were added using published equilibrium constants (Jeong et al., 2011). The measured pH and redox potential, as well as the initial concentrations of Fe²⁺, SO₄²⁻, and HS⁻ were input for each experiment. Concentrations of cations, anions, lactate, and buffer in the media were accounted for, and both Fe²⁺/Fe³⁺ and HS⁻/SO₄²⁻ redox couples were enforced. The model was then used to determine saturation indices (SI = log(Q/K)) (Stumm and Morgan, 2012), and SI values equal to or greater than zero indicate mineral formation is favored.

3.10 One-Dimensional Numerical Model Development

A 1D diffusion-reaction model was developed and coded in MATLAB to simulate flow cell effluent concentrations, and the corresponding depth profiles in the LPZ. Operator splitting was used to separately solve for transport and reactions (Barry et al., 1996; Steefel and MacQuarrie, 1996). For diffusive transport, a standard second-order finite difference spatial approximation and first-order fully implicit temporal approximation were used. The MATLAB routine used to solve the coupled system of equations was ODE15s, a fully implicit variable-order solver that is well suited for stiff systems.

Biological dechlorination of TCE to cis-DCE, to VC, and to ethene (biotic pathway indicated in **Figure 2.2**) was considered, and reactions were modeled using Dual Monod kinetics. Based on initial modeling of the diffusion profiles of donor and acceptor without reaction, it was apparent that both donor and acceptor could be limiting in the clay profile during the experiment. Previous work (Bagley, 1998; Cupples et al., 2004b) has shown that Dual Monod kinetics is well suited when both substrates have the potential to limit biological reactions. Abiotic reactions of TCE and cis-DCE with reactive minerals were considered and modeled using 1st order kinetics (with respect to TCE or cis-DCE). Reactive mineral concentrations were assumed to remain constant because rates of abiotic reduction in complementary batch experiments appeared to be constant for over 3 months (Berns et al., 2019).

As noted above, it is assumed that lactate is completely fermented and oxidized to acetate, H₂, and propionate in the HPZ, and this is supported by groundwater effluent measurements. The fermentation products diffuse into the LPZ, and it is also assumed that only acetate and hydrogen serve as electron donors for dechlorination. Propionate is not a direct electron donor for dechlorination, and hydrogen produced from propionate fermentation was determined to be insignificant due to the high concentrations of acetate available. TCE degraders are known to use both acetate and hydrogen, whereas cis-DCE and VC degraders use only hydrogen. Therefore, it is assumed that one microbial community uses either hydrogen or acetate for the first dechlorination step, and a second community uses only hydrogen, but can facilitate all three dechlorination steps. This assumption is based on previous work (Lee et al., 2007), which concludes that dechlorinating communities can be generally split into two groups: (1) organisms from a wide range of genera that can use acetate and/or hydrogen to dechlorinate TCE to cis-DCE, and (2) organisms, specifically *Dehalococcoides*, that use hydrogen to dechlorinate from TCE to ethene.

The governing equation is shown in Equation 3.1. Solute concentration C_i is in units of $\mu\text{moles per liter of fluid}$, $D_{eff\ i}$ is the effective diffusion coefficient in porous media; reaction terms (r_i) vary for each species (i). The full set of reaction terms are shown in Equations 3.2 – 3.10. The reaction term for Cis-DCE (Eqn. 3.3) is used as an example to describe relevant terms. The Cis-DCE is formed biologically from reaction of TCE with either acetate or hydrogen, is degraded biologically with hydrogen to form VC, and reacts abiotically to form acetylene. In this equation, $r_{ut\ TCE\ H_2}$, $r_{ut\ TCE\ ac}$ are the maximum utilization rates for TCE growing on hydrogen and acetate respectively, $r_{ut\ DCE}$ is the maximum utilization rate for DCE growing on only hydrogen, k_{TCE} , k_{DCE} , and k_{H_2} are the half saturation constants, and $k_{Abio\ DCE}$ is the pseudo first order rate constant for abiotic reaction of DCE. Two biological communities for TCE are indicated. The X_1 community represents the concentration of cells that use both acetate and hydrogen as electron donors (for the first dechlorination reaction), and X_2 represents the concentration of cells that use hydrogen only (for all three dechlorination reactions). An initial concentration of cells throughout the LPZ was assumed and the model allowed for cell growth and decay over time.

$$\frac{dC_i}{dt} = D_{eff\ i} \frac{d^2 C_i}{dz^2} + r_i \quad (3.1)$$

$$\begin{aligned} \frac{dC_{TCE}}{dt} = & -r_{ut\ TCE\ h_2} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H_2}}{C_{H_2} + k_{H_2}} \right) - r_{ut\ TCE\ h_2} X_2 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H_2}}{C_{H_2} + k_{H_2}} \right) - \\ & r_{ut\ TCE\ ac} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{Acetate}}{C_{Acetate} + k_{Acetate}} \right) - k_{Abio\ TCE} C_{TCE} \end{aligned} \quad (3.2)$$

$$\begin{aligned} \frac{dC_{DCE}}{dt} = & r_{ut\ TCE\ h_2} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H_2}}{C_{H_2} + k_{H_2}} \right) + r_{ut\ TCE\ h_2} X_2 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H_2}}{C_{H_2} + k_{H_2}} \right) + \\ & r_{ut\ TCE\ ac} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{Acetate}}{C_{Acetate} + k_{Acetate}} \right) - r_{ut\ DCE} X_2 \left(\frac{C_{DCE}}{C_{DCE} + k_{DCE}} \right) \left(\frac{C_{H_2}}{C_{H_2} + k_{H_2}} \right) - k_{Abio\ DCE} C_{DCE} \end{aligned}$$

$$(3.3)$$

$$\frac{dC_{VC}}{dt} = r_{ut\ DCE} X_2 \left(\frac{C_{DCE}}{C_{DCE} + k_{DCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - r_{ut\ VC} X_2 \left(\frac{C_{VC}}{C_{VC} + k_{VC}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) \quad (3.4)$$

$$\frac{dC_{C2H4}}{dt} = r_{ut\ VC} X_2 \left(\frac{C_{VC}}{C_{VC} + k_{VC}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) + k_{acet \rightarrow eth} C_{C2H2} \quad (3.5)$$

$$\frac{dC_{C2H2}}{dt} = k_{AbioTCE} C_{TCE} + k_{AbioDCE} C_{DCE} - k_{acet \rightarrow eth} C_{C2H2} \quad (3.6)$$

$$\begin{aligned} \frac{dC_{H2}}{dt} = & -r_{ut\ TCE_h2} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - r_{ut\ TCE_h2} X_2 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - \\ & r_{ut\ DCE} X_2 \left(\frac{C_{DCE}}{C_{DCE} + k_{DCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - r_{ut\ VC} X_2 \left(\frac{C_{VC}}{C_{VC} + k_{VC}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - k_{acet \rightarrow eth} C_{C2H2} \end{aligned} \quad (3.7)$$

$$\frac{dC_{Acetate}}{dt} = -\frac{1}{4} r_{ut\ TCE_ac} X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{Acetate}}{C_{Acetate} + k_{Acetate}} \right) \quad (3.8)$$

$$\frac{dX_1}{dt} = \mu_1 X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) + \mu_1 X_1 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{Acetate}}{C_{Acetate} + k_{Acetate}} \right) - k_d X_1 \quad (3.9)$$

$$\begin{aligned} \frac{dX_2}{dt} = & \mu_2 X_2 \left(\frac{C_{TCE}}{C_{TCE} + k_{TCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) + \mu_2 X_2 \left(\frac{C_{DCE}}{C_{DCE} + k_{DCE}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) + \\ & \mu_2 X_2 \left(\frac{C_{VC}}{C_{VC} + k_{VC}} \right) \left(\frac{C_{H2}}{C_{H2} + k_{H2}} \right) - k_d X_2 \end{aligned} \quad (3.10)$$

The boundary conditions (BCs) for the 1D diffusion-reaction model are summarized in **Figure 3.8**, and the initial conditions for all species are zero concentration in the LPZ matrix. The TCE concentration was set equal to its aqueous solubility limit (9.74 mM) at the base of the LPZ, and acetate and hydrogen were held constant at the LPZ/HPZ interface. Their values were determined from the HPZ effluent data, and electron donor balance calculations. The BCs for all other compounds at the LPZ/HPZ interface were set to zero, based

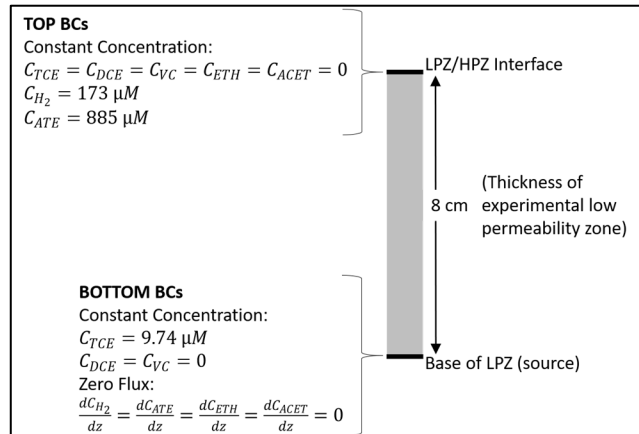


Figure 3.8. Boundary conditions for 1D diffusion-reaction model used to simulate flow cell.

on the assumption that flow in the HPZ is fast (residence time is 35 hours) relative to diffusion time scales in the LPZ (days to weeks) for TCE and its products. The BCs for other chlorinated compounds at the base of the LPZ were set to zero, assuming Tenax acts as a sink. Zero-flux BCs were used for ethene, acetylene, and acetate at the base of the LPZ, which is based on the assumption that these compounds do not sorb to Tenax.

Parameters used in the model are provided in the results section, and biological parameters fall in the range of previously reported parameters. The abiotic rate constant for cis-DCE in the model was assumed to be 1/10th of the TCE rate constant (per batch experiment data), and the first order rate constant for acetylene conversion to ethene ($k_{acetylene}$ for Fe-oxides) from Jeong et al. (2007) was used. Model validation was done using an analytical solution for diffusion and reaction; validation results are reported in the publication from Berns et al. (2019). There are seven fitting parameters, and confidence intervals were determined for each of the them to gain an understanding of model sensitivity to these fitting parameters. The model was simulated for 180 days – the same length of time the experiment was conducted.

3.11 Computationally Efficient Numerical Model Simulation Using RT3D

A new Immobile Zone (IMZ) Model was implemented within the commercial software RT3D to provide a tool for practitioners to simulate diffusion and reaction in low permeability zones more computationally efficiently. The governing equations are derived assuming the simple case of a thick clay layer (LPZ) underlying a sand layer (HPZ) (Figure 3.9) for a conservative tracer. The conservation of mass for transport of a nonreactive solute is given by Eq. 3.11.

$$R \frac{\partial C}{\partial t} = -v_x \frac{\partial C}{\partial x} + \frac{\partial}{\partial x} \left((D_{eff} + \alpha_L v_x) \frac{\partial C}{\partial x} \right) + \frac{\partial}{\partial z} \left((D_{eff} + \alpha_{TV} v_x) \frac{\partial C}{\partial z} \right) \quad (3.11)$$

where C is the tracer concentration (mg/mL), t is time (d), x is horizontal coordinate, z is vertical coordinate (cm), D_{eff} is effective diffusion coefficient (cm^2/d), α_L is longitudinal dispersivity (cm), v_x is the horizontal pore water velocity (cm/d), α_{TV} is transverse dispersivity (cm), and R is the retardation factor. In deriving Eq. (3.11) we assumed advection merely occurs in the x direction. The presented equations are based on a 2D cross-section domain, but the approach can also be applied in 3D. If the hydraulic conductivity in the sand is much higher than in clay (flow in the latter is negligible), then the time scale over which the mass gets transported through the HPZ is much shorter than the time scale of diffusion within the LPZ. Thus, it is reasonable to assume that diffusion is primarily vertical (longitudinal concentration gradient is negligible in diffusion dominated areas); therefore, the conservation of mass in the LPZ reduces to Eq. (3.12).

$$R' \frac{\partial C'}{\partial t} = \frac{\partial}{\partial z} \left(D_{eff} \frac{\partial C'}{\partial z} \right) \quad (3.12)$$

where C' and R' refer to the concentration and retardation factor in the LPZ. Finite volume discretization of Eq. (3.12) turns the PDE into a system of ODEs (Eq. 3), known as the Method of Lines (MOL).

$$R' \frac{dC'_j}{dt} = \frac{1}{\Delta z_j} \left(D_{eff} \frac{C'_{j+1} - C'_j}{z_{j+1} - z_j} - D_{eff} \frac{C'_j - C'_{j-1}}{z_j - z_{j-1}} \right), j = 1, \dots, N \quad (3.13)$$

in which, z_j is the coordinate center of j^{th} immobile zone, and Δz_j is the thickness of j^{th} immobile zone. Note that Eq. 3.13 can be interpreted as sequential mass transfer between a series of immobile species, if C'_j is interpreted as an immobile species concentration in grid block j .

For CASE I, the following set of first-order reactive equations (Eq. 3.14-3.17) represent diffusive transport of conservative tracer in the LPZ:

$$R' \frac{dC'_m}{dt} = \frac{1}{\Delta z_m} \left(D_{eff} \frac{C'_{im,1} - C'_m}{z_1 - z_m} \right) = k_0 (C'_{im,1} - C'_m) \quad (3.14)$$

$$R' \frac{dC'_{im,1}}{dt} = \frac{1}{\Delta z_{im,1}} \left(D_{eff} \frac{C'_{im,2} - C'_{im,1}}{z_2 - z_1} - D_{eff} \frac{C'_{im,1} - C'_m}{z_1 - z_m} \right) = k_2 (C'_{im,2} - C'_{im,1}) - k_1 (C'_{im,1} - C'_m), j = 1 \quad (3.15)$$

$$R' \frac{dC'_{im,j}}{dt} = \frac{1}{\Delta z_{im,j}} \left(D_{eff} \frac{C'_{im,j+1} - C'_{im,j}}{z_{j+1} - z_j} - D_{eff} \frac{C'_{im,j} - C'_{im,j-1}}{z_j - z_{j-1}} \right) = k_{j+1} (C'_{im,j+1} - C'_{im,j}) - k_j (C'_{im,j} - C'_{im,j-1}), j = 2 \sim N - 1 \quad (3.16)$$

$$R' \frac{dC'_{im,N}}{dt} = \frac{1}{\Delta z_{im,N}} \left(0 - D_{eff} \frac{C'_{im,N} - C'_{im,N-1}}{z_N - z_{N-1}} \right) = -k_N (C'_{im,N} - C'_{im,N-1}), j = N \quad (3.17)$$

The subscripts m and im,j relate to ‘mobile’ and the j^{th} ‘immobile’ components, respectively. Note that we use ‘immobile species’ or ‘immobile components’ terminology throughout this paper instead of ‘immobile zones’ because each immobile zone is defined as a chemical component in RT3D. The above equations show how the first-order mass transfer rate coefficients are related to the diffusion coefficient and the grid size. **Table 3.3** explicitly shows the expressions for computing k_j . **Figure 3.9** illustrates the equivalence between LPZ diffusion in the full model and sequential mass-transfer in the IMZ-RT3D. Note that immobile zones are spread along the length of LPZ and only contribute to transport of solutes in the LPZ by vertical diffusion. When a lens of LPZ is embedded in HPZ, Eq. (3.17) will be modified to Eq. (3.18), where last immobile zone is in contact with the mobile species.

$$R' \frac{dC'_{im,N}}{dt} = \frac{1}{\Delta z_{im,N}} \left(D_{eff} \frac{C'_m - C'_{im,N}}{z_m - z_N} - D_{eff} \frac{C'_{im,N} - C'_{im,N-1}}{z_N - z_{N-1}} \right) = k_{N+1}(C'_m - C'_{im,N}) - k_N(C'_{im,N} - C'_{im,N-1}), j = N \quad (3.18)$$

If the mobile concentration at the bottom of lens, i.e. C'_m is Eq. (3.18), is equal to mobile concentration at the top of lens, i.e. C'_m in Eq. (3.14), one could benefit from the symmetry of the problem and model only half thickness of the lens. Even though this method needs less number of immobile zones, it requires manual adjustments to account for the mass stored and exchanged in the other half of the lens. Another method, used in this paper, is to involve both top and bottom rows around the LPZ in the reaction module. This way the first immobile zone (Eq. 3.15) and the last immobile zone (Eq. 3.18) will exchange mass with mobile species on top and bottom, respectively. This automatically accounts for the mass exchanged and stored over the total thickness of the LPZ. The latter method will be flexible and accurate when the mobile concentration on top of the lens is different than the bottom, e.g. when two sources of contamination with different strengths are released on top and bottom of the lens.

It is worth noting that when all immobile zones have identical thickness, Eq. (3.15-3.17) will be represented by three mass transfer coefficients, k_0 , k_1 , and k_2 . Any variability in thickness of immobile zones adds to the number of reaction rates, i.e. k_j , in RT3D.

Table 3.3. Definition of k_j s in Eq. (3.14-3.16)

	k_0 in Eq. (4)	k_1 in Eq. (5)	k_2 in Eq. (5)	k_{j+1} in Eq. (6)	k_j in Eq. (6)
k_j	$\frac{D_{eff}}{\Delta z_m(z_1 - z_m)}$	$\frac{D_{eff}}{\Delta z_{im,1}(z_1 - z_m)}$	$\frac{D_{eff}}{\Delta z_{im,1}(z_2 - z_1)}$	$\frac{D_{eff}}{\Delta z_{im,j}(z_{j+1} - z_j)}$	$\frac{D_{eff}}{\Delta z_{im,j}(z_j - z_{j-1})}$
=					

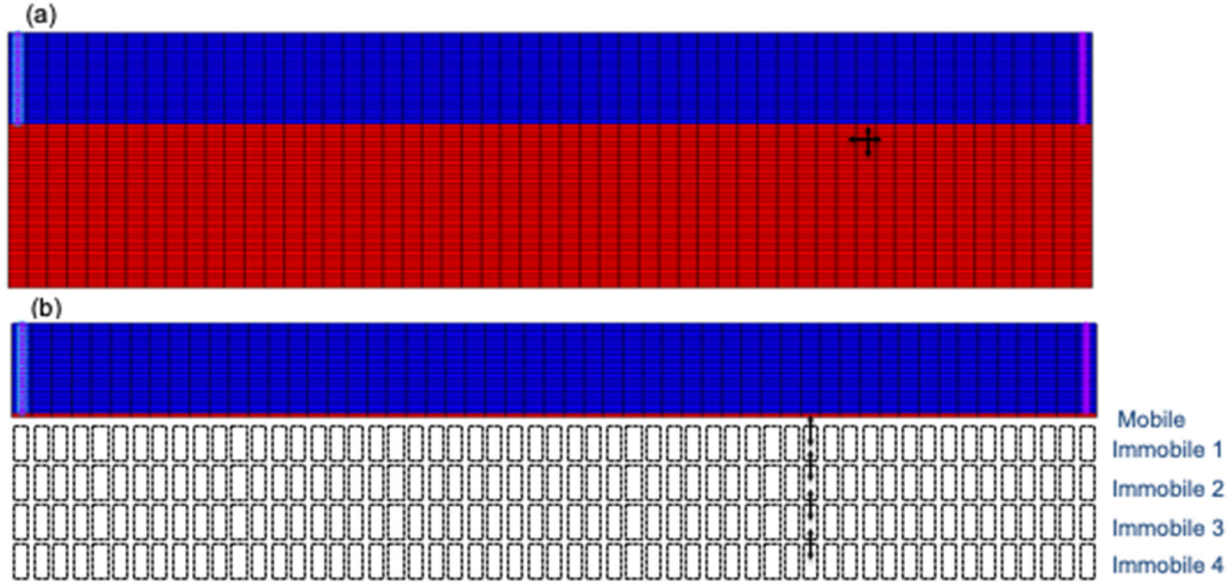


Figure 3.9. Schematic of (a) full-RT3D model, and (b) 4 immobile-zone RT3D (IMZ-RT3D) model. The number of immobile zones are determined based on calibration by less than 10% change in error.

RT3D allows for user-defined arbitrary reactions that can be localized in space. We deactivated the LPZ cells in the full-RT3D model domain (Figure 1), and then integrated sequential mass transfer between ‘mobile’ species in the grid blocks along the LPZ boundary and the ‘immobile’ species representing each discrete slice of the LPZ. Transport of mobile components in the HPZ was handled by the advection-dispersion PDE solver in RT3D, and transport between immobile components was computed via the reaction module (ODE solver). A no-flux boundary condition at the bottom of the LPZ was applied via Eq. (3.17), which was for the immobile component corresponding to the LPZ region at the boundary (CASE I). It is worth noting that the mobile component (C'_m) is the linkage that establishes diffusive transport of solute from HPZ to LPZ during forward diffusion (or vice versa during back diffusion) in Eq. (3.15) or Eq. (3.18). Under anaerobic conditions and in the presence of sufficient electron donor and dechlorinating bacteria, TCE can sequentially transform to dichloroethylene (DCE), vinyl chloride (VC), and then ethene (Berns et al., 2019). Assuming dual Monod kinetics, Eq. (3.14)-(3.17) are modified as follows for reaction of TCE with molecular hydrogen to form DCE.

$$R \frac{dTCE_m}{dt} = \frac{1}{\Delta z_m} \left(D_{eff} \frac{TCE_{im,1} - TCE_m}{z_1 - z_m} \right) \quad (3.19)$$

$$R \frac{dTCE_{im,1}}{dt} = \frac{1}{\Delta z_{im,1}} \left(D_{eff} \frac{TCE_{im,2} - TCE_{im,1}}{z_2 - z_1} - D_{eff} \frac{TCE_{im,1} - TCE_m}{z_1 - z_m} \right) - q_{tce} X \frac{TCE_{im,1}}{TCE_{im,1} + K_{s,TCE}} \frac{H_{im,1}}{H_{im,1} + K_{s,H}}, j = 1 \quad (3.20)$$

$$R \frac{dTCE_{im,j}}{dt} = \frac{1}{\Delta z_{im,j}} \left(D_{eff} \frac{TCE_{im,j+1} - TCE_{im,j}}{z_{j+1} - z_j} - D_{eff} \frac{TCE_{im,j} - TCE_{im,j-1}}{z_j - z_{j-1}} \right) - q_{tce} X \frac{TCE_{im,j}}{TCE_{im,j} + K_{S,TCE}} \frac{H_{im,j}}{H_{im,j} + K_{S,H}}, j = 2, \dots, N-1 \quad (3.21)$$

$$R \frac{dTCE_{im,N}}{dt} = \frac{1}{\Delta z_{im,N}} \left(-D_{eff} \frac{TCE_{im,N} - TCE_{im,N-1}}{z_N - z_{N-1}} \right) - q_{tce} X \frac{TCE_{im,N}}{TCE_{im,N} + K_{S,TCE}} \frac{H_{im,N}}{H_{im,N} + K_{S,H}}, j = N \quad (3.22)$$

in which, H is molecular hydrogen concentration (mol H₂/L), X is biomass concentration (cell/L), q_{tce} is the maximum substrate utilization rate (mol TCE/cell.d), $K_{S,H}$ is hydrogen half-saturation constant (mol H₂/L), and $K_{S,TCE}$ is TCE half-saturation constant (mol TCE/L). Biomass concentration is assumed to be constant and equal to 1.0×10^9 cell/L. A similar set of equations is used for hydrogen and DCE formation. Also, additional reactions can be added for further transformation of DCE. In deriving Eq. (3.19-3.22), we assume reactions occur only in the LPZ, advective transport occurs in the HPZ, and transport in the LPZ is merely due to molecular diffusion in the vertical direction. This is reasonable if the time scale for transport in the HPZ is fast relative to the timescale for mixing and reaction, and/or if mixing of two solute species primarily occurs in the LPZ. The IMZ-RT3D model, however, is easily adaptable to consider reaction in the HPZ, and/or other boundary or initial conditions.

Model Scenarios and Input Parameters:

The IMZ-RT3D model is tested for the four scenarios illustrated in **Figures 3.9** and **3.10**. In CASE I, an 18 cm LPZ layer underlies a 10 cm HPZ (**Figure 3.9**), in CASE II, a thin 8 cm LPZ lens (length to thickness ratio of 11.25:1) is surrounded by HPZ in a 28 x 110 cm domain (**Figure 3.10**). In CASE III, a thicker 16 cm LPZ lens (length to thickness ratio of 5:1) is surrounded by HPZ in a 28 x 110 cm domain. In CASE IV, multiple LPZs of varying geometry are surrounded by HPZ in a 84 x 107 cm domain. CASE I represents field sites in Parker et al. (2004), while CASE IV is adapted from Chapman et al. (2012). For all cases, a solute is injected from the left boundary into the sand layer for a specific time period, during which it diffuses into the LPZ. Next, either solute-free water or a second solute is injected from the left boundary into the sand layer, allowing the initial solute to diffuse out of the LPZ, and in some cases react in the LPZ with the second solute.

For each scenario, MODFLOW is first run to obtain the velocity field for RT3D. The velocity field is then used as input to RT3D, where the LPZ cells in the IMZ-RT3D model are set inactive. Simulations are then performed for either a conservative tracer or TCE reacting with hydrogen, and results from the IMZ-RT3D are compared to those from fully discretized domains. For the tracer, solute is injected from the left boundary for 22 days and then flushed for 100 days. For TCE transformation in CASE I-IV, 10 mM of TCE is injected from the left boundary for 22 days, and then 1 mM of hydrogen is injected from the left boundary for 100 days. During this time, TCE is biotically transformed to DCE in the LPZ via dual-Monod kinetics, considering constant bacterial

concentration. The boundary conditions used in MODFLOW/ RT3D are presented in **Table 3.4**. Model input parameters are presented in **Table 3.5** and they are primarily adapted from two sources (Berns et al., 2019; Chapman et al., 2012).

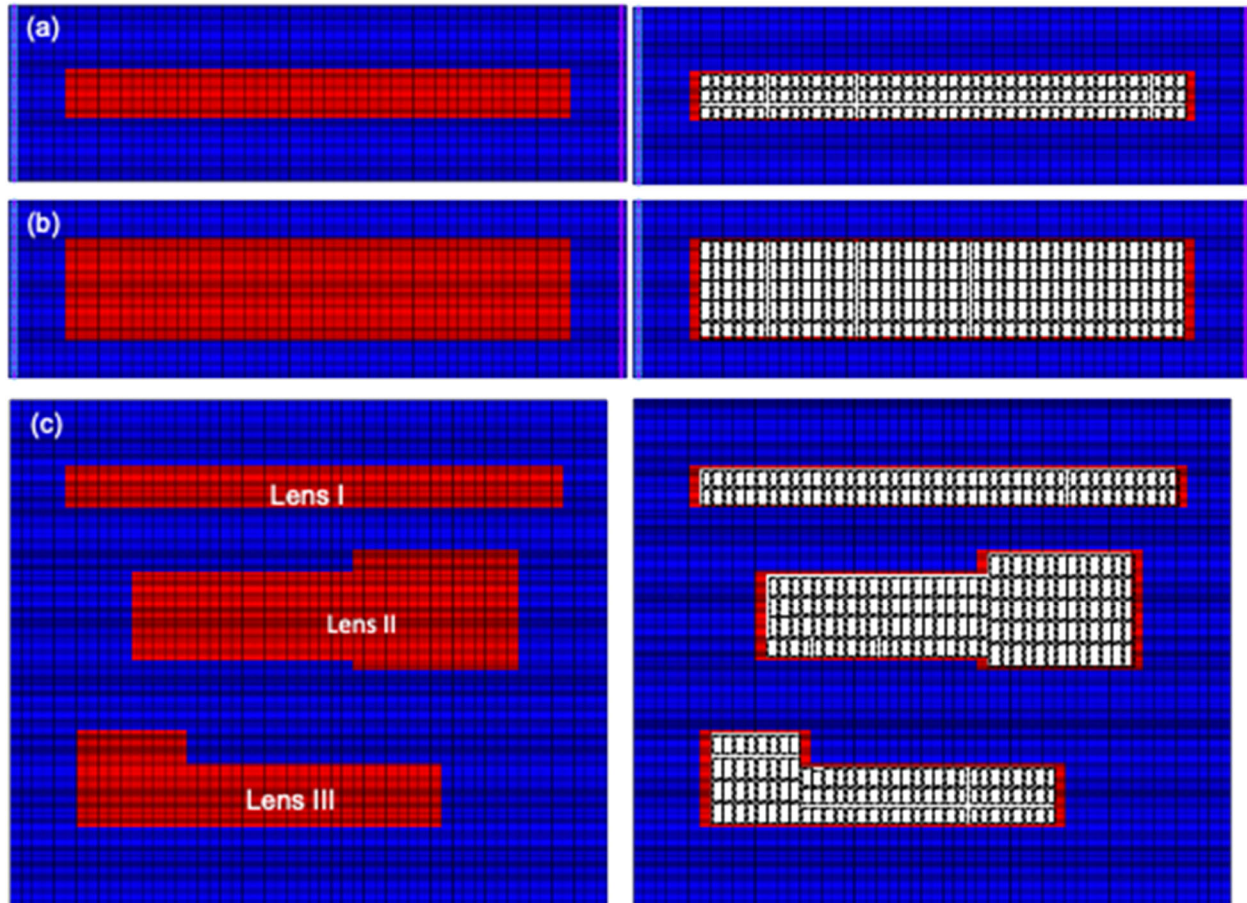


Figure 3.10. Schematic of (a) thin LPZ lens- CASE II, (b) thick LPZ lens- CASE III, (c) multiple LPZs- CASE IV: full-RT3D model (left), IMZ-RT3D model (right).

Model Implementation

In order to implement the IMZ-RT3D model, 1) grid cells within the LPZ are made inactive in RT3D except those along the boundary in direct contact with HPZ grid cells, and 2) each mobile species is coupled to N immobile components with N equal to the number of immobile zones in the LPZ. The transport and reaction within the LPZ are incorporated into the RT3D code with the user-defined reaction module. Mesh independency of the fully discretized version for a single low permeable lens (CASE II) was evaluated and grid cells of 2 x 0.5 cm were chosen.

Table 3.4. Boundary conditions imposed on full models in MODFLOW/RT3D

Boundary	Left	Right	Top	Bottom
MODFLOW	Specified head	Specified head	Zero flux	Zero flux
RT3D	Specified concentration	Zero dispersive flux	Zero flux	Zero flux

Table 3.5. Input properties of tracer and reaction scenarios.

	Two-layer (CASE I)	Single thin lens (CASE II)	Single thick lens (CASE III)	Multiple lens (CASE IV)	References
Physical *					
K _{LPZ} (cm/d)	8.640e-04				Chapman et al., 2012*
K _{HPZ} (cm/d)	2.074e03	2.074e03	2.074e03	2.070e03	Chapman et al., 2012*
θ _{LPZ}	0.60				Chapman et al., 2012
θ _{HPZ}	0.45				Chapman et al., 2012
LPZ depth (cm)	18	8	16	-	-
Total depth(cm)	28	28	28	84	Chapman et al., 2012*
L (cm)	110	110	110	107	Chapman et al., 2012*
D _{eff} (cm ² /min)	7.815e-1				Berns et al., 2019**
α _L (cm)	2				Chapman et al., 2012
α _v (cm)	0.1				Chapman et al., 2012
ρ _{bulk} (g/mL)	1.95				Chapman et al., 2012
Q _{in} (mL/min)	1.50				Chapman et al., 2012
Tracer					
C _{in,tracer}	0.0011				Chapman et al., 2012

T_{inj} (d)	22	Chapman et al., 2012
T_{flush} (d)	100	Chapman et al., 2012
Reaction **		
C_{in,H_2} (mole/L)	0.001	Berns et al., 2019**
$C_{in,TCE}$ (mole/L)	0.01	Berns et al., 2019**
$T_{inj,TCE}$ (d)	22	Chapman et al., 2012
T_{inj,H_2} (d)	100	Chapman et al., 2012
q (mole/cell.d)	$1.0e-12$	Berns et al., 2019**
X (cell/L)	$1.0e+09$	***
$K_{s,TCE}$ (mole/L)	$1.0e-05$	Berns et al., 2019**
K_{s,H_2} (mole/L)	$7.0e-09$	Berns et al., 2019**

* Physical values used for individual cases (other than CASE IV) can vary slightly from reference values and are noted in the text as needed.

** Reaction values used for individual cases can vary slightly from the reference values and are noted in the text as needed.

*** Constant biomass is assumed.

4.0 Results and Discussion

4.1. Pease AFB Sediment and Groundwater Characterization Results

X-ray diffraction results for Pease AFB clay and sand are presented in **Table 4.1**. For the clay, quartz is the dominant mineral, followed by feldspar. Minerals present at low amounts (<10%) but still above 1% are biotite, glauconite, illite, chlorite, montmorillonite, ankerite, and riebeckite. Minerals present in trace amounts (<1%) are nontronite, goethite, magnetite, and sphalerite. Results for the sand are similar, with quartz and feldspar dominating mineralogy, and almost the same minerals at low and trace amounts. The exceptions are magnetite and sphalerite, which for one or two replicates are above 1%.

Table 4.1. Pease AFB clay and sand mineralogical results from XRD. Material from three cores for each material type were evaluated to show variability.



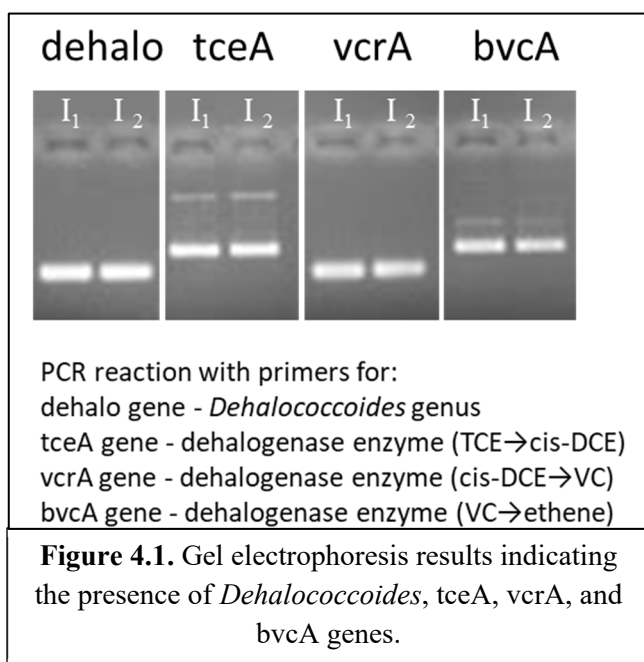
Mineral	Description	Clay - Batch (Core)			Sand - Batch (Core)	
		AcA (L7-P3)	AcB (L6-P3)	AcC (L9-P3)	AsA (L3-P2)	AsB (L15-P1)
Quartz	Framework Silicate (or oxide)	45.7	56.6	35.9	35.2	45.5
Albite	Feldspar	22.7	21.9	20.8	10.1	22.8
Orthoclase	Feldspar	6.5	8.5	8.3	16.3	11.3
Biotite	Phyllosilicate (Mica)	4.1	1.7	6.1	1.9	2.2
Glauconite	Phyllosilicate (mica)	3.6	2.4	4.2	1.2	2.9
Illite/Muscovite	Non-expanding clay/phyllsilicate	6.6	1.0	15.1	2.7	5.7
Chlorite	Phyllosilicate	4.9	3.1	4.6	6.9	1.0
Montmorillonite	Soft phyllosilicate (Smectite)	1.8	1.1	2.8	6.1	0.6
Nontronite	Phyllosilicate (Smectite)	0.5	0.4	0.7	0.9	1.0
Goethite	Oxide (hydroxide)	0.1	0.0	0.0	0.3	0.1
Magnetite	Oxide (spinel)	0.9	0.3	0.1	3.2	1.7
Ankerite	Carbonate	2.2	1.4	0.3	2.7	1.2
Riebeckite	Amphibole (double chain silicate)	0.3	1.0	1.0	1.8	3.8
Sphalerite	(Fe,Zn) Sulfide	0.2	0.7	0.2	10.8	0.2

Groundwater quality measurements for Pease AFB are presented in **Table 4.2**. The pH is near circumneutral (7.7), inorganic carbon is low (48.1 mg/L), and organic carbon is intermediate (40.4 mg/L). Cations are dominated by sodium (87.2 mg/L), with lesser but still significant amounts of calcium, potassium, and magnesium. Trace amounts of iron and manganese are present. Anions are dominated by chloride and sulfate, at approximately equal concentrations (35 mg/L). Trace amounts of nitrate, bromide, and fluoride are also present. The Pease AFB groundwater was pumped from the sand unit, and the low iron reflects the oxidizing conditions in this groundwater.

Table 4.2. Pease AFB groundwater quality measurements.

pH			Carbon Content (mg/L)	
7.7			IC	TOC
			48.1	40.4

Cations			Anions	
Element	Concentration	Units	Element	Concentration (mg/L)
Na ⁺	87.2	mg/L	Cl ⁻	35.4
Ca ²⁺	11.7	mg/L	SO ₄ ²⁻	35.5
K ⁺	7.1	mg/L	NO ₃ ⁻	trace
Mg ²⁺	6.0	mg/L	Br ⁻	trace
Fe (total)	182.0	µg/L	F ⁻	trace
Mn (total)	2.6	µg/L		



4.2. Dechlorinating Culture Characterization

Primers from Behrens et al. (2008) were used to confirm the presence of the *Dehalococcoides* genus and the dehalogenase enzymes in the dechlorinating culture (DCC) used in batch and flow cell experiments. Gel results are shown in **Figure 4.1**. In each case, the replicate results show high intensity bands at the location indicated for the appropriate gene. Hence, the DCC contains the *Dehalococcoides* genus, as well as the tceA gene, vcrA gene, and bvcA genes.

Initial batch tests were performed to confirm that the dechlorinating culture was active for

TCE dechlorination all the way to ethene. As previously noted, the Pease AFB groundwater was used as the media, amended with 1 mM lactate and 100 µM TCE. Results are shown in Figure 4.2. TCE is completely removed in the first 10 days, followed by DCE in another 10 days. VC peaks near day 23, and ethene is the sole transformation product after 30 days. These results show that the DCC is active for TCE reduction through ethene in approximately 30 days under well-mixed batch conditions.

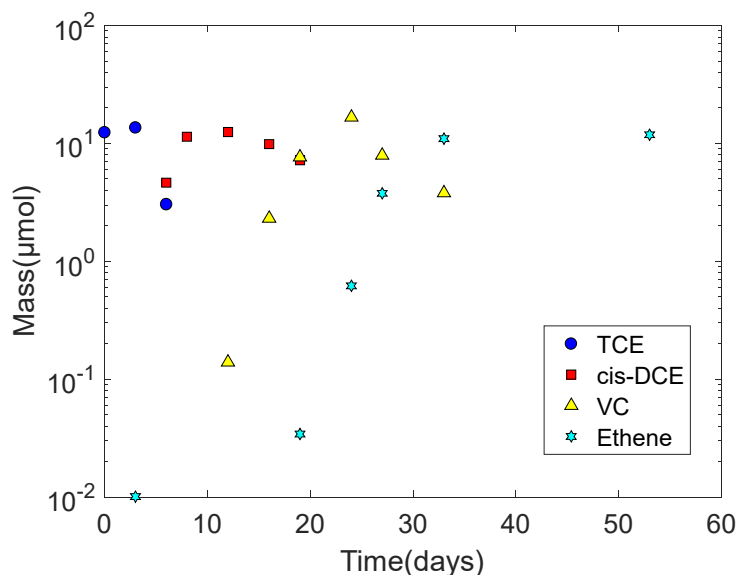


Figure 4.2. Batch experimental results showing sequential reductive dechlorination for the DCC.

4.3. Batch Results of Abiotic Reduction and Oxidation with Natural Sediments

4.3.1 Solids Characterization

Abiotic reduction experiments were initially performed with the Pease AFB clay and two additional solids, referred to as Soils 1 -3 in **Table 4.3**. The magnetic susceptibility value for Soil 1 is approximately 15-times greater than for Soils 2 and 3, suggesting that the presence of magnetic minerals is substantially greater in Soil 1 than the other soils. The ferrous mineral content, as determined using the 1,10-phenanthroline method, is much greater in Soils 2 and 3 than in Soil 1. Comparison of the ferrous minerals identified via XRD and the ferrous mineral content among the 3 soils suggests that the ferrous mineral content is most sensitive to the riebeckite and/or chlorite content of the soil, and that ferrous minerals such as antigorite and illite contribute little to the ferrous mineral content as measured via the 1,10-phenanthroline method.

Abiotic reduction experiments were next performed with the Pease AFB and four additional solids, referred to as Sediments 1-5 in **Table 4.3**. Sediment texture and mineralogy varied among the 5 sediments. Ferrous mineral content varied nearly 3 orders of magnitude among the sediments, with ferrous mineral content generally greater in the clayey sediments than in the sandy sediments. The ferrous mineral content did not appear to be associated with the presence of ferrous-containing minerals identified via XRD (i.e., biotite, siderite, illite, and ankerite), suggesting that the ferrous content of these minerals varied greatly among the sediments. It is also possible that the 1,10-phenanthroline method used to determine the ferrous iron content did not sufficiently quantify the ferrous iron associated with the minerals identified via XRD.

Table 4.3. Mineral properties for Soils 1 through 3. XRD, magnetic susceptibility, ferrous content, and sieve results are provided. Only minerals with at least 1% content are shown.

Property	Soil 1	Soil 2	Soil 3
% Mineral Content (XRD analysis)	Dickite (26%); Kaolinite (26%); Antigorite (25%); Quartz (12%); Illite/mica (8.5%); Hematite (1.8%)	Albite (30%); Quartz (23%); Illite/Mica (11%); Orthoclase (7.4%); Chlorite (6.7%); Dolomite (6.4%); Riebeckite (5.8%); Goethite (4.7%); Kaolinite (2.3%); Illite/Smectite (1.8%)	Quartz (46%); Albite (25%); Orthoclase (6.1%); Chlorite (3.8%); Illite/Smectite (2.9%); Calcite (2.0%); Riebeckite (1.2%)
% Clay	44	78	17
% Silt	50	21	44
% Sand & Gravel	6	1	39
Magnetic Susceptibility (m³/kg)	1.1 x 10 ⁻⁵ / 1.1 x 10 ⁻⁵	6.7 x 10 ⁻⁷	7.7 x 10 ⁻⁷
Ferrous mineral content (mg/kg)	3.8 / 4.5	4200	1800
pH	4.9	7.2	9.7

Table 4.4. Characteristics of the sediment. Only minerals present in at least 1% are shown for the XRD analysis.

Property	Sediment 1	Sediment 2	Sediment 3***	Sediment 4****	Sediment 5
% Mineral Content (XRD)	illite (41); albite (30); quartz (25); siderite (1.6); anhydrite (1.5)	illite (33); quartz (27); ankerite (16); albite (16); kaolinite (3.5); calcite (3.0); siderite (1.0)	illite (29); quartz (23); ankerite (18); albite (17); kaolinite (6.9); calcite (5.4)	albite (42); illite (29); quartz (12); anhydrite (16);	Quartz (34); Albite (33); Orthoclase (22); biotite (9.3); apatite (1.5)
% Clay	37	23	23	2.1	13
% Silt	55	26	22	2.2	13
% Sand & Gravel	8	51	55	96	74
Magnetic Suscept. (m³/kg)	3.9 x 10 ⁻⁷	3.5 x 10 ⁻⁷	2.1 x 10 ⁻⁷	6.1 x 10 ⁻⁷	3.4 x 10 ⁻⁶
Ferrous mineral content (mg/kg)	2570	160	337	45	3.3
Diss. iron* (mg/L)	0.01	0.02	0.01	<0.01	0.02
Tot. Org. C (mg/kg)	< 750	1,500	3,900	< 750	< 750
pH*, **	7.2 / 7.2	7.8 / 7.8	7.6 / 7.3	6.8 / 6.8	7.0 / 7.0

* in supernatant water of anaerobic samples measured at the end of the batch experiment

** First pH value is from anaerobic vials, and the second is from the aerobic vials

*** Sediment 3 was collected from the same location as sediment 2, but approximately 1m deeper

**** Sediment 4 was collected from a sandy layer immediately overlying sediment 1

4.3.2 Batch Results for Anaerobic Conditions with Soils 1-3

For Soil 1, no detectable levels of PCE were present in the unspiked samples, and no detectable levels of biotic PCE/TCE dechlorination products were observed under any conditions. Note that biotic only means that the soil was not autoclaved; in no cases were soils amended with biological growth media or dechlorinating bacteria. For Soil 1, low levels of acetylene were initially ($t=1$ day) present in the PCE-spiked samples for both the abiotic and biotic conditions, but no increases over time in acetylene were observed in either condition. No acetylene was observed in the Soil 1 sample without the PCE spike. Thus, this acetylene may have been present due to a rapid but transient abiotic dechlorination reaction (acetylene is a known abiotic dechlorination product, and is not expected to be generated biologically), or more likely may have been present as an impurity or transformation product with the same elution time as acetylene associated with the PCE spiking solution. The absence of any of the expected biotic PCE dechlorination products (i.e., DCE, vinyl chloride, ethene) at any temperature suggests that heating did not stimulate any measurable biological reduction of PCE for this soil.

Butane was the only gaseous product that was generated in Soil 1, where the generated butane levels were calculated based on the difference between the PCE spiked and unspiked samples. Butane generation was most clearly noted at 55° C. Butane generation was only observed in the abiotic condition, and not the biotic condition. The absence of butane in the biotic condition likely was due to subsequent butane biotransformation.

For Soil 2, accumulation of PCE abiotic dechlorination products were observed at each temperature. The generation of PCE dechlorination products for the PCE-spiked Soil 2 conditions is presented in Figure 1. The results indicate that the reduced gas transformation products consisted of a mixture of acetylene, butane, ethene, and trace levels of propane. Reduced gases in all bottles were at least 5-times less in the non-spiked samples than in the PCE-spiked samples, confirming that the bulk of the reduced gases generated were from the spiked PCE and not from any dechlorination reactions that had occurred from historic exposure (prior to sample collection) of the soil to chlorinated ethenes. Similar to Soil 1, acetylene was present at the initial sampling event for all temperatures in Soil 2; this acetylene may have been the result of an initial rapid and transient reaction, or (more likely) it was the result of an impurity or transformation product associated with the PCE spiking solution. Gas product generation in Soil 2 was approximately 100-times greater in Soil 2 than in Soil 1.

Acetylene and ethene have been identified as ferrous iron-induced abiotic dechlorination products of chlorinated ethenes in several studies (Lee and Batchelor, 2002a, Truex et al., 2007; Schaefer et al., 2013). Butane, propane and/or other short chain (3 or more carbons) hydrocarbons have been identified as likely abiotic coupling products from (chloro)acetylene or ethene transformation on zerovalent iron surfaces (Arnold and Roberts, 2000; Elsner et al., 2008) and in ferrous rock

minerals (Schaefer et al., 2015). With the exception of ethene, which can be generated abiotically or biotically from PCE or TCE (Maymó-Gatell et al., 1997; Schaefer et al., 2009), the other generated reduced gases observed in this study have not been reported as biotic PCE transformation products, thereby reinforcing their generation via abiotic processes.

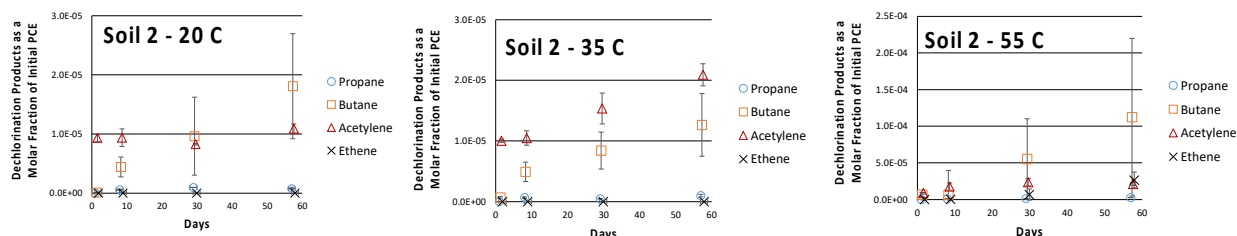


Figure 4.3. Reduced gas PCE dechlorination products as a molar fraction of the initial PCE added to the vials (moles dechlorination product / moles initial PCE). Results are shown for Soil 2 at 20° C (top), 35° C (middle) and 55° C (bottom) under Abiotic conditions. Values were calculated by subtracting the concentration measured in the samples that were not spiked with PCE from the concentration measured in the PCE-spiked samples. Average values are shown. Error bars represent the 95% confidence interval. The y-axis scale was increased for the 55° C condition. Data are plotted with a slight off-set to improve the clarity of the error bars.

Assuming the acetylene present at the initial sampling event was an artifact of the spiking solution, butane was the most abundant dechlorination product measured at both 20 and 35 degrees C in Soil 2, which is consistent with the results obtained for Soil 1. For Soil 2, both acetylene and butane showed an approximately linear increasing trend over time, thereby indicating that abiotic dechlorination of PCE was ongoing throughout the 8-week incubation period, yielding these reduced gases as transformation products.

At 55° C, butane remained the dominant dechlorination product for both Soils 1 and 2. For Soil 2, butane increased at a rate approximately 4-times greater than at the lower temperatures. Ethene generation in Soil 2 also became significant by 29 days at 55° C. By day 57, ethene generation surpassed that of acetylene, and acetylene levels began to decline. No ethene generation was observed at the lower temperatures. These results suggest that the elevated temperature facilitated the reduction of acetylene to ethene in Soil 2 by increasing the rate or extent of hydrogenation reactions, and increased the rate of butane generation by increasing the rate or extent of higher coupling reactions.

The dominant presence of higher coupling products (i.e., butane) in this study differs from observations in several studies using zerovalent iron, where higher coupling hydrocarbon species accounted for only a trace fraction of the chlorinated ethene or acetylene transformation products in the presence of zerovalent iron (Arnold and Roberts, 2000; Elsner et al., 2008). A notable exception is a study by Gao et al. (2015), where up to 50% of the TCE that was dechlorinated resulted in generation of coupling products from ball milled zerovalent iron. Ball milled zerovalent

iron has a much higher abundance of iron oxides present on the surface compared to native zerovalent iron (Li et al., 2009), thus the presence of iron oxide mixtures may facilitate the formation of coupling products. In rock containing ferrous minerals, propane accounted for approximately 20 to 25% of abiotically dechlorinated TCE in ferrous mineral containing rock (Schaefer et al., 2015), thus ferrous oxide and hydroxide minerals present in natural solids may have a greater propensity to form these higher coupling compounds than unoxidized zerovalent iron.

Lowry and Reinhard (2001) observed a substantial increase in coupling reactions when hydrogen levels were decreased below approximately 100 μM in hydrodechlorination reactions between TCE and Pd catalysts. Hydrogen levels measured in Soils 1 and 2 at the end of the experiments were below the analytical detection limit of 0.082 μM in the bulk water phase (based on headspace sampling and Henry's Law). Thus, limited availability of hydrogen in the natural clay systems may have allowed coupling reactions to further outcompete hydrogenation reactions at the natural ferrous mineral surfaces, although bulk water hydrogen levels may not have been representative of hydrogen levels near the active mineral surfaces. Others (Schrack et al., 2002; Fennelly and Roberts, 1998) showed substantial generation of alkane and alkene coupling products using bimetallic iron particles, suggesting that coupling reactions may be enhanced in the presence of bimetallic mineral surfaces. The mineralogy for Soils 1 and 2 is complex and consists of multiple minerals; thus, these soil multi-metallic mineral surfaces may facilitate coupling reactions.

4.3.2.1 PCE Removal Rates as a Function of Temperature

The mole fraction of PCE abiotically transformed for Soil 2 based on the sum of the dechlorination transformation products generated in Figure 1 was calculated. PCE removal in Soil 2 was greatest at 55° C, and only a small (<0.1%) fraction of PCE was removed over the duration of the experiments. The fractional molar PCE removal for Soil 2 is approximately two orders of magnitude greater than the fractional molar PCE removal for Soil 1 at 55° C.

Due to the presence of the vapor phase (approximately 8 mL) in the vials, Henry's Law partitioning of the PCE occurred between the water and gas phases. To determine the true kinetics of PCE dechlorination, properly accounting for a reduction in the observed rate due to water-gas partitioning of PCE, the following mass balance was employed for PCE in the vials:

$$\frac{\partial C_w}{\partial t} = \frac{k_{\text{bulk}}}{R_e} C_w \quad (4.1)$$

$$K_{\text{bulk}} = -k \left(\frac{\rho k_m}{\varepsilon_w} \right) \quad (4.2)$$

$$R_e = \frac{\varepsilon_v H_{\text{PCE}} + \varepsilon_w}{\varepsilon_w} \quad (4.3)$$

where C_w is the PCE concentration in the aqueous phase, t is time, k_{bulk} is the bulk first-order abiotic dechlorination rate constant, R_e accounts for the reduction in observed rate due to gas-liquid partitioning, k is the intrinsic first-order abiotic dechlorination rate constant, ρ is the bulk soil density, k_m is the PCE linear sorption coefficient on to the reactive mineral surfaces, and H_{PCE} is the dimensionless Henry's Law constant, which is a function of temperature with values of 0.76, 1.6, and 4.1 at 20, 35, and 55° C experiments, respectively (Sander, 1999). PCE sorption to the bulk soil was minimal, as determined by parallel experiments performed at 20° C comparing PCE loss in the vials with and without soil present, so a soil sorption term is omitted from Eq. 4.3. Since the trace adsorption of PCE to the reactive minerals cannot be independently determined, the reaction rate constant is assessed in terms of k_{bulk} rather than k . Equation 4.3 was solved for each temperature in the Soil 2 experiments, with R_e values of 1.2, 1.5, and 2.9 for the 20, 35, and 55° C experiments, respectively; these R_e values account for observed reduction in the dechlorination reaction rate due to PCE partitioning into the vapor phase. First order kinetics were assumed, consistent with several previous abiotic dechlorination studies with ferrous minerals (Butler et al., 2013; He et al., 2015; Schaefer et al., 2015). Measured PCE dechlorination products were used to determine the mass of PCE removed.

The regressed values of k_{bulk} are provided in **Table 4.5**. Regression results indicate that the data are well described by the first-order model, and that k_{bulk} increases with temperature. It is noted that a zero-order model also would have well-described the experimental data, but previous studies have suggested that ferrous mineral enhanced dechlorination is a first-order process (Schaefer et al., 2013; He et al., 2015). The correction for leakage at 55° C resulted in less than a 35% change in the regressed first-order rate constants.

Table 4.5. Regressed first order PCE or TCE bulk rate constants (k_{bulk} in Eq. 4.1) for Soils 1 through 3. Due to the limited amount of transformation and scatter in the data, first order rate constant were not calculated for the 20 and 35 C conditions for Soil 1. The calculated k_{bulk} scaled for *in situ* soil conditions, which are based on the rate constants observed for Soil 2, also are provided for each temperature. The $\pm$ values indicate the 95% confidence intervals. NA=not applicable.

Temperature (°C)	k_{bulk} (day ⁻¹)	R ²
Soil 1 - PCE		
<i>Observed in Vials (regression results)</i>		
55	$1.7 \pm 0.84 \times 10^{-7}$	0.75
Soil 2 - PCE		

<i>Observed in Vials (regression results)</i>		
20	$8.3 \pm 0.82 \times 10^{-7}$	0.98
35	$10 \pm 1.8 \times 10^{-7}$	0.94
55	$134 \pm 7.8 \times 10^{-7}$	0.99
<i>Average Value Scaled for in situ Soil:Water Ratio</i>		
20	2.0×10^{-5}	NA
35	2.4×10^{-5}	NA
55	32×10^{-5}	NA
Soil 3 - TCE		
<i>Observed in Vials (regression results)</i>		
20	$45 \pm 0.10 \times 10^{-7}$	0.99
<i>Average Value Scaled for in situ Soil:Water Ratio</i>		
20	11×10^{-5}	NA

To further assess dechlorination reaction kinetics in Soil 2, Eqs. 4.1 through 4.3 were applied to the formation of the individual transformation products generated (i.e., acetylene, ethene, butane, and propane). For assessment purposes, acetylene and ethene were lumped together, as ethene is an immediate hydrogenation product of acetylene. Figure 2 shows the contribution of each of the gases on the fractional removal of PCE, and how first order kinetics provide a reasonable prediction of the data for each of the gases. Figure 3 shows the regressed rate constants as a function of inverse temperature for the generated gases. The Arrhenius equation is used to further assess kinetics:

$$k_{\text{bulk}} = A \exp \left[\frac{-E}{RT} \right] \quad (4.4)$$

where E is the activation energy, T is temperature (K), R is the gas constant, and A is a pre-exponential factor. **Figure 4.4** shows that the generation of acetylene+ethene is well described by Eq. 5. The activation energy of 91 kJ/mol (via regression of Eq. 4.4) is substantially greater than the dechlorination activation energies of 32 to 45 kJ/mole observed for zerovalent iron (Su and Puls, 1999; Lien and Zhang, 2007), likely causing the ferrous minerals present in the natural soils examined to be far less reactive towards chlorinated ethenes than Fe⁰. While Eq. 4.4 describes the temperature effects on acetylene/ethene generation, it does not accurately describe the generation of the coupling products butane and propane as a function of temperature, and subsequently the overall PCE dechlorination kinetics (first order rate constants were over-predicted by Eq. 4.4 by

up to a factor of 3.5). The failure of the Arrhenius expression as defined in Eq. 4.4 has been noted when recombination and free radical reactions occur (Gardiner Jr., 1977; Upadhyay, 2006). The coupling reactions that generate butane and propane from PCE (C-C bond formation) are combination-type reactions and are likely radical induced via a reductive process (Lowry and Reinhard, 2001; Schrick et al., 2002; Shang et al., 2017). For such reactions, modified forms of the Arrhenius equation have been developed that show the non-linear functionality similar to that shown for the coupling product data in Figure 3 (Upadhyay, 2006). A more detailed mechanistic kinetic interpretation of these coupling reactions as a function of temperature is beyond the scope of this study.

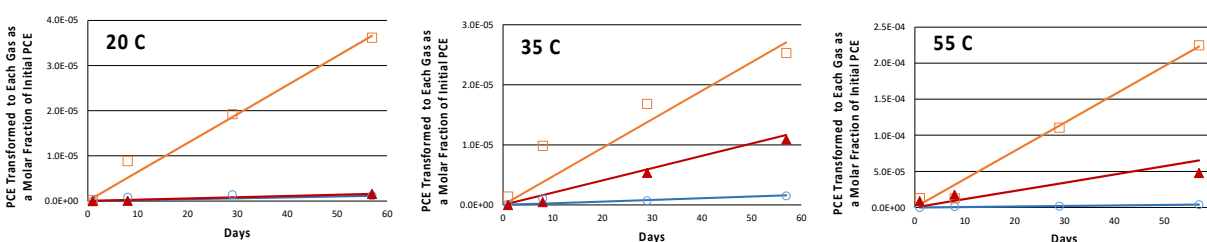


Figure 4.4. Contribution of each gas (□=butane, ▲=acetylene+ethene, ○=propane) in Soil 2 to the cumulative PCE dechlorination calculated based on the generation of reduced gas daughter products at each temperature, and plotted as the fraction of PCE transformed. Averages of the triplicate samples are shown. Lines represent the first-order kinetic model (Eqs. 4.1-4.3) applied to the individual gases.

Alternate explanations for the observed butane and propane formation kinetics, and deviation from the Arrhenius expression in Eq. 4.4, also are possible, but are less likely than the explanation provided above. For example, increased PCE adsorption with increasing temperature could result in the non-linear coupling products behavior shown in Figure 3, as this would cause the magnitude or the slope to increase with increasing temperature due to the enhanced PCE attachment to mineral surfaces. However, previous studies have suggested that chlorinated ethene sorption to mineral surfaces is slightly exothermic (Goss and Eisenreich, 1996; Werth and Reinhard, 1997), and thus PCE is expected to adsorb less as temperature increases, which would cause the opposite deviation from non-linearity shown for the coupling products in Figure 3. In addition, such PCE sorption behavior would likely impact all gaseous products, not just the coupling products. Thus, the deviation of the coupling product generation from the Arrhenius model (Eq. 4.4) is likely not due to sorption effects.

It is also plausible, but unlikely, that diffusional mass transfer within small clay pores was the cause of the observed bulk deviation from the Arrhenius equation (Eq. 4.4) at 55° C. The aqueous PCE diffusion coefficient increases by approximately a factor of 2 between 20 and 55° C (Cussler, 1994). Heating can alter the pore structure of the clays (Romero et al., 2001), which can further

enhance the diffusion coefficient with temperature. If the active mineral sites were located in micropores, the increase in temperature at 55° C may also have facilitated the activation-driven diffusion processes in the micropores (Werth et al., 2000). These mass transfer effects would all lead to an increase in the magnitude of the slope with increasing temperature, consistent with the coupling product trend in Figure 3, if mass transfer to mineral surfaces played a role in controlling the observed dechlorination kinetics. However, such mechanisms would impact all gaseous products, not just the coupling products, unless the mineral sites responsible for coupling reactions were different from those responsible for the other (acetylene and ethene) products, with only the minerals responsible for coupling products located within clay micropores.

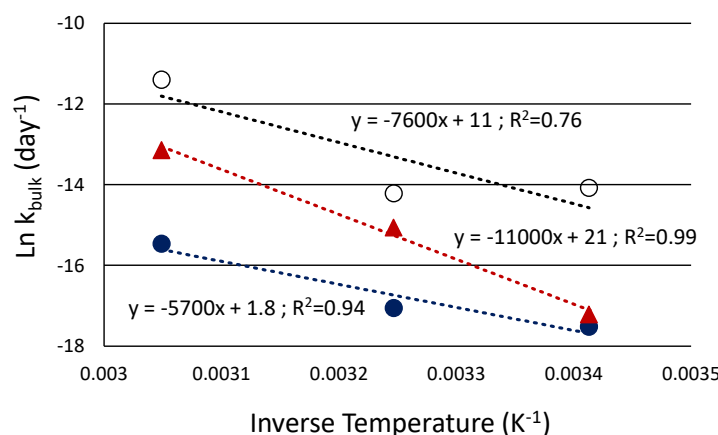


Figure 4.5. Values of k_{bulk} for generation of butane (○), propane (●), and acetylene+ethene (▲) as a function of temperature. The dashed lines represent the regression of the Arrhenius equation to the data.

4.3.2.2 TCE Dechlorination in Soil 3

Results for Soil 3 are provided in Table 3 and Figure S7. Although TCE was used for Soil 3, previous studies showed that the observed dechlorination rate constant for TCE is within a factor of 2 of that observed for PCE (Lee and Batchelor, 2002a,b). Thus, it is reasonable to expect that TCE dechlorination behavior in natural solids is within a factor of 2 (with respect to k_{bulk}) as PCE, so comparison among the 3 soils is warranted. Acetylene was the only observed dechlorination product for Soil 3, indicating that the dechlorination mechanism was abiotic despite not gamma-irradiating the soil. The absence of higher coupling products for Soil 3 is not readily explained. Because previous studies have shown that coupling product formation proceeds via (chloro)acetylene (Elsner et al., 2008), it is unlikely that the presence of acetylene and absence of butane is attributable to the use of TCE (rather than PCE) as the parent compound in the Soil 3 experiments. Rather, the difference in dechlorination daughter product composition is likely due

to the complexities associated with the mineral phase and mineral distribution in the soil; this is discussed further below.

4.3.2.3 Mineral Impacts on Abiotic Dechlorination

The values for k_{bulk} in Table 3 do not appear to be correlated to magnetic susceptibility, although previous studies have been careful to note that magnetic susceptibility is only appropriate as an indicator of abiotic dechlorination when the magnetic susceptibility is controlled by magnetite (USEPA, 2009; Weidemeier et al., 2017), which is not the case for the soils used in this current study; this current study is consistent with that assessment. Soil 1, which has a magnetic susceptibility approximately 15-times greater than the other soils, did not exhibit any measureable abiotic dechlorination at 20 and 35° C, and approximately 2 orders of magnitude less abiotic dechlorination than Soils 2 and 3 at 55°. Ferrous mineral content appeared to be a better indicator for abiotic dechlorination among these 3 soils, as Soils 2 and 3 had elevated ferrous mineral contents and measureable abiotic dechlorination. Riebeckite and/or chlorite likely are the ferrous minerals responsible for the observed abiotic dechlorination in Soils 2 and 3, while illite likely is the ferrous mineral responsible for the limited abiotic dechlorination in Soil 1.

Riebeckite minerals (Wittenham Gorge, Australia, and Longvale Quarry, California) were purchased from the Mineralogical Research Co. (San Jose, CA), and chlorite minerals (Green Fine Grained Chlorite Schist Metamorphic Rock) were purchased from American Educational Products (Cat# 5241-01A) to test, using the same methodology used for the soils, if these minerals facilitated the abiotic dechlorination of PCE. No generation of PCE transformation products was observed in these mineral tests. However, the minerals used were dry and stored under aerobic conditions for an unknown period of time prior to the laboratory dechlorination testing, so the mineral surfaces may have become oxidized, and the tested riebeckite and chlorite minerals may not have been representative of the minerals present in the clays. Ferrous mineral analysis performed on the chlorite showed that the ferrous mineral content was only 31 mg/kg, indicating that the chlorite mineral tested was not representative of the ferrous-rich chlorite that is likely present in Soils 2 and 3. Hence, further work is needed to unambiguously determine the ferrous iron minerals responsible for abiotic PCE reduction.

The 20° C data show that Soil 3 had a much greater k_{bulk} than Soil 2, despite the fact that Soil 2 had a greater ferrous mineral content. Because the XRD data show that the ferrous minerals in Soil 3 are present at equal or greater quantities in Soil 2, it is speculated that either the specific nature of the individual ferrous minerals, the local pH near the surface of the clay minerals, and/or the pore scale distribution of the minerals are the cause of the increased abiotic dechlorination activity and lack of coupling products observed in Soil 3. It has been shown that abiotic dechlorination activity for chlorinated ethenes can increase with pH (Lee and Batchelor, 2002b). Thus, the elevated pH for Soil 3 may have facilitated enhanced abiotic dechlorination despite the fact that

the ferrous mineral content was slightly less than that of Soil 2. The elevated pH for Soil 3 may also have contributed to the transformation product distribution, as previous studies using FeS have suggested that elevated pH results in an increase in the fractional conversion of TCE to acetylene (Butler and Hayes, 2001).

The clay mineralogy also may have played a role in the observed difference between Soils 2 and 3. Soil 3 has a much smaller clay content and a much higher coarse-grained fraction than Soils 1 and 2. Additional mineral testing showed that >90% of the ferrous mineral content was associated with the silt and clay fraction of Soil 3, and an additional batch test performed on the sand component of Soil 3 showed that no measurable abiotic dechlorination was observed in the sandy material associated with Soil 3. Thus, the difference in behavior with respect to ferrous iron and dechlorination are likely associated with the fine grained particles in Soils 2 and 3. For Soil 3, chlorite is the primary clay mineral by mass, which likely accounts for an appreciable fraction of the ferrous mineral content. For Soil 2, illite is the primary clay by mass, which is typically much more deficient in ferrous iron than chlorite. Mass transfer limited access to the (presumably) ferrous-rich chlorite clay by intermingled illite clay in Soil 2 serves as a plausible explanation for the lower k_{bulk} in Soil 2 than in Soil 3. Alternately, Soil 2 contains kaolinite, which is a known weathering/oxidation product of chlorite (Jackson, 1963). Ferrous-rich chlorite clays in Soil 2 could be bounded by kaolinite, which would also potentially serve as a means to limit the dechlorination reaction via diffusional mass transfer.

4.3.2.4 Environmental Implications

The solid:liquid ratio in the experimental vials was substantially less than those encountered for in situ soils. The term ρ/ϵ_w in Equation 4.2, assuming a bulk clay density of 1.8 g/cm³ and a water porosity of 0.4 under typical field conditions, would be approximately 24-times greater than in the vials, resulting in values of k_{bulk} that are 24-times larger than those measured in this study (Table 3). At 20° C, assuming first-order kinetics and the k_{bulk} for in situ conditions in Table 3, 315 years would be required for PCE concentrations to decrease by 90% via abiotic dechlorination alone in Soil 2. This estimated timeframe assumes that the dechlorination rate constant remains constant, which previous studies in rock have shown may be a reasonable assumption for a time scale of decades (Schaefer et al., 2015). At 55° C, 20 years would be required for PCE concentrations to decrease by 90% via abiotic dechlorination alone. Thus, for Soils 1 and 2, mild heating is not expected to result in treatment timeframes that are economically attractive.

For Soil 3, assuming the TCE bulk rate constant for in situ conditions in Table 3, 48 years would be required for TCE concentrations to decrease by 90% via abiotic dechlorination alone. Thus, these rates would likely be an important to include in conceptual site models incorporating natural attenuation process. If heating to 55° C also were to increase the dechlorination bulk rate constant of TCE in Soil 3 by 20-times, then the time needed for TCE concentrations to decrease by 90%

under solid:liquid ratios typical of field conditions at 55° C would be 3.2 years. Similarly, using the same assumption for DCE with a bulk dechlorination rate constant of 0.0015 day⁻¹ measured at 20° C for a shallow sandy sediment containing magnetite (Ferrey et al., 2004), heating to 55° C would result in 90% DCE removal in approximately 76 days. Thus, there is reasonable potential that mild heating of natural sediments with high intrinsic rates of ferrous mineral induced dechlorination reactions can be used for cost-effective remediation.

4.3.3 Batch Results for Anaerobic Conditions with Sediments 1-3

Generation of the observed reduced gas transformation products for sediments 1 through 3 is shown in **Figure 4.6**; no generation of reduced gases were observed for sediments 4 and 5. Acetylene or ethene were only present in the vials spiked with TCE, and not in the vials without amended TCE or in the no-sediment controls. Acetylene is not a known biotic reductive dechlorination product of TCE, and therefore serves as an indicator of abiotic dechlorination. While ethene can be both a biotic and abiotic TCE dechlorination product, the absence of measurable VC and DCE accumulation suggests that the ethene is likely an abiotic transformation product (Schaefer et al., 2009; Cápiro et al., 2015). For sediments 1 through 3, results show that abiotic generation of the TCE dechlorination products was occurring throughout the duration of the experiment, as evidenced by the increasing trends in acetylene and ethene. However, the lack of a replicate for the sediment 3 acetylene data at 106 days warrants interpreting this final data point with caution. The abiotic generation of reduced gases such as acetylene and ethene under anaerobic conditions is consistent with many previous studies using either TCE or PCE (e.g, Lee & Batchelor, 2002a; Schaefer et al., 2013; Schaefer et al., 2017; Schaefer et al., 2015).

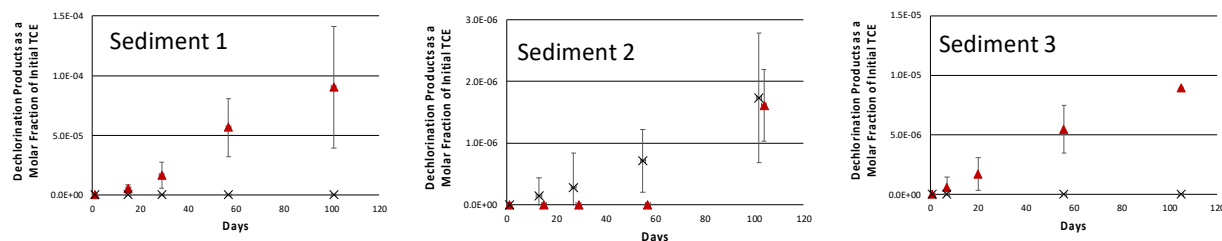


Figure 4.6. Dechlorination products acetylene and ethene as a molar fraction of the initial TCE (3 mM) present in the anaerobic experiments for sediments 1 through 3. The scale on the y-axis varies among the sediment types due to the large difference in dechlorination product concentrations. Error bars represent the 95% confidence intervals. For sediment 3, 1 of the 3 replicates consistently had acetylene concentrations below the analytical quantification limit, and thus was excluded. Also, for the 106 day sampling point for sediment 3, substantial TCE and acetylene leakage was observed in one replicate, and thus was omitted. Ethene data for Sediment 2 are offset by two days to provide clarity for the error bars. ▲=acetylene, X=ethene

In addition to the reduced gas generation shown in **Figure 4.6**, sediment 1 also exhibited generation of glycolic/acetic acid; generation of glycolic/acetic acid was only observed in the TCE-

amended sediment 1 samples. The generation of this TCE oxidation product is further explored in subsequent sections, as the role of trace oxygen levels on hydroxyl radical formation is examined. It is noted that the analytical method used could not distinguish between glycolic acid and acetic acid. Glycolic or acetic acids have been observed as oxidative abiotic chlorinated ethene dechlorination products in studies using crushed rock and in electrochemical systems (Yu et al., 2018, Yuan et al., 2012). No sustained generation of OAs was observed for the other sediments. However, quantification limits for the OAs were much greater than for those of the reduced gases - approximately 5×10^{-3} as a molar fraction of the TCE present - so low-level OA generation at a magnitude similar to that exhibited by acetylene could have occurred. Similarly, no generation of CO_2 was observed in any experiment, but CO_2 levels of 2×10^{-3} to 52×10^{-3} (depending on the background CO_2 levels) as a molar fraction of the TCE present would have been required for detection. Regardless, for all sediments, the fraction of TCE transformed for the duration of the experiment was a very small (<0.01) fraction of the initial TCE present in the vials.

4.3.3.1 Batch Results for Aerobic Conditions

For the aerobic experiments, no generation of any reduced gasses was observed for any of the sediments. Generation of both formic/glyoxylic acid and glycolic/acetic acid, relative to the non-TCE spiked controls, are shown in **Figure 4.7** for sediments 1 through 3; no other OAs were present, and no OA generation was observed in sediments 4 and 5. No OAs were generated in the non-TCE-amended samples for sediment 1. For sediment 2, glyoxylic/acetic acid was generated in the non-TCE amended samples, but at levels lower than in the TCE-amended samples. Similarly, for sediment 3, OAs were generated in the non TCE-amended samples, but at levels lower than in the TCE-amended samples.

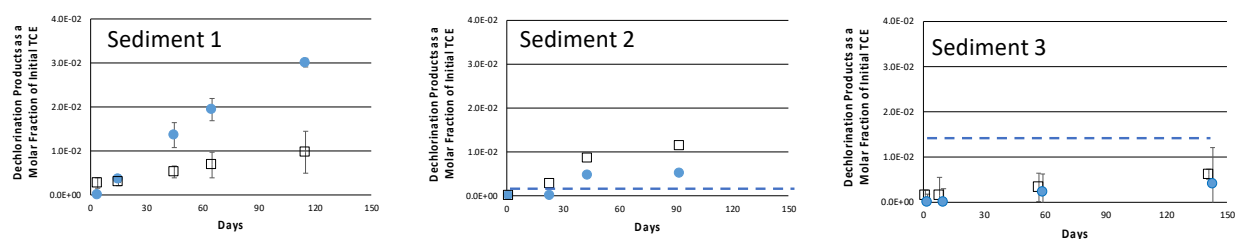


Figure 4.7. Dechlorination products (OAs) as a molar fraction of the initial TCE (3 mM) present in the aerobic experiments for sediments 1 through 3. Error bars represent the 95% confidence intervals. The horizontal dashed line represents the CO_2 background levels determined in the sediment samples that were not amended with TCE. For sediment 1, the CO_2 quantification limit is 0.07. No increases in CO_2 in the TCE-amended samples were observed relative to the CO_2 levels in the non-TCE amended treatments. Data for Sediment 3 are offset by two days to provide clarity for the error bars. ●=formic/glyoxylic acid, □=glycolic/acetic acid.

The generation of the OAs in **Figure 4.7** indicates that oxidative abiotic dechlorination of TCE occurred, similar to previous observations under aerobic conditions (Pham et al., 2008; 2009; Liu

et al., 2017). While only a small molar fraction of the TCE was transformed during the study (e.g., 0.04 in sediment 1), the increasing trends in OAs for sediments 1 through 3 throughout the duration of the experiment indicate that transformation of the TCE was ongoing. For sediment 2, the triplicate samples needed to be combined for OA analysis because elevated sulfate concentrations (approximately 500 mg/L) required pretreatment using anion exchange resin prior to analysis, which required an increased sample volume. No CO₂ generation above background levels (CO₂ background levels indicated in Figure 4.4) was observed. The elevated background CO₂ level for sediment 1 is likely due to off-gassing from siderite (Duckworth & Martin, 2004). Previous kinetic studies (Pham et al., 2008; 2009) have shown that significant CO₂ generation in the aerobic systems was unlikely due to the sustained elevated TCE concentrations.

4.3.3.2 Hydroxyl Radical Generation

Hydroxyl radical generation for sediments 1 and 3 (aerobic conditions) and sediment 1 (anaerobic) are shown in **Figure 4.8**. Results clearly show the formation of hydroxyl radicals, particularly when aerobic conditions were maintained. In addition to the data shown in **Figure 4.8**, aerobic sediment 1 samples prepared without the formaldehyde-forming reagent used to quantify hydroxyl radicals showed no hydroxyl radical formation (<0.3 μ M cumulative hydroxyl radical). This confirms that any trace levels of unexpected background formaldehyde present in the sediments was not responsible for the observed hydroxyl radical generation.

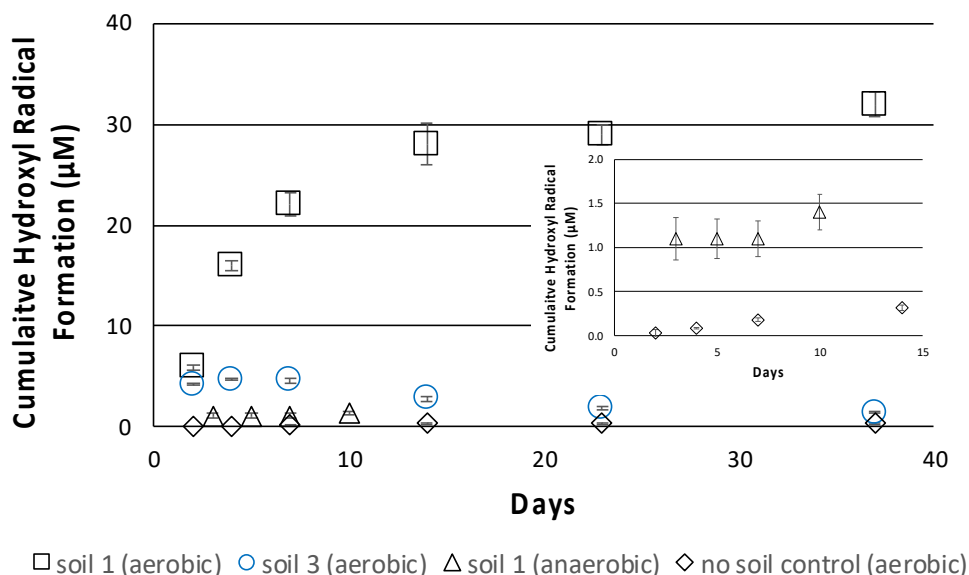


Figure 4.8. Cumulative hydroxyl radical generation. Error bars represent 95% confidence intervals. The inset shows the low levels of hydroxyl radicals generated in the anaerobic sediment 1 samples are clearly greater than in the controls.

Under anaerobic conditions in sediment 1, relatively low levels of hydroxyl radicals were formed. This formation may have occurred due to very low levels of oxygen present in the water (although oxygen headspace levels indicated that oxygen was non-detect, the detection limit for dissolved oxygen, based on oxygen headspace levels and Henry's law, was 26 μM). This is further explored in the sub-section below discussing the role of oxygen on dechlorination mechanisms.

The methodology used to quantify hydroxyl radical generation has been used for short (<1 day) time scales (Tai et al., 2004), and the stability of the formed complexes and ability to appropriately quantify slow hydroxyl generation over longer time scales has not been verified. Thus, the results shown in **Figure 4.8** should be viewed as semi-quantitative, particularly after the first few days. The apparent decreases in the cumulative hydroxyl radical levels suggest that the formaldehyde complex formed likely was not stable over the timeframe of the experiment; thus, the data shown in **Figure 4.8** likely underestimates the long-term hydroxyl radical generation. However, early time (<5 day) results 1) confirm that hydroxyl radicals are in fact being formed, and 2) provide a relative comparison of hydroxyl radical generation among the sediment types and conditions tested.

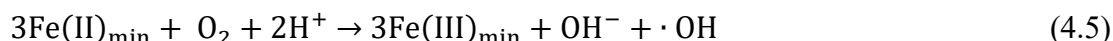
4.3.3.3 The Role of Oxygen on Dechlorination Mechanisms and Transformation Products

In the presence of dissolved oxygen (at dissolved oxygen concentrations greater than 120 μM), no reduced gas generation from the abiotic reductive dechlorination of TCE was observed; reduced gas formation was only observed in the anaerobic experiments when the dissolved oxygen levels were less than 26 μM . The absence of measurable reduced gas generation in the presence of elevated levels of dissolved oxygen likely was due to competitive sorption/reaction with oxygen on ferrous mineral surfaces and/or rapid oxidation of the reduced gases at the ferrous mineral surface via formation of hydroxyl radicals. The latter, however, is unlikely, as trace (approximately 100-times less than the acetylene or ethene observed in the anaerobic experiments) levels of ethane present in the background sediment 1 samples under aerobic conditions remained approximately constant throughout the duration of the experiments. This ethane was present but not generated (or, increasing) during the aerobic study. Thus, the relatively high hydroxyl radical levels formed under aerobic conditions had no measurable impact on the ethane levels.

Consistent with previous studies using pyrite minerals (Pham et al., 2008; 2009), the generation of formic/glyoxylic acids is indicative of sustained hydroxyl radical generation in sediments 1 through 3 under aerobic conditions. The generation of glycolic/acetic acid also is consistent with observed abiotic TCE dechlorination via hydroxyl radicals (Yuan et al., 2012). The relatively constant rate of OA generation in sediments 1 through 3 further suggests that hydroxyl radicals were generated at a relatively constant rate, and that the apparent decrease in hydroxyl radical generation rate was in fact due to the previously discussed long-term instability of the formaldehyde complex. Similar results were observed by Pham et al. (2009), where hydroxyl

radical generation measured using a fluorescence-based approach essentially ceased after 3 days, but hydroxyl radical driven oxidation of dichloroacetic acid continued with no substantial decrease in rate for up to 10 days. Thus, these results highlight the difficulty in quantifying slow and long-term rates of hydroxyl radical generation in experimental systems.

Both the generation of hydroxyl radicals and glycolic/acetic acid under the anaerobic condition for sediment 1 suggest that low levels (<26 μM) of oxygen likely were present in the anaerobic treatments; sediment 1 was the most reactive material tested in this study, and thus likely was most susceptible to hydroxyl radical formation at these low oxygen levels. Oxygen levels at least as low as 17 μM have been shown to facilitate hydroxyl radical induced oxidation of TCE in the presence of pyrite (Pham et al., 2008). In addition, the following net reaction for generation of hydroxyl radicals from the oxidation of ferrous minerals is assumed (Kong et al., 2015; Rimstidt & Vaughan, 2003):



where $\text{Fe(II)}_{\text{min}}$ and $\text{Fe(III)}_{\text{min}}$ are mineral phase ferrous and ferric iron (respectively). The net reaction shown in Eq. 4.5 was originally developed for pyrite minerals where electron transfer between the sulfide and iron mineral recycled the oxidized $\text{Fe(III)}_{\text{min}}$ back to $\text{Fe(II)}_{\text{min}}$ (Rimstidt & Vaughan, 2003), but herein we conservatively assume that such recycling does not occur because the ferrous minerals present in the sediments are not pyrite (although it is possible that trace levels of pyrite are present, but were not detected by XRD). From Eq. 4.5, and regardless of the iron recycling, one mole of molecular oxygen is consumed per mole of hydroxyl radical produced. Assuming formation of the observed OAs requires 1 mole of hydroxyl radical (Li et al., 2007), the amount of oxygen present in the sediment 1 “anaerobic” system was approximately 4-times that needed to generate the observed OA transformation products if oxygen levels were approximately half of the quantification level for oxygen (this accounts for Henry’s Law partitioning of oxygen from the headspace to the water).

To further demonstrate that the OA generation was due to the abiotic oxidation of TCE by hydroxyl radicals, the aerobic experiment for sediment 1 was repeated, but with the addition (100 mM) of known hydroxyl radical scavenger 2-propanol (Kwan & Voelker, 2003). This experiment, performed over a period of 47 days, showed that there was no measurable generation of any OAs, thereby serving as another line of evidence that OA generation was due to the formation of hydroxyl radicals.

4.3.3.4 Kinetics

Consistent with previous approaches for the anaerobic dechlorination of chlorinated ethenes by ferrous minerals (Butler & Hayes, 1999; Schaefer et al., 2013; Schaefer et al., 2017; Pham et al.,

2008), TCE reductive dechlorination in the anaerobic systems is described by the following pseudo first-order expression:

$$\frac{\partial C}{\partial t} = \frac{k_{an}}{R} C \quad (4.6)$$

$$k_{an} = k'_{an} \frac{\rho K}{\varepsilon_w} \quad (4.7)$$

$$R = \frac{\varepsilon_v H + \varepsilon_w}{\varepsilon_w} \quad (4.8)$$

where C is the aqueous phase TCE concentration (mM), t is time (days), R is the retardation factor due to gas-water partitioning, k_{an} is the bulk pseudo first order rate constant (d^{-1}), k'_{an} is the intrinsic pseudo first order rate constant (d^{-1}), ρ is the bulk density ($kg\ L^{-1}$), K is the linear TCE adsorption coefficient to both reactive and unreactive sites ($L\ kg^{-1}$), ε_v and ε_w are the dimensionless gas and water porosities in the vial (respectively), and H is the dimensionless Henry's Law constant for TCE. Since the trace adsorption of TCE to the reactive minerals cannot be independently determined, the observed reaction rate constant is assessed in terms of k_{an} rather than k'_{an} . For Eq. 4.6, the generation of acetylene + ethene is used to determine the change in TCE concentration (C). This approach has been employed previously (Schaefer et al., 2017).

Results from the regression of k_{an} in Eqs. 4.6 through 4.8 to the anaerobic data are provided in **Table 4.6**. The pseudo first-order model provides a reasonable description of the data and the range of k_{an} values observed are consistent with those observed previously in clayey sediments (Schaefer et al., 2017). While a first-order model described the data well, it is noted that a zero-order model also would have provided a reasonable description of the data.

Table 4.6. First order TCE abiotic dechlorination rate constants determined in vials for sediments 1 through 3 for both reductive (k_{an}) and oxidative (k_{ox}) mechanisms. Reductive processes were measurable under anaerobic conditions only. With the exception of sediment 1, oxidative processes were measurable only in the aerobically prepared experiments. The $\pm$ values represent the 95% confidence intervals, and the R^2 values for the regressions are shown in parenthesis. Rate constants scaled to a typical in situ groundwater sediment:water ratio also are provided.

Sediment*	Mechanism	Rate Constant in Vials (k_{an} or k_{ox}) (d^{-1})	Rate Constant Scaled for in situ sediment:water Ratio (d^{-1})
Sediment 1	Reductive	$1.3 \pm 0.16 \times 10^{-6}$ (0.98)	$3.1 \pm 0.38 \times 10^{-5}$
	Oxidative	$3.1 \pm 0.20 \times 10^{-4}$ (0.99)	$7.4 \pm 0.48 \times 10^{-3}$
	Oxidative (anaerobic)	$0.15 \pm 0.024 \times 10^{-4}$ (0.92)	$0.36 \pm 0.024 \times 10^{-3}$

Sediment 2	Reductive	$2.6 \pm 0.92 \times 10^{-8}$ (0.83)	$6.2 \pm 0.28 \times 10^{-7}$
	Oxidative	$2.0 \pm 0.58 \times 10^{-4}$ (0.85)	$4.8 \pm 1.4 \times 10^{-3}$
Sediment 3	Reductive	$1.6 \pm 0.19 \times 10^{-7}$ (0.94)	$3.8 \pm 0.46 \times 10^{-6}$
	Oxidative	$0.94 \pm 0.12 \times 10^{-4}$	$2.3 \pm 0.48 \times 10^{-3}$
(0.97)			

* No measurable dechlorination occurred for sediments 4 and 5.

Eqs. 4.6 through 4.8 also are applied to the oxidative dechlorination of TCE, where TCE dechlorination is determined via the accumulation of formic/glyoxylic acid and glycolic/acetic acid. It is assumed 1 mole of TCE was dechlorinated per mole of OA generated (Pham et al., 2009; Li et al., 2007; Yan & Schwartz, 2000). Application of this first-order model to describe the abiotic oxidative dechlorination of TCE assumes that the oxidant level (or, rate of hydroxyl radical generation) remains constant throughout the duration of the experiment. For the oxidative dechlorination, k_{an} in Eq. 4.6 is substituted with k_{ox} . Regression results for the oxidative abiotic dechlorination of TCE are provided in **Table 4.6**. Regression results show that the pseudo first-order model (or a zero-order model) provided a reasonable description of the data, thereby suggesting that the rate of oxidant generation remained relatively constant throughout the duration of the experiment, as previously discussed.

The solid:liquid ratio in the experimental vials was much less than those for saturated *in situ* sediments. The term ρ/ϵ_w in Eq. (4.7), using the bulk density and volume fraction of water in the vials, is 24-times less than the expected value of ρ/ϵ_w for *in situ* conditions, assuming a bulk clay density of 1.8 g/cm^3 and a water porosity of 0.4 for typical *in situ* conditions. This scaling results in values of k_{an} and k_{ox} that are 24-times larger than those measured in this study; these field-normalized values of k_{an} and k_{ox} are provided in **Table 4.6**.

The values for k_{ox} in **Table 4.6** are 2 to 3 orders of magnitude greater than the values of k_{an} (for sediments 1 through 3, where measurable dechlorination was observed). The values of k_{ox} are greater than k_{an} likely due to the fact that oxygen is more reactive with the ferrous iron surfaces than TCE, thus the oxygen-facilitated generation of hydroxyl radicals and subsequent oxidation of TCE has much faster kinetics than the TCE-ferrous iron reductive reaction. While values of k_{ox} were 2 to 3 orders of magnitude greater than k_{an} , the values of k_{ox} obtained in this study were 3 to 4 orders of magnitude less than those observed using pyrite for abiotic dechlorination of TCE (Pham et al., 2008; 2009). This large difference likely is due to the increased ferrous iron content and/or the available reactive surface of the minerals (and subsequent hydroxyl radical generation) associated with the pyrite minerals, and perhaps also due to the presence of oxidant sinks (e.g., organic carbon) associated with the natural sediments.

Using the observed rate of hydroxyl generation over the first 3 to 4 days as a relative measure of the long-term hydroxyl radical generation rate among sediment types and conditions, an increasing rate of hydroxyl radical generation corresponds to an increasing value of k_{ox} . Thus, the TCE oxidation rate observed for different sediments, and for different oxygen levels, serves as another line of evidence that the OA formation was due to TCE oxidation via hydroxyl radicals. Comparison of the total estimated molar hydroxyl radical generation (assuming the rates measured during the first 3 to 4 days are sustained through the duration of each experiment) to the molar generation of OAs shows that 3 to 4 moles of hydroxyl radicals are required per mole of TCE transformed to OAs. This is much greater than the theoretical 1:1 ratio (Li et al., 2007). This discrepancy is likely due to the fact that alternate hydroxyl radical sinks (e.g., natural organic carbon, other reduced metals) contributed to the oxidant demand. However, it's possible that the initial rates of hydroxyl radical generation overestimate the long-term generation rate.

Previous studies using pyrite (Pham et al., 2008; 2009) have shown that the first order oxidative transformation rate constants for TCE, glycolic acid, and formic acid are similar (i.e., within a factor of 2). The concentration of TCE was approximately two orders of magnitude greater than the concentrations of these OAs. Assuming a first-order kinetic model dictates rates of TCE and OA oxidation via hydroxyl radicals, and that rate constants for these compounds are similar, OA oxidation will be very slow compared to their formation from TCE. The absence of measurable CO_2 generation in sediment 2 (**Figure 4.6**), where the background CO_2 levels were sufficiently low (less than 10 mole % of the OAs) to detect mineralization of the OAs, is consistent with this model.

In addition, the previous study using pyrite minerals (Pham et al., 2009) showed that the first order rate constant for oxidative TCE transformation to formic acid was 12-times greater than the first order rate constant for TCE to glyoxylic acid. Consistent with this finding, Li et al. (2007) showed that the rate constant associated with hydroxyl transformation of TCE to formic acid was 10-times greater than that for transformation to glyoxylic acid. Thus, it is likely formic acid (as opposed to glyoxylic acid) is the actual transformation product in **Figure 4.6**.

To form formic/glyoxylic acid after the initial hydroxyl radical attack on TCE, it has been shown that dissolved oxygen is required, as the dissolved oxygen reacts with carbon centered radicals to form peroxy radicals (Li et al., 2007). For natural solids where reactive mineral surfaces typically reside in clay micropores, oxygen diffusion into these reactive zones is likely limited, and dissolved oxygen is likely to be rapidly consumed via reactions with ferrous minerals (Eq. 4.5), resulting in removal of dissolved oxygen that would otherwise react with intermediate oxidized TCE species. Consistent with this speculated dependence on oxygen availability for TCE abiotic dechlorination, abiotic dechlorination of DCE in a natural sandstone resulted in the formation of glycolic and acetic acids (both of which have been observed as oxidative TCE products) under

conditions described as anaerobic (Darlington et al., 2013). Also consistent with this speculated mechanism are the dechlorination results for sediment 1. Under “anaerobic” conditions, no formic/glyoxylic acid was generated, but only glycolic/acetic acid. Under aerobic conditions ($> 120 \mu\text{M}$ dissolved oxygen), formic/glyoxylic acid was the primary TCE transformation product. Together, these results suggest that oxygen levels play a role in determining the TCE oxidative transformation pathway in natural sediments.

Based on the information provided in the previous sections, the abiotic TCE dechlorination pathways via ferrous minerals shown in Figure 1 are proposed; these pathways are based on results obtained herein, as well as TCE chemical oxidation studies performed by others (Schaefer et al., 2017; Li et al., 2007; Yan & Schwartz, 2000; Halasz et al., 2010). Reduced gases are produced via the anaerobic reductive dechlorination pathway. Based on data presented herein, accumulation of reduced gases was only observed when the dissolved oxygen concentrations were less than $26 \mu\text{M}$, although it is unclear if reduced gas formation could be observed between $26 \mu\text{M}$ dissolved O_2 and $120 \mu\text{M}$ dissolved O_2 (above which no reduced gas formation was observed). OAs are produced via the oxidative dechlorination pathway. The OAs generated depend on the oxygen levels present. The data presented herein show that when dissolved oxygen levels are maintained above $120 \mu\text{M}$, formic/glyoxylic acids were predominantly formed. When the oxygen levels were limited ($<120 \mu\text{M}$, and even less than $26 \mu\text{M}$), glycolic acid was predominantly formed. While a more precise determination of the dissolved oxygen levels that mark these transition points were not part of the scope of the current study, these results serve as a preliminary guide and highlight the mechanisms involved in the abiotic dechlorination transformations. Ultimately, upon depletion of the TCE source, CO_2 is the expected end-product of the oxidative pathway, as the produced OAs likely will be mineralized (Li et al., 2007).

Limited ($<120 \mu\text{M}$) levels of dissolved oxygen lead to the formation of glycolic acid. It has been shown that it is only at this point (i.e., after this carbon centered radical intermediate is formed) in the oxidation pathway from TCE to OAs that dissolved oxygen plays a role in the transformation process (excluding the role of oxygen in the formation of the hydroxyl radicals, as shown in Eq. 4.5) (Li et al., 2007); thus, it is assumed that dissolved oxygen availability would impact this point in the oxidative pathway. While the initial step of the hydrolysis of the carbon centered radical is speculated (**Figure 4.9**), the subsequent elimination of the chlorine atom has been observed (Kwan & Voelker, 2003), as has the hydrolysis of the glycolaldehyde to glycolic acid (Halasz et al., 2010).

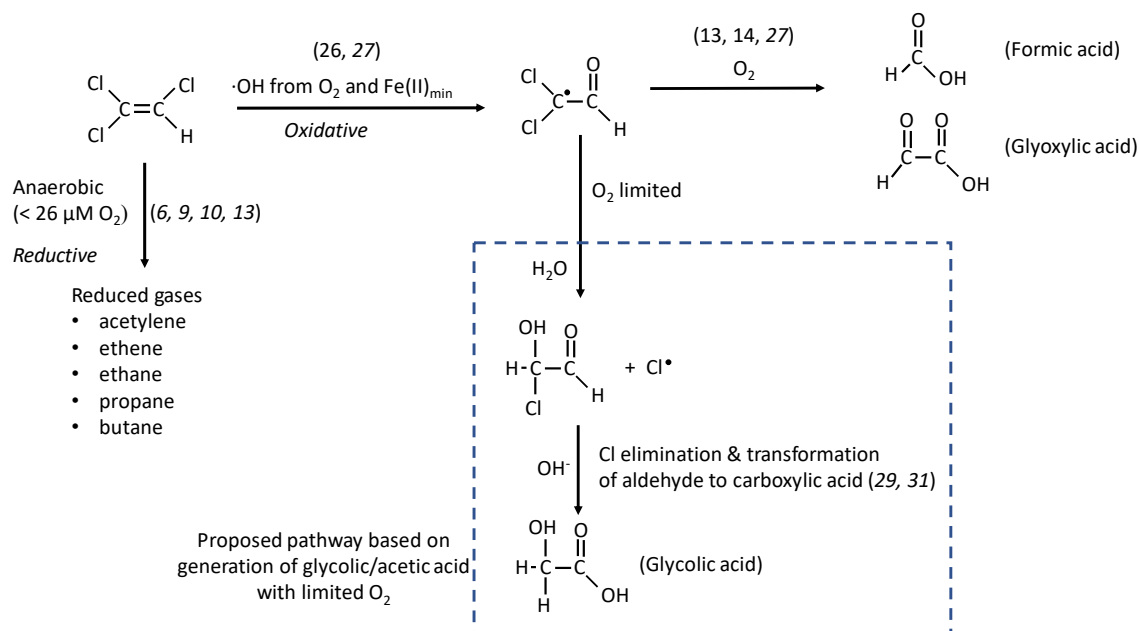


Figure 4.9. Proposed abiotic dechlorination pathway for TCE as a function of available O_2 . The dashed box represents the speculated oxidative pathway under O_2 -limited conditions, leading to the formation of glycolic acid. The final transformation from the aldehyde to the carboxylic acid is similar to reactions observed by others (Yan & Schwartz, 2000; Halasz et al., 2010). Reductive transformations were observed when the dissolved O_2 was less than $26 \mu\text{M}$, but not at dissolved O_2 levels above $120 \mu\text{M}$.

4.3.3.5 Mineralogy and Ferrous Iron Capacity

For the anaerobic experiments, less than 0.003% of the ferrous iron present in sediments 1 through 3 were consumed in the transformation of TCE to acetylene or ethene (assuming 3 moles of iron were required per mole of acetylene/ethene formed). Thus, consistent with previous findings (Schaefer et al., 2013; Schaefer et al., 2015), the reductive dechlorination reactions for these sediments are expected to persist for decades in aquifers impacted with chlorinated ethenes. In contrast, assuming 3 moles of ferrous iron were oxidized to form 1 mole of hydroxyl radical (Eq. 4.5) at the rates observed over the first 3 to 4 days, the ferrous iron consumption in sediments 1 and 3 were 11% and 38%, respectively. These ferrous consumption values likely represent maximum values, as the actual ferrous iron consumption may have been less. For example, it has been observed for pyrite that electron transfer from adjacent anodic sulfide sites reduce $\text{Fe(III)}_{\text{min}}$ to $\text{Fe(II)}_{\text{min}}$ during oxidation of the ferrous minerals (Rimstidt & Vaughan, 2003). It is possible that similar processes occur for the controlling ferrous mineral species (e.g., siderite, ankerite) present in the sediments studied herein.

TCE reductive dechlorination rate constants (k_{an}) increased with increasing ferrous iron content, as shown in **Figure 4.10**; **Figure 4.10** also includes data from a previous study using PCE and clayey sediments (Schaefer et al., 2017). Similarly, values of k_{ox} are plotted as a function of the ferrous mineral content (bottom of **Figure 4.10**). These data suggest that abiotic dechlorination is a function of the ferrous mineral content, thereby serving as another line of evidence that the observed reductive and oxidative dechlorination reactions are due to abiotic interactions with the ferrous minerals. Results in **Figure 4.10** also suggest the potential for using ferrous mineral content as a screening tool for determining if abiotic dechlorination of TCE or PCE may occur. Not shown in this figure (to preserve scale in the top figure) is a sediment from our previous study (Schaefer et al., 2017) exhibiting an anomalously high reductive abiotic dechlorination rate constant ($4.5 \times 10^{-6} \text{ d}^{-1}$ with a ferrous mineral content of 1800 mg kg^{-1}). The pH of this sediment was 9.7, which is much higher than the circumneutral sediment pH for all the other sediments plotted in Figure 2 (top). This result suggests that pH may also be an important factor when considering the abiotic reactivity of naturally occurring ferrous minerals. Increased abiotic reductive dechlorination with elevated pH has been previously observed with green rusts (Lee & Batchelor, 2002b). The reason for this observation with respect to TCE dechlorination is unclear, but it may be related to dissolved ferrous iron sorbed at the iron mineral surfaces (Gregory et al., 2004) and/or increased surface deprotonation at elevated pH values (USEPA, 2009). We note that similar plots of the k_{an} and k_{ox} as a function of the magnetic susceptibility show no observable correlation. Thus, for the sediments and reductive/oxidative transformation products examined in this study, magnetic susceptibility was not an indicator of abiotic dechlorination.

Sediments 1 through 5 contain minerals that likely include ferrous iron, including biotite, siderite, illite, and ankerite (**Table 4.4**). Trace (<1%) levels of chlorite also are present in sediments 1 through 3. The siderite, ankerite, and trace levels of chlorite present in sediments 1 through 3 (but absent in sediments 4 and 5) suggest that these specific minerals play a role in facilitating the observed abiotic dechlorination reactions. However, the levels of these minerals in sediments 1 through 3 do not appear to correlate to their ferrous mineral content or abiotic dechlorination activity. It remains unclear as to exactly which specific ferrous mineral(s) contribute to both the ferrous iron content (as measured via the 1,10-phenanthroline method) and the abiotic dechlorination. Also, based on the trends observed in **Figure 4.10**, it is reasonable to speculate that the same ferrous minerals that contribute to the ferrous iron content are responsible for both the reductive and oxidative dechlorination, since in both cases oxidation of ferrous minerals is contributing to reactivity. Additional mineral evaluation and testing are needed to confirm this hypothesis; such testing is beyond the scope of this current study.

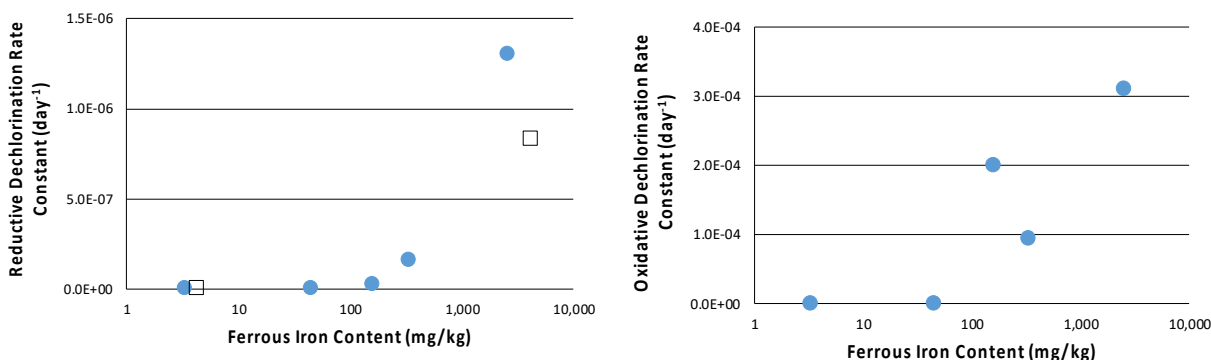


Figure 4.10. Left - Reductive first order dechlorination rate constants (k_{an}) plotted as a function of sediment ferrous iron content in the anaerobic experiments. ●=TCE data, □=PCE data from (10). Right - Oxidative TCE first order dechlorination rate constants (k_{ox}) plotted as a function of sediment ferrous iron content in the aerobic experiments.

4.3.3.6 Potential Environmental Impacts

These abiotic dechlorination processes may play an important role at some chlorinated ethene impacted sites. The corresponding half-life for the field-normalized anaerobic (reductive) rate constant in sediment 1 is 60 years, suggesting that these slow dechlorination processes could be a very important in conceptual site models where long-term natural attenuation processes are likely to play an important role. The field-normalized values for k_{ox} (with corresponding half-lives ranging from 0.25 to 0.83 years for sediments 1 through 3) suggest that this oxidative dechlorination process can play a very substantial role in chlorinated ethene attenuation. However, it is currently unclear how depletion of ferrous minerals will limit overall TCE removal in natural systems. For sediment 1, assuming k_{ox} decreases linearly with the decrease in ferrous iron due to oxygen consumption, TCE removal of approximately 95% would be attained before the ferrous iron is depleted; for sediment 3, less than 50% TCE removal would be attained before the ferrous iron is depleted. These estimates are likely conservative because they assume that electron transfer from adjacent anodic mineral sites do not replenish ferrous mineral levels, and that the reaction order is truly first-order with respect to TCE. If (as previously noted) the reaction order was in fact a zero-order process, then the potential for TCE removal in dilute TCE plumes would be orders of magnitude greater than calculated above, as the bench scale experiments were performed using an elevated TCE concentration of approximately 3 mM.

Furthermore, it is likely that low *in situ* oxygen levels (the ferrous minerals appear to be associated with anoxic low permeability clays) will limit abiotic oxidative dechlorination. However, it is plausible that the oxidative dechlorination could occur near the interface of aerobic zones, as oxygen diffuses towards ferrous minerals in anoxic zones. Additional study is needed to determine the extent to which these oxidative dechlorination processes naturally attenuate chlorinated ethenes

(both long- and short-term) and mitigate back-diffusion from clays, and also the extent to which addition of oxygen to the subsurface may enhance these processes.

Finally, while determination of abiotic TCE dechlorination based on OAs and reduced gases under the controlled conditions of this study was feasible, such determination would be problematic in the field. Formation of OAs and reduced gases (e.g., ethene) from biotic processes, as well as the subsequent biotic transformation of OAs and reduced gases formed from abiotic TCE dechlorination, would limit the effectiveness of directly measuring these compounds *in situ* for determining TCE abiotic dechlorination rates. In addition, the elevated (3 mM) TCE concentrations used in the bench-scale testing facilitated the generation of transformation products. Quantifying these transformation products in more dilute plumes likely would be challenging.

4.4 Batch Results of Iron Sulfide Mineral Reactivity for TCE in The Presence of Sulfate Reducing Bacteria

Abiotic sulfide experiments were conducted without *D. vulgaris* to evaluate TCE transformation with iron sulfide precipitates at different Fe:S ratios without biological effects, and results are shown in **Figure 4.11**. Associated rate constants are presented in **Table 4.7**. Acetylene was the dominant product in all experiments, and acetylene production was measured over time to determine pseudo first order TCE transformation rates. The rate of TCE transformation to acetylene is significantly greater (e.g., 3.17×10^{-5} 1/day) at a Fe:HS⁻ ratio of 2:1 than at ratios of 20:1 and 50:1 ($< 8 \times 10^{-6}$ 1/day). This corresponds to prior work that has shown measurable chlorinated ethene transformation between Fe:S ratios of 2:1 and 1:2. For example, Jeong et al. (2011) observed higher *cis*-DCE transformation rates at an Fe:S ratio of 2:1 compared to 1:1 and 1:2. Hyun and Hayes (2009) observed higher TCE transformation rates at an Fe:S ratio of 1:1 compared 1:2 and 2:1. Important to note, however, is that absolute TCE transformation rates via reaction with abiotically formed iron sulfides in this work are 2-3 orders of magnitude lower than in prior work (Butler and Hayes, 2001, 1999; Jeong and Hayes, 2007). There are many possible reasons for these lower rates; prior studies had shorter experiments, higher solids and TCE loadings, different reaction media, and higher pH values. Regardless, the trend of higher reactivity at Fe:S ratios closer to 1:1 are consistent.

Biotic sulfate experiments were set up similar to abiotic sulfate experiments, except they were amended with sulfate and *D. vulgaris* to evaluate TCE transformation by iron sulfide precipitates influenced by the presence of sulfate reducing bacteria. Results are shown in **Figure 4.11B**. Greater than 90% of the sulfate was reduced to sulfide within 7 days by *D. vulgaris*. Acetylene was again the dominant TCE product in all experiments and was used to determine pseudo first order TCE transformation rates. The rate of TCE transformation to acetylene is almost identical to the abiotic experiments at the Fe:S ratio of 2:1. However, this transformation rate increases with Fe:S ratios up to maximum at 50:1 (1.13×10^{-4} 1/day), and then decreases again at 100:1. Both

abiotic and biotic experiments without iron ($X:HS^-$ and $X:SO_4^{2-}$) showed no acetylene production. No prior work has shown that TCE transformation with iron sulfide mineral precipitates is favored at elevated Fe:S ratios in the presence of SRB.

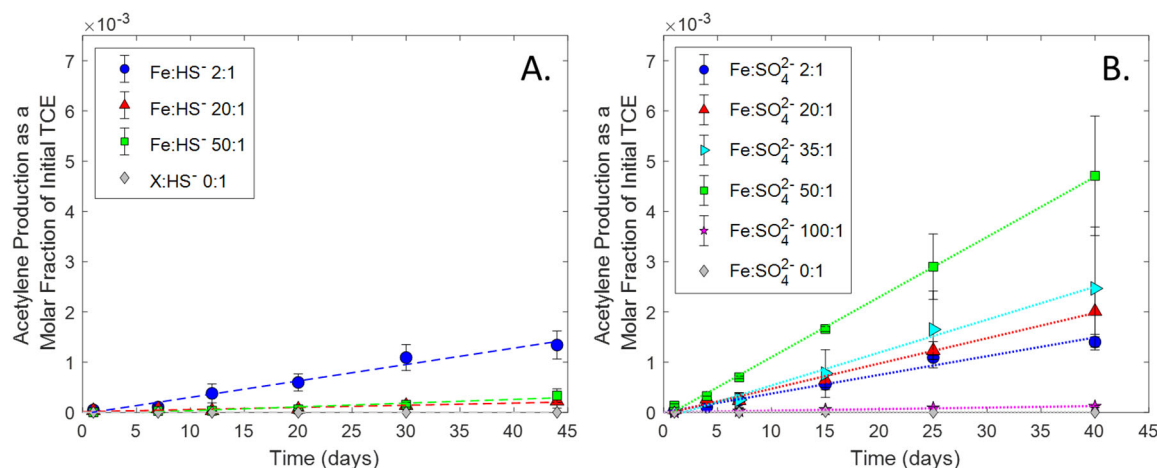


Figure 4.11. Acetylene production from TCE transformation for (A) abiotic sulfide experiments with 1mM sulfide, and (B) biotic sulfate experiments with 1 mM SO_4^{2-} and *D. vulgaris*. Different series represent different molar ratios of aqueous ferrous iron to either SO_4^{2-} or HS^- . The controls without iron are indicated in the legend with an X (grey diamonds).

Table 4.7. Rate constant summary for TCE transformation to acetylene under abiotic and biotic conditions. Sample names with an X indicate no sulfur or iron.

Set	Sample	Average TCE Transformation Rate
Abiotic Sulfide	Fe2+:HS- 2:1	3.17×10^{-5} ($\pm 3.97 \times 10^{-6}$)
	Fe2+:HS- 20:1	4.67×10^{-6} ($\pm 2.72 \times 10^{-6}$)
	Fe2+:HS- 50:1	7.51×10^{-6} ($\pm 2.27 \times 10^{-6}$)
Biotic Sulfate	Fe2+:SO42- 2:1	3.63×10^{-5} ($\pm 2.73 \times 10^{-6}$)
	Fe2+:SO42- 20:1	5.01×10^{-5} ($\pm 1.13 \times 10^{-5}$)
	Fe2+:SO42- 35:1	6.71×10^{-5} ($\pm 3.25 \times 10^{-5}$)
	Fe2+:SO42- 50:1	1.13×10^{-4} ($\pm 2.58 \times 10^{-5}$)
	Fe2+:SO42- 100:1	3.13×10^{-6} ($\pm 4.66 \times 10^{-7}$)
Abiotic Sulfate/Sulfide	Fe2+:SO42- 50:1	no reaction
	Fe2+:HS-:SO42- 50:0.5:0.5	2.08×10^{-5} ($\pm 1.96 \times 10^{-6}$)
Microbial Product	Fe2+:HS- 50:1 Autoclaved	5.44×10^{-5} ($\pm 8.50 \times 10^{-7}$)
	Fe2+:HS- 50:1 Filtered	1.47×10^{-4} ($\pm 3.18 \times 10^{-5}$)
	Fe2+:X 50:X Filtered + Iron	no reaction
	X:X 0:0 Filtered Culture	no reaction
Select Groundwater Experiments	Fe2+:SO42- 2:1	2.67×10^{-5}
	Fe2+:SO42- 20:1	1.34×10^{-4}
	Fe2+:SO42- 50:1	no reaction

4.4.1 Sulfate is not Responsible for the Enhanced TCE Transformation with Iron Sulfide in the Presence of Sulfate Reducing Bacteria

Abiotic sulfate/sulfide experiments were performed to evaluate if the elevated rate constant observed in the biotic sulfate experiment at the Fe:S ratio of 50:1 is due to the presence of sulfate, and not SRB. This was done by mimicking initial and intermediate sulfate/sulfide concentrations expected during the biological sulfate reduction experiment. Before sulfate reduction occurs, only 1 mM SO_4^{2-} is present. When half of the sulfate is reduced to sulfide, 0.5 mM of both SO_4^{2-} and HS^- are present. In both cases, 50 mM Fe^{2+} is present. Results showing TCE transformation to acetylene are shown in **Figure 4.12A**. There is no TCE transformation when only sulfate is present. The TCE transformation rate constant in the half sulfide, half sulfate experiment is 2.07×10^{-5} 1/day, which is 2.75 times that of the abiotic sulfide at the Fe:S ratio of 50:1 (7.51×10^{-6} 1/day), and ~65% that the abiotic sulfide experiment at the Fe:S of 2:1 (3.17×10^{-5} 1/day). Interestingly, the rate constant of the half sulfide, half sulfate experiment is approximately 6 times the biotic sulfate experiment at 100:1 (3.13×10^{-6} 1/day), which has the same $\text{Fe}^{2+}:\text{SO}_4^{2-}$ ratio. While the elevated TCE transformation constant observed in the half sulfide, half sulfate experiment is higher than the abiotic sulfide at 50:1, it is still only 18% of that determined for the biotic sulfate experiment at the Fe:S ratio of 50:1. Hence, the presence of sulfate appears to have some beneficial effect relative to the abiotic system, but it is not responsible for the relatively high TCE transformation rate observed in the biotic sulfate experiment at the Fe:S ratio of 50:1.

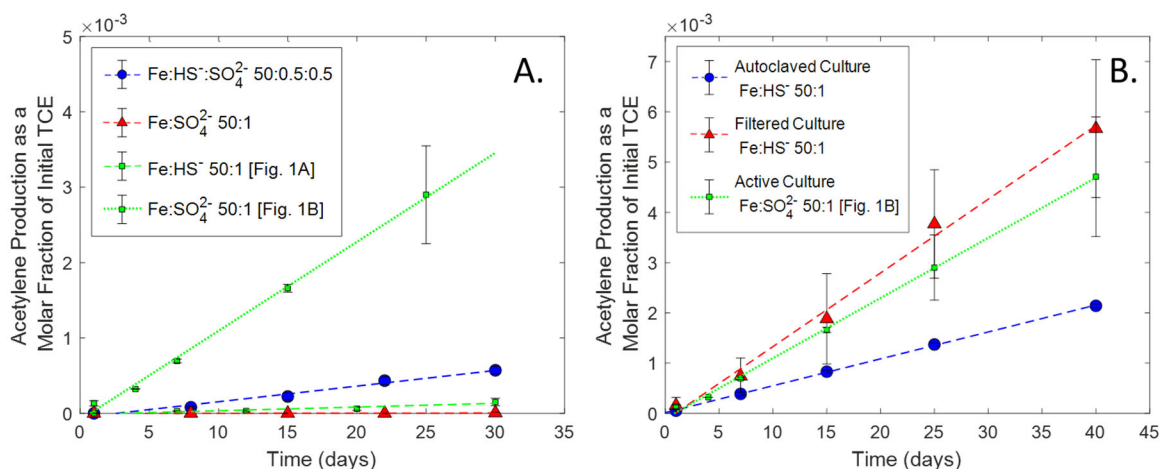


Figure 4.12. Acetylene production from TCE transformation in experiments with (A) Fe:S molar ratios of 50:1 with different sulfur sources (sulfide, sulfate, or sulfate/sulfide), and (B) Fe:S molar ratios of 50:1 for autoclaved and filtered *D. vulgaris* cultures with sulfide, compared to an active sulfate-reducing culture. The abiotic Fe:HS⁻ 50:1 series and biotic 50:1 Fe:SO₄²⁻ series from Figure 3.1 are plotted for comparison (small green squares, dashed lines abiotic and dotted lines biotic). Note difference in axes on Figure 3.2A.

4.4.2 Microbial Exudates But Not Cells Are Necessary for Biologically Enhanced TCE Reaction with Iron Sulfide Precipitates

Microbial product experiments were conducted to determine if active SRB are required to obtain the elevated TCE transformation rates in the biotic sulfate experiments at the Fe:S ratio of 50:1, or if only inactive SRB cells or cell exudates are required. Results are shown in **Figure 4.12B**. The TCE transformation rate constant with autoclaved cell cultures is only $\sim 1/3$ that of the biotic sulfate experiment at the 50:1 Fe:S ratio. However, the TCE transformation rate constant with the filtered cell culture is approximately the same. The experiments with filtered *D. vulgaris* cultures indicate that soluble microbial products produced by *D. vulgaris* are enhancing TCE transformation to acetylene. The lower production of acetylene with the autoclaved cell cultures suggests microbial exudates or lysis products important to TCE transformation may be denatured or destroyed during autoclaving. Microbial product experiments without sulfur or without iron and sulfur showed no TCE reactivity, indicating that the microbial exudates alone do not promote TCE transformation, and iron sulfide minerals are required.

4.4.3 Groundwater Biotic Experiments

Groundwater biotic experiments were performed to evaluate the potential for TCE transformation enhancement by the SRB in a natural matrix; like the biotic sulfate experiments, both lactate and *D. vulgaris* were added to promote sulfate reduction. Experiments were conducted at nine different $\text{Fe}^{2+}:\text{SO}_4^{2-}$ ratios, and results are shown in **Figure 4.13**.

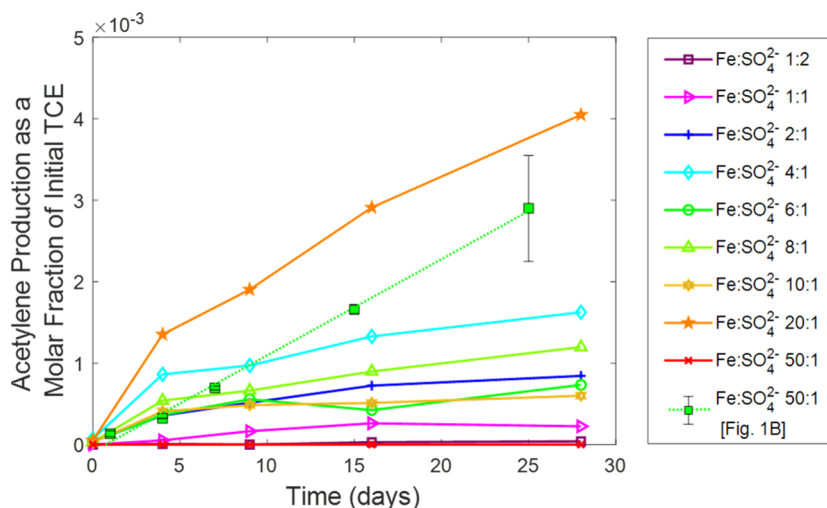


Figure 4.13. TCE transformation to acetylene in groundwater with Fe^{2+} , SO_4^{2-} , and *D. Vulgaris*. Biotic sulfate data from Figure 12B is plotted for comparison (green squares with dotted line).

The buffering capacity of the groundwater was lower than the tris-buffered experiments, resulting in a larger pH range from 7.48 at a $\text{Fe}^{2+}:\text{SO}_4^{2-}$ ratio of 1:2 to 5.87 at a $\text{Fe}^{2+}:\text{SO}_4^{2-}$ ratio of 50:1. TCE transformation to acetylene is greatest at the 20:1 Fe:S ratio, and comparable to the biotic sulfate

50:1 ratio. TCE transformation is less at the 2:1 Fe:S ratio, and comparable to the abiotic sulfide result at 2:1. TCE transformation to acetylene drops to almost zero at the 50:1 Fe:S ratio. However, the pH drops to 5.87 for the 50:1 groundwater experiment, which likely influences the reducing capacity of mineral surface sites. These experiments support enhancement of TCE reduction by iron sulfide precipitates in the presence of sulfate reducing bacteria in a natural groundwater matrix.

4.4.4 Mackinawite is Favored to Precipitate in the Biotic Sulfate Experiments Only

Saturation indices (SIs) from equilibrium modeling were determined for selected minerals. TCE transformation rates are plotted against mackinawite and ferrihydrite SIs in **Figure 4.14**; these minerals were chosen as representative iron sulfide and iron oxide minerals, respectively, and other iron sulfide and iron oxide minerals follow similar trends. Reduction potentials in the simulations were fixed at experimentally measured values. In the biotic sulfate experiments, mackinawite is favored to form at Fe:S ratios of 35:1 and 50:1 (red diamonds and triangles, **Figure 4.14**), but not at higher and lower values. Sulfate green rust is favored to precipitate at all Fe:S ratios in biotic sulfate experiments, though the saturation indices are slightly elevated at higher at ferrous iron loading above 2 mM. In contrast, ferrihydrite is not favored to form at Fe:S ratios of 35:1 and 50:1, but is favored at higher and lower values (**Figure 4.14B**).

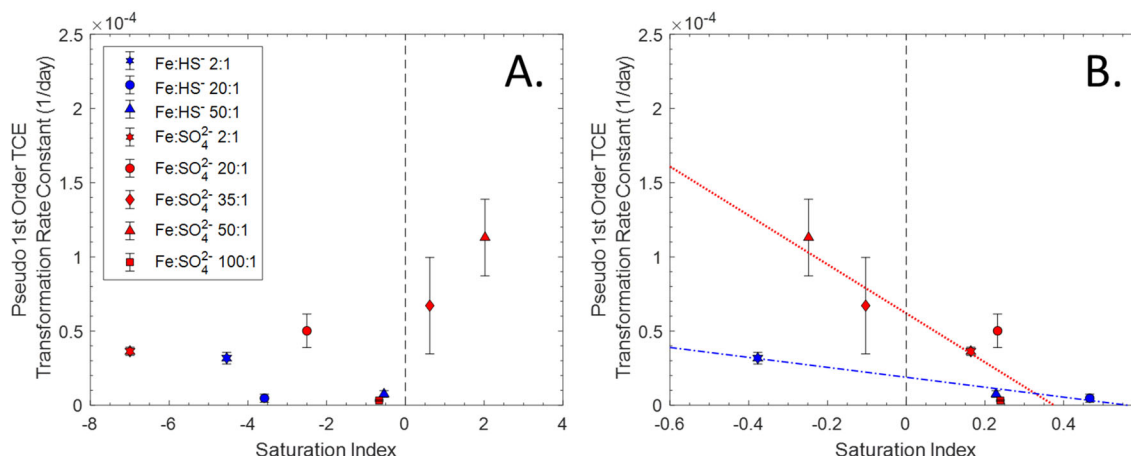


Figure 4.14. Pseudo first order TCE transformation rate constants for TCE transformation to acetylene plotted against saturation indices for (A) mackinawite and (B) ferrihydrite for biotic sulfate (red markers) and abiotic sulfide (blue markers). Dashed black line is a saturation index of zero and precipitation is favored above (to the right of) this line.

In the abiotic sulfide experiments, mackinawite is not favored to precipitate at any Fe:S ratios, while ferrihydrite is only favored at 20:1 and 50:1. In the abiotic sulfate/sulfide experiment, the sulfate-containing green rust is favored in both the all sulfate and half sulfate half sulfide experiments, and ferrihydrite is favored in both experiments, while mackinawite is not favored

under either condition. The favored presence of mackinawite in only the biotic sulfate experiments at intermediate (i.e., 35:1 and 50:1) Fe:S ratios suggests this mineral is a key to the higher reactivity of TCE transformation rate constants in the biotic sulfate experiments.

4.4.5 SEM, EDS, ICP-OES, XRD, and XPS Data Do Not Indicate Clear Trends Between Solid Properties and TCE Reactivity in Abiotic and Biotic Experiments

Dark solid precipitates were collected from both abiotic sulfide and biotic sulfate experiments and characterized for morphology and chemical composition. SEM results are shown in **Figure 4.15**. Platy structures comparable to mackinawite are evident (Herbert et al., 1998; Picard et al., 2018), and there is no apparent difference between abiotic sulfide and biotic sulfate experiments.

Both ICP-OES (on digested samples) and EDX elemental values for total Fe:S ratio are reported in **Table 4.8**, and trends are similar for the approaches. The Fe:S ratios increase with initial aqueous phase ferrous iron loading, although the measured ratios are less than the initial aqueous phase ratios. The latter indicates that high Fe^{2+} concentrations remained in the aqueous phase after mineral formation. A ratio of 1:1 is expected for pure mackinawite. If this mackinawite is present in the 50:1 biotic sulfate experiment as suggested by geochemical modeling, then high concentrations of Fe^{2+} are present on the solid surface or other minerals are present.

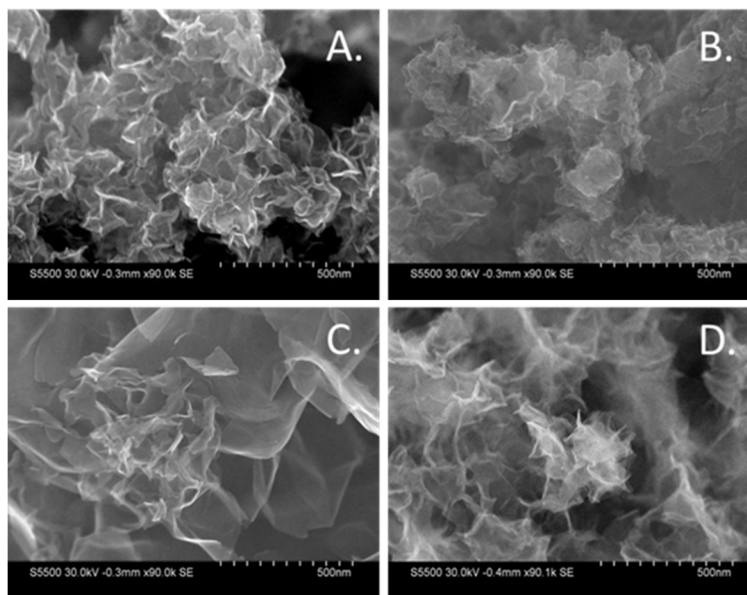


Figure 4.15. SEM images showing iron sulfide morphology of abiotic, (A) $\text{Fe}^{2+}:\text{HS}^-$ 2:1 and (B) $\text{Fe}^{2+}:\text{HS}^-$ 50:1, and biotic, (C) $\text{Fe}^{2+}:\text{SO}_4^{2-}$ 2:1 and (D) $\text{Fe}^{2+}:\text{SO}_4^{2-}$ 50:1, experiments. Similar morphology was observed for the different conditions.

Table 4.8. Comparison of Fe:S ratios with different characterization methods.

Set	Analysis	Fe:S Ratio		
		2:1	20:1	50:1
Biotic (sulfate)	Fe:S XPS	1.35	2.29	5.89
	Fe:S EDX	2.11	--	9.45
	Fe:S ICP	0.91	1.43	8.6
Abiotic (sulfide)	Fe:S XPS	2.09	4.52	5.42
	Fe:S EDX	0.92	--	4.61
	Fe:S ICP	1.2	2.35	3.46

XPS was used to probe Fe:S ratios at the precipitate surfaces. As with ICP-OES and EDX, Fe:S ratios of solids from XPS increase with higher Fe^{2+} loading and are generally above 1. Comparisons of Fe:S ratios among all methods show no obvious trends correlated to TCE reactivity. Efforts were made to obtain XRD diffraction patterns for these samples to further probe the presence of mackinawite, but no defined peaks were observed. The samples either had limited crystallinity or the crystallites were too small to provide measurable diffraction patterns.

XPS spectra were also used to interpret oxidation states and binding of iron and sulfur species on the solid surfaces, and results are shown in **Figures 4.16** and **4.17**. Peaks for S2p consistent with reduced sulfur (S(II)) and poly sulfides (S_n^{2-}) (Fantauzzi et al., 2015; Mullet et al., 2002) were observed for all biotic sulfate and abiotic sulfide samples. A transition from more reduced to oxidized sulfur at higher iron loading in biotic sulfate experiments is shown for ratios of 2:1, 20:1, and 50:1 in **Figure 4.16**. For the biotic sulfate experiments, peaks consistent with SO_4^{2-} (binding energy ~ 169 eV) were only apparent at the highest Fe:S ratios (i.e., 50:1 and 100:1), but not in the 35:1 experiment with similar TCE reduction activity as the 50:1 experiment. Four different iron bonding environments are indicated in the Fe 2p_{3/2} data (**Figure 4.17**): Fe(II)-S, Fe(II)-O, Fe(III)-S, and Fe(III)-O (Lan and Butler, 2016). All four are present in both abiotic sulfide and biotic sulfate experiments, with some enrichment of Fe(II)-S in the abiotic sulfide 2:1 Fe:S ratio experiment. Hence, clear trends in XPS results with TCE reactivity are not apparent.

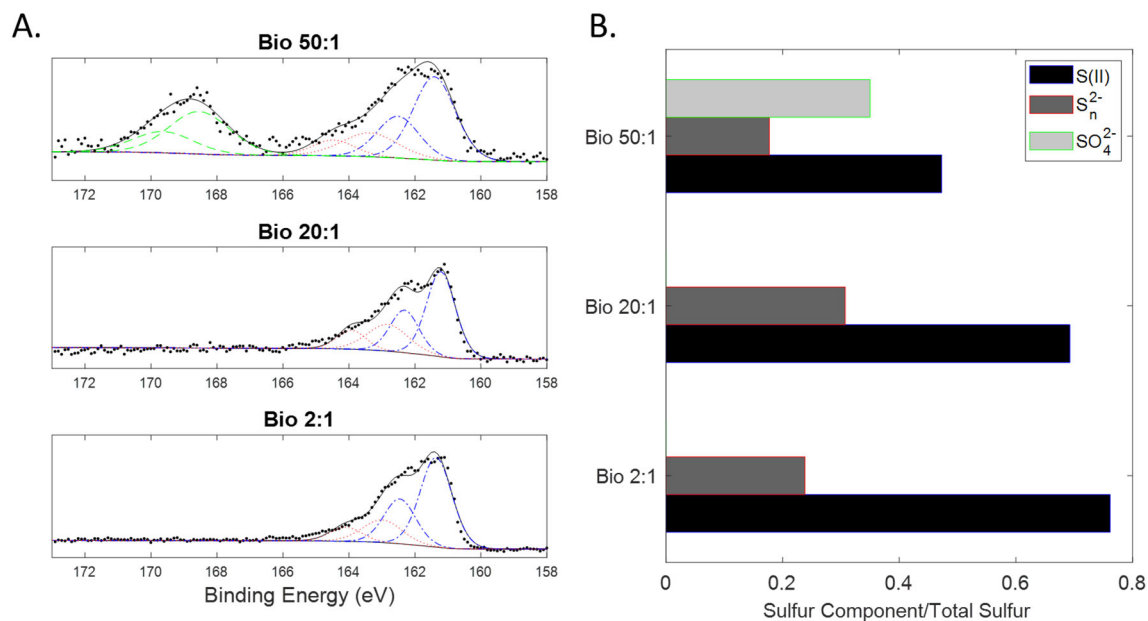


Figure 4.16. XPS data for sulfur in biotic sulfate experiments presented as (A) XPS spectra for S_{2p} with component fits [green = sulfate, red = polysulfides and/or elemental sulfur, blue = S(II)], and (B) XPS spectra components normalized by total sulfur.

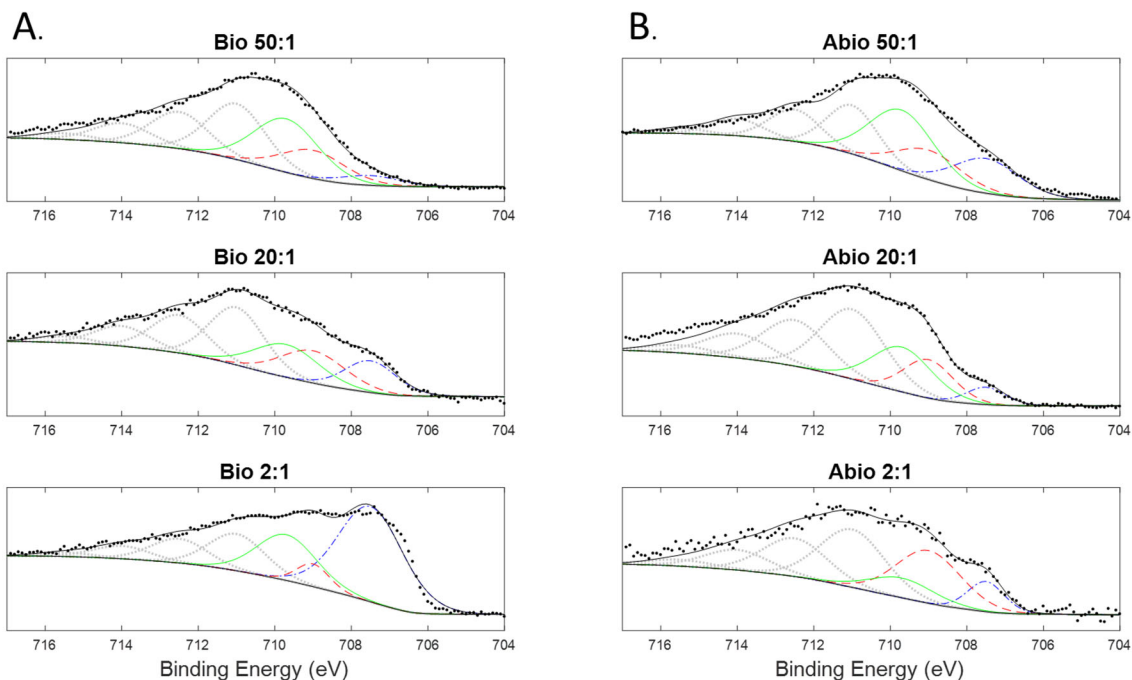


Figure 4.17. XPS data for iron Fe_{2p3/2} in (A) biotic sulfate experiments and (B) abiotic sulfide experiments [blue dot-dash = Fe(II)-S, red dash = Fe(II)-O, green line = Fe(III)-S, and grey dots = Fe(III)-O].

4.4.6 Reduction Potential Is a Possible Indicator of TCE Reactivity

Aqueous iron concentrations and reduction potentials were measured at the end of each experiment and results are reported in **Figure 4.18** versus TCE pseudo first order rate constants. Aqueous phase ferrous iron loadings increase with initial iron loading as expected. They are correlated to TCE reactivity (**Figure 4.18A**) when only the biotic experiments are considered up to the 50:1 Fe:S ratio, but not when the 100:1 ratio is considered. When both abiotic and biotic experiments are considered, no correlation is evident.

Reduction potentials are more negative with increasing ferrous iron for biotic sulfate experiments for ratios from 2:1 to 50:1 (red circles, **Figure 4.18B**), but show an increase at 100:1 (which correlates with a decrease in TCE transformation rate). The decrease in reduction potential at 100:1 was attributed to less mackinawite precipitation at higher iron loading relative to iron oxides. A trend is not apparent for the three abiotic sulfide data points (blue diamonds, **Figure 4.18B**). When all abiotic and biotic experiments are considered together, there is a weak relationship ($R^2=0.66$) between reduction potential and TCE reactivity, and the lack of correlation among the three abiotic sulfide data points may be due to the relatively less negative reduction potentials of these experiments (where the dependence on TCE reactivity is weaker).

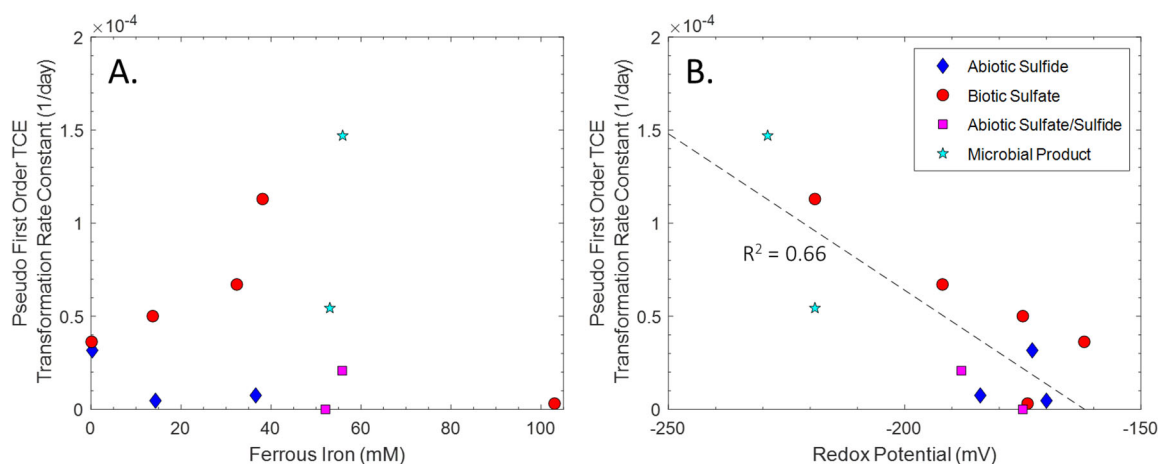


Figure 4.18. TCE transformation rate constants for abiotic sulfide, biotic sulfate, abiotic sulfate/sulfide, and microbial product experiments plotted against (A) aqueous ferrous iron and (B) reduction potential.

4.4.7 Discussion

The results provide compelling evidence that the presence of exudates from sulfate reducing bacteria enhance the reduction of TCE to acetylene with iron sulfide precipitates formed at initial Fe:S solution ratios from 20:1 to 50:1, but not at lower or higher ratios. The presence of sulfate or bacterial cells do not appear necessary for this enhancement, and exudates from sulfate reducing

cells alone cannot promote TCE reaction. Solids characterization results (SEM, EDS, ICP-OES, XRD, XPS) do not provide key insights, though these techniques may provide more relevant insight in experiments with shorter experimental times in which the precipitates are subjected to less aging. However, geochemical modeling results indicate that only in the biotic sulfate experiments at these intermediate Fe:S ratios (Fe:S 35:1 and 50:1) is mackinawite favored to precipitate while ferrihydrite is not (**Figure 4.14**). This suggests that TCE transformation is enhanced by iron sulfide mineral precipitation but limited under conditions that preferentially promote formation of iron oxides which are less reactive toward TCE.

Reduction potential measurements provide another key piece of evidence that is in agreement with geochemical modeling results. They show a weak correlation between higher TCE reduction rates and more negative reduction potentials. More negative reduction potentials are consistent with more mackinawite and less iron oxides (Rickard, 1969b; Rickard and Luther, 2007). Hence, reactivity with mackinawite, and the lack of iron oxides, appears to be key to high TCE reactivity in the presence of sulfate reducing bacteria exudates.

Microbial exudates likely include electron transfer mediators (ETMs) that are in their reduced states and acting as a redox buffer to lower the reduction potential of the medium, thereby favoring mackinawite formation over iron oxides. There is evidence from XPS studies that TCE is reduced by Fe(II) in mackinawite, either resulting in the release of sulfide to media, or the formation of mixed Fe(II)/Fe(III) greigite (Lan et al., 2016). Related studies have identified ferrihydrite, an amorphous Fe(III) hydroxide, as a mineral product resulting from reactions with chlorinated solvents and mackinawite (Lan and Butler, 2016). Therefore, the sulfate reducing ETMs are likely reducing Fe(III) that forms during TCE reaction and maintaining mackinawite. This reaction mechanism is illustrated in **Figure 4.19**, and in the absence of SRB the pool of sulfate reducing bacteria ETMs is limited due to the lack of regeneration.

There has been substantial work evaluating possible electron donating SRB exudates. *D. vulgaris* can biosynthesize cobalamin (vitamin B₁₂), a range of different cytochromes, riboflavin, and other redox active compounds listed in **Table 4.9**. The reduction potential of these ETMs makes any of them feasible reductants for ferric iron formed during reaction of iron sulfide with TCE. We also note that the compounds listed in **Table 4.9** are not necessarily unique to *D. vulgaris*. Most have been identified in other bacteria, including dissimilatory metal reducing bacteria (DMRB) common to the subsurface (Canstein et al., 2008; Field et al., 2000; Marsili et al., 2008; Meyer et al., 2004; Newman and Kolter, 2000). Hence, the role of *D. vulgaris* in this work is likely not unique, but can be carried out by many subsurface bacteria. While the role of ETMs in contaminant transformation is widely accepted, this study is the first known report of sulfate reducing bacteria utilizing soluble species to sustain low reducing conditions and maintain reduced biogenic iron sulfide minerals for abiotic TCE transformation.

Table 4.9. Reduction potentials for TCE and some potential ETMs in water at pH 7 and 25°C.

Reaction (Oxidized/Reduced)	Reduction Potential (mV)	Reference
$\text{Fe}^{3+}/\text{Fe}^{2+}$	770	Bard et al. (1985)
cob(III)alamin/cob(II)alamin	200	Lexa et al. (1977)
ubiquinone ox/red	113	Thauer et al. (1997)
Fe(III)-porphyrin/Fe(II)-porphyrin	65	Schwarzenbach et al. (1990)
$\text{Fe}(\text{OH})_3/\text{FeCO}_3$ [HCO_3^-] = 10^{-3} M	-50	Schwarzenbach et al. (2005)
menaquinone ox/red	-74	Thauer et al. (1997)
FMN/FMN H_2	-190	Thauer et al. (1997)
FAD/FAD H_2	-220	Thauer et al. (1997)
cytochrome c_3 ox/red (<i>D. vulgaris</i>)	-290	Yagi & Maruyama (1971)
FeS ox/red	-300 *	Kirsch et al. (2011)
ferredoxin ox/red	-390	Madigan et al. (2012)
cob(II)alamin/cob(I)alamin	-400-600	Wohlfarth & Diekert (1997)

* approximate value determined from FeS slurry

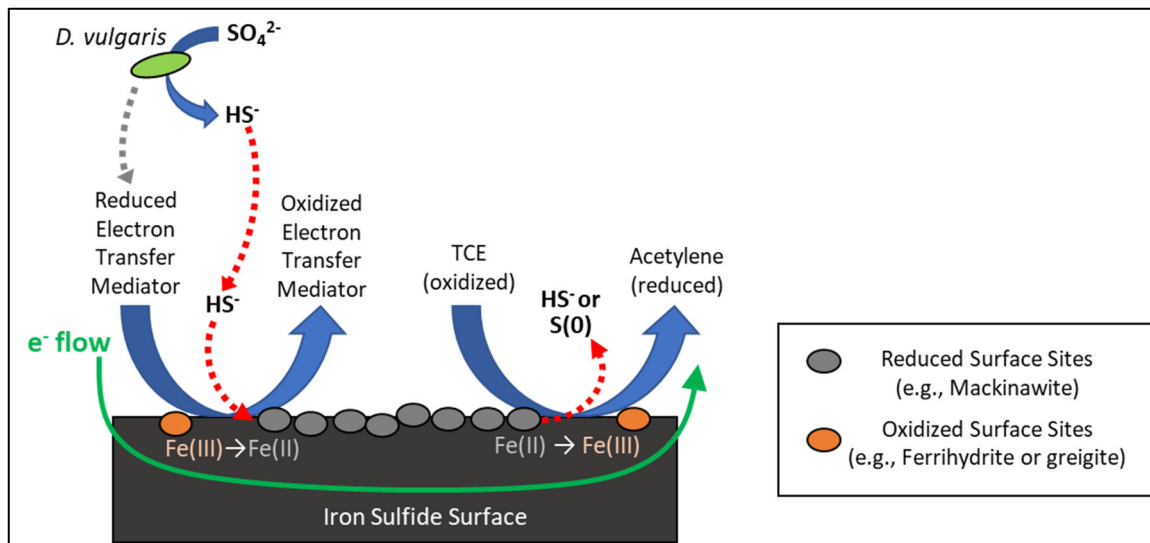


Figure 4.19. Schematic illustrating the role of sulfate reducing bacteria (*D. vulgaris*) and electron transfer mediators on maintaining reducing conditions favorable for iron sulfides with high reactivity for TCE.

4.4.8 Environmental Significance

The results from this work are encouraging for environmental contaminant remediation. All experiments preferentially selected for acetylene as a product. While very low fractions of initial TCE were transformed to acetylene, there was not significant production of other partially dechlorinated products such as *cis*-DCE, *trans*-DCE, and vinyl chloride, which are more mobile than TCE. This study highlights the potential for enhancing the TCE transformation kinetics with microbial redox-active species, while still selecting for innocuous, fully dechlorinated products. These findings are relevant for a range of environmental remediation approaches from monitored natural attenuation to more active approaches that supplement iron, sulfur, or carbon sources. While the main study presented was conducted in a buffered system, the results presented in **Figure 4.12** show that soluble redox-active species from SRB can promote TCE transformation in groundwater with less buffering capacity, though the groundwater results reiterate the importance of either controlling the pH or accounting for pH effects in remedial design. Lower pH improves stability of $\text{Fe}^{2+}(\text{aq})$, but also results in more protonated, less nucleophilic surface sites on the surface of mineral precipitates, which ultimately yields lower TCE transformation rates.

Previous studies have shown the potential for biologically enhanced chlorinated solvent transformation with different microbial metabolisms and iron sulfide. Gander et al. (2002) showed that 1,1,1-trichloroethane transformation with mackinawite was enhanced in the presence of a methanogenic consortium. Butler et al. (2013) demonstrated that microcosms supplemented with Fe(II) and sulfate resulted in enhanced TCE degradation and noted that biotic and abiotic processes contributed to faster TCE transformation kinetics. Finally, Huo et al. (2016) showed higher carbon tetrachloride transformation rates in the presence of *Shewanella putrefaciens* relative to abiotic iron sulfide controls. The current study expands on this previous work and looks specifically at the role of SRB, redox potential, and electron transfer mediators on TCE transformation.

This study demonstrated that lower redox potentials – likely promoted by soluble ETMs from SRB – enhance TCE transformation, and that TCE-reactivity depends on the presence of an iron-sulfide precipitate. It was also demonstrated that higher concentrations of ferrous iron can promote more efficient TCE transformation kinetics in the presence of SRB, but excessively high ferrous iron concentrations can lead to a decrease in reactivity. Future work should further explore the role of ETMs in TCE transformation in biogenic iron sulfide systems as well as focus on the complex interactions of mineral precipitation, redox potential, and microbial metal reduction under different sulfur loading and biomass concentrations. Overall, interactions with ferrous iron and microbial redox-active species can have significant implications in many natural environments, and this work adds to our understanding of these processes during biological sulfate reduction.

The transition from iron-reducing to sulfate-reducing conditions is relevant in groundwater, but also in lacustrine and marine sediments, engineered biofilters, and anaerobic terrestrial sediments.

Interactions between iron and microbial redox-active species could control the transformation of different contaminants in groundwater, sediments, and drinking water. Elevated ferrous iron concentrations in the presence of microbial redox-active species may also have implications for carbon and other nutrient cycling. Future studies should focus on gaining improved mechanistic insight into reductive reactions promoted by biogenic iron sulfides in a wider range of environmental systems.

4.5 Batch Results of Iron Sulfide Mineral Reactivity for TCE in The Presence of Iron Reducing Bacteria

TCE reduction results for the biotic ferrihydrite experiments are presented in **Figure 4.20**. Acetylene was the only transformation product detected. Its production increases as the Fe:S ratio increases from 1:1 to 2:1, to 5:1, but then decreases 10:1 (magenta stars) for two replicates. One replicate at 10:1 – denoted outlier – shows acetylene production at rates comparable to the 2:1 ratio experiment (open stars). As will be shown later, the difference in these replicates is the lack of iron reduction activity in the two that showed no TE transformation, and this will yield important insights. The pseudo first order TCE transformation rate constants are summarized in **Table 4.10**. The results indicate that the mass of oxidized iron (i.e., ferrihydrite) that is being reduced influences TCE transformation rates. By contrast, the abiotic ferrihydrite experiments conducted in parallel showed no acetylene production over time.

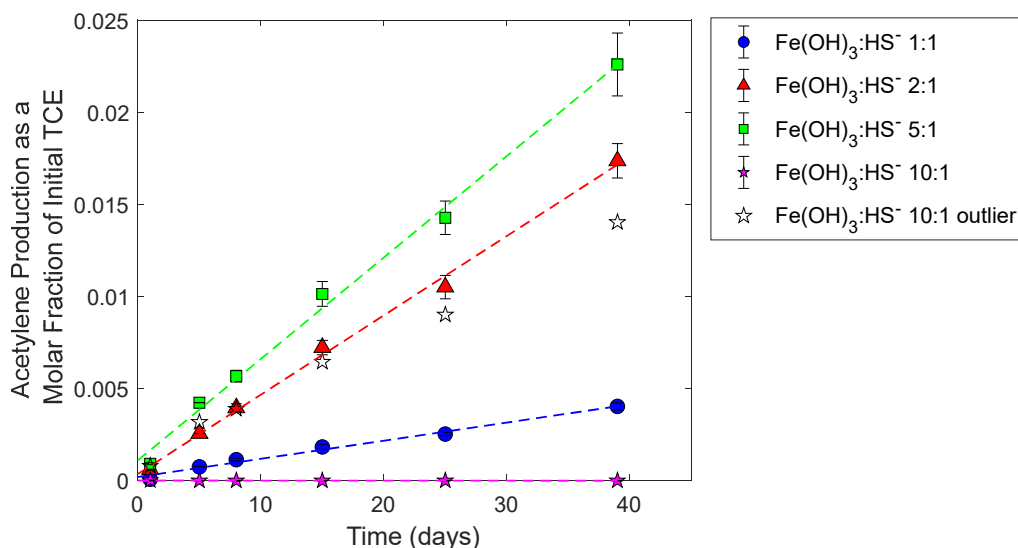


Figure 4.20. Acetylene production from TCE transformation in biotic ferrihydrite experiments at different Fe(OH)₃:HS⁻ ratios in the presence of 1 mM sulfide. Two replicates at the 10:1 Fe(OH)₃:HS⁻ ratio showed no acetylene production; the third replicate at 10:1 showed TCE transformation to acetylene, and is denoted “outlier” (open star symbols).

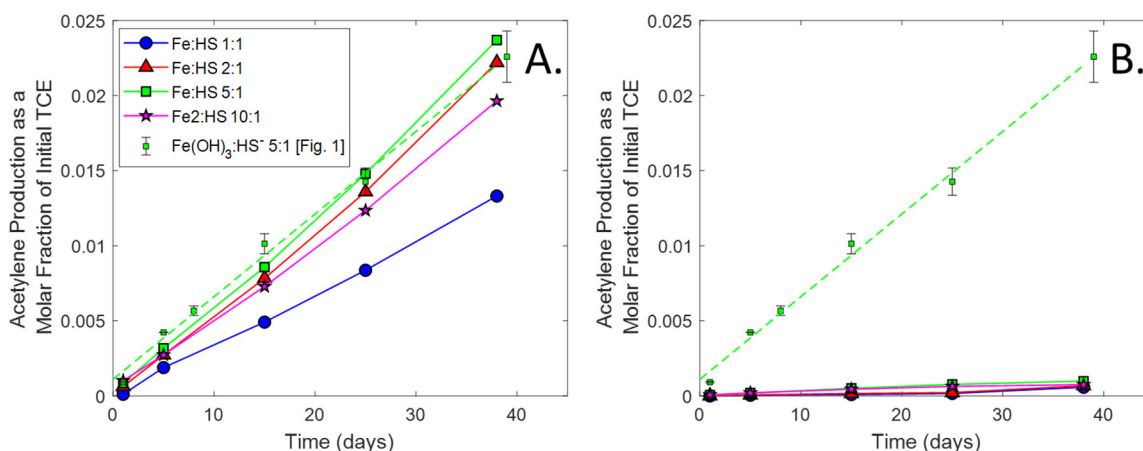


Figure 4.21. Acetylene production from TCE transformation in experiments with Fe^{2+} as the iron source (A) in biotic ferrous iron experiments – with *S. oneidensis* – and (B) in abiotic ferrous iron experiments. Green squares with small markers indicate acetylene production from the $\text{Fe}(\text{OH})_3\text{:HS}^-$ 5:1 biotic ferrihydrite experiment presented in Figure 4.1, and are plotted for comparison.

The biotic and abiotic ferrous iron experiments were conducted to determine if the TCE transformation observed in the biotic ferrihydrite experiments is controlled by active metabolic ferrihydrite reduction, or simply the presence of cellular material or Fe^{2+} . Acetylene was again the only transformation product from TCE, and the results are presented in **Figure 4.21**. In both biotic and abiotic ferrous iron experiments, acetylene production increases from 1:1, to 2:1, to 5:1, and then decreases at 10:1. However, compared to the biotic ferrihydrite experiments, acetylene production in abiotic ferrous iron experiments (**Figure 4.21B**) is an order of magnitude lower, while the acetylene production in the biotic ferrous iron experiment is similar (**Figure 4.21A**). Ratios of pseudo first order rate constants between the biotic ferrous iron and biotic ferrihydrite experiments are 3.54, 1.34, 1.1, and 1.51 at Fe:S ratios of 1:1, 2:1, 5:1, and 10:1 (for outlier), respectively. These ratios indicate that the transformation rates are relatively close at most ratios, but that biotic ferrous iron experiments may yield more acetylene at an iron:sulfur ratio of 1:1 than the biotic ferrihydrite experiments at 1:1. This discrepancy at the 1:1 ratio may be related to limited availability of iron at the solid surface in the 1:1 biotic ferrihydrite experiment, and future work should explore this difference with more focused experiments. These important results indicate that biotic systems with *S. oneidensis* promote faster TCE transformation kinetics, whether the source of iron is ferrihydrite or ferrous iron. Hence, iron reducing bacteria can promote enhanced abiotic TCE transformation with either solid or aqueous phase iron sources.

Table 4.10. Rate constant summary for TCE transformation to acetylene under abiotic and biotic conditions with ferrihydrite and Fe^{2+} .

Set	Sample	Average TCE Transformation Rate Constant (1/day)
Biotic Ferrihydrite & Sulfide	Fe(OH)3:HS- 1:1	9.87×10^{-5} ($\pm 4.03 \times 10^{-6}$)
	Fe(OH)3:HS- 2:1	4.31×10^{-4} ($\pm 2.43 \times 10^{-5}$)
	Fe(OH)3:HS- 5:1	5.54×10^{-4} ($\pm 4.20 \times 10^{-5}$)
	Fe(OH)3:HS- 10:1	No reaction
	Fe(OH)3:HS- 10:1 _{out}	3.31×10^{-4}
	Fe(OH)3:X 1:0	No reaction
	Fe(OH)3:X 10:0	No reaction
	X:HS- 0:1	No reaction
Biotic Ferrous Iron & Sulfide	Fe2+:HS- 1:1	3.50×10^{-4}
	Fe2+:HS- 2:1	5.79×10^{-4}
	Fe2+:HS- 5:1	6.13×10^{-4}
	Fe2+:HS- 10:1	5.02×10^{-4}
Abiotic Ferrous Iron & Sulfide	Fe2+:HS- 1:1	1.42×10^{-5}
	Fe2+:HS- 2:1	1.66×10^{-5}
	Fe2+:HS- 5:1	2.55×10^{-5}
	Fe2+:HS- 10:1	1.85×10^{-5}

4.5.1 XRD and XPS Results Suggest Mackinawite as the Reactive Mineral Species for TCE

XRD results are presented in **Figure 4.22**. The XRD patterns indicate a transition from mackinawite (FeS) at lower Fe:S ratios to a mix of mackinawite and siderite (FeCO_3), or only siderite, at higher Fe:S ratios. Siderite dominates the XRD spectra in the 5:1 and 10:1 (outlier) Fe:S ratio experiments, as well as the 10:0 experiment with no sulfur. Small mackinawite peaks are likely present in the 5:1 and 10:1 experiments, but the increasing peak size for siderite makes this difficult to determine. Hence, the 5:1 Fe:S ratio experiment that shows the most reactivity for TCE may have intermediate amounts of mackinawite and siderite present, making it difficult to determine whether either mineral is more reactive. Also of note is the XRD result for the 10:1 Fe:S experiment that was unreactive; it shows only 2-line ferrihydrite is present, indicating that limited iron reduction and biological activity occurred in these experiments. This may be due to more ferrous iron sorbing to the iron oxide surface and preventing further iron reduction (Roden and Urrutia, 2002).

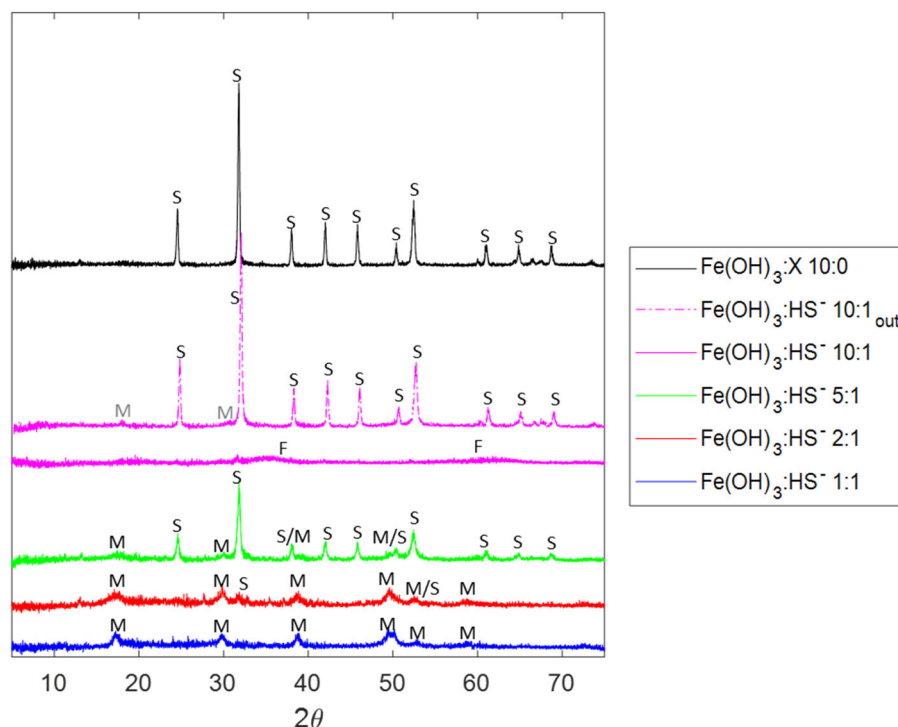


Figure 4.22. XRD patterns of biotic ferrihydrite experiments. Peaks for mineral species denoted with a letter for that mineral [S = siderite, M = Mackinawite, and F = ferrihydrite]. Slash lines indicate an overlap of peaks for different minerals, and light-colored text suggests peaks may be present.

XPS results are presented in **Figures 4.23** and **4.24**. The iron Fe 2p_{3/2} peaks in **Figure 4.23** indicate that the biotic ferrihydrite experiments at Fe:S ratios of 1:1, 2:1, and 5:1 have relatively more reduced iron (i.e., Fe(II)-S, Fe(II)-O), and less oxidized iron as Fe(III)-O, than the abiotic ferrihydrite experiments. The Fe(II)-S is consistent with the occurrence of mackinawite in the XRD spectra, while the Fe(II)-O is consistent with the occurrence of siderite. The XPS results indicate that both minerals are present in biotic ferrihydrite experiments from 1:1 to 5:1, with no apparent trend in their amounts. Interestingly, the abiotic ferrihydrite experiment at the 1:1 H:S ratio showed a strong peak for Fe(II)-S bonds, indicating that reduced iron-sulfur species also precipitated in the abiotic system, yet they were unreactive for TCE. Sulfur 2p data supports the presence of mackinawite in biotic ferrihydrite experiments at Fe:S ratios of 1:1, 2:1, and 5:1.

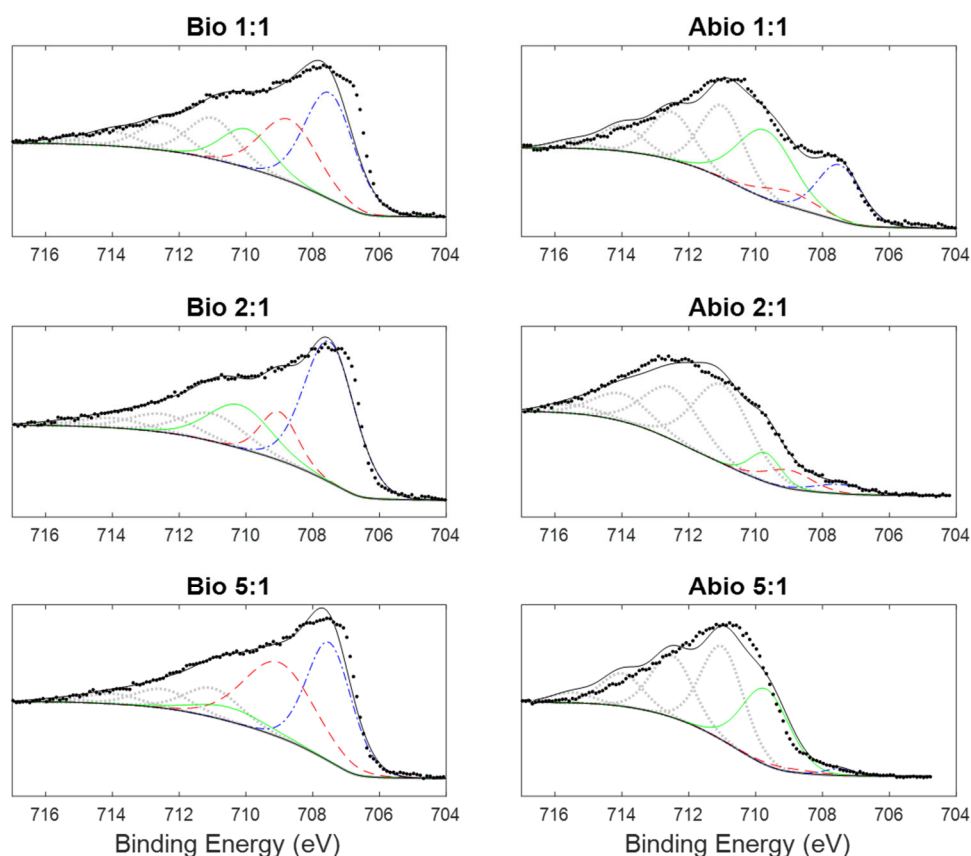


Figure 4.23. XPS spectra of iron $2p_{3/2}$ for biotic (Bio) and abiotic (Abio) ferrihydrite experiments at $\text{Fe}(\text{OH})_3\text{:HS}^-$ ratios of 1:1, 2:1, and 5:1. [blue dot-dash = Fe(II)-S, red dash = Fe(II)-O, green line = Fe(III)-S, and grey dots = Fe(III)-O]. Peaks interpreted as follows: peaks at approximately 707 eV represent Fe(II)-S bonds, (likely mackinawite (Herbert et al., 1998)), multiplet peaks with the main peak at approximately 711 eV represent Fe(III)-O (likely ferrihydrite (Descostes et al., 2000; Lan and Butler, 2016; Mullet et al., 2002)), and peaks between 708 eV and 710.5 represent Fe(II)-O or Fe(III)-S bonds (either siderite or greigite (Descostes et al., 2000; Lan et al., 2016; Mullet et al., 2002)).

Iron XPS $2p_{3/2}$ data are presented separately for the different 10:1 Fe:S experiments in **Figure 4.24**, as well as the biotic control with no sulfur (i.e., 10:0). Like the other biotic ferrihydrite experiments, the outlier at the 10:1 Fe:S ratio has relatively large peaks for Fe(II)-S and Fe(II)-O that are not present in the other 10:1 experiments. The peak for Fe(II)-O is also present in the biotic 10:0 experiments, which showed no TCE reactivity. The results support the interpretation that incomplete iron reduction occurred in the two biotic ferrihydrite replicates at the 10:1 Fe:S ratio. The results also suggest that mackinawite associated with Fe(II)-S, and not siderite associated with Fe(II)-O, is the primary mineral species reactive for TCE.

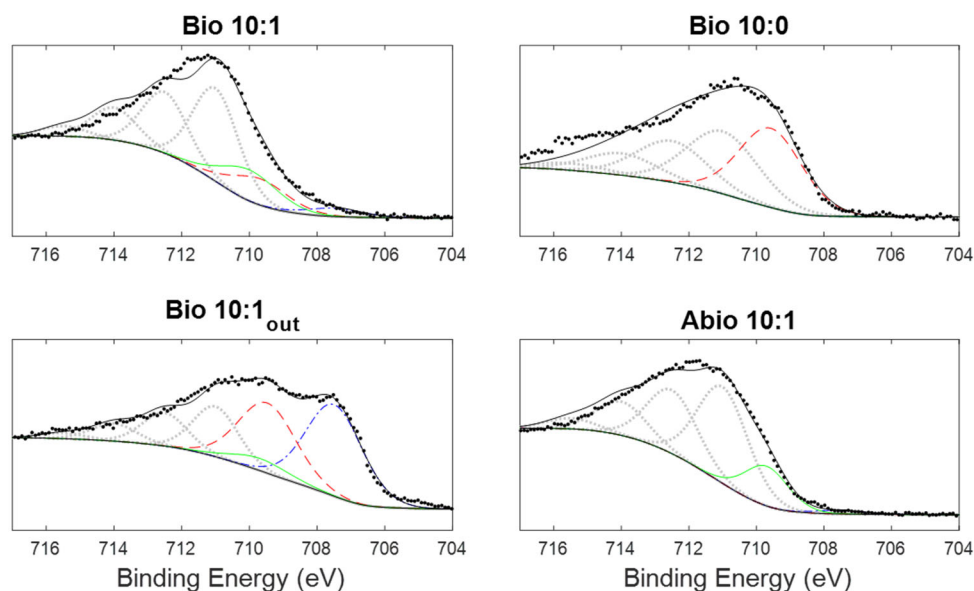
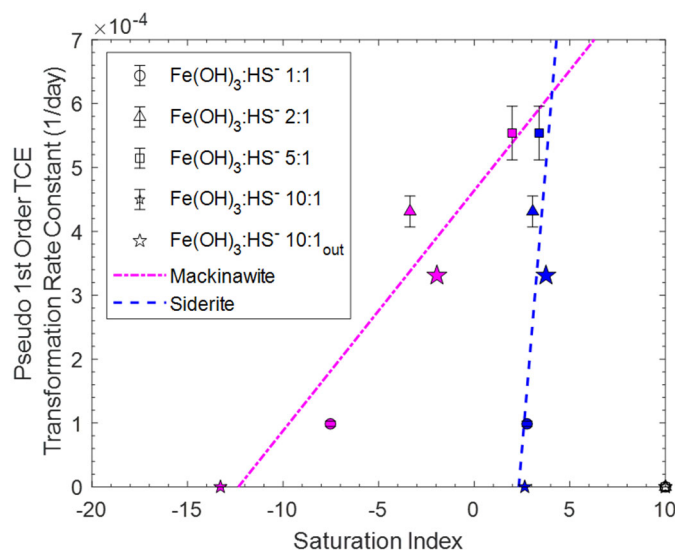


Figure 4.24. XPS spectra of iron 2p_{3/2} for biotic (Bio) and abiotic (Abio) ferrihydrite experiments at a Fe(OH)₃:HS⁻ ratio of 10:1 or 10:0 (no sulfide) [blue dot-dash = Fe(II)-S, red dash = Fe(II)-O, green line = Fe(III)-S, and grey dots = Fe(III)-O].

4.5.2 Geochemical Modeling Suggests Mackinawite as Reactive Mineral Species for TCE

Saturation indices ($\log IAP/K_{sp}$, IAP =ion activity product, K_{sp} =solubility product) were determined from equilibrium calculations in Visual MINTEQ. In the biotic ferrihydrite experiments, mackinawite transitions from being undersaturated at Fe:S ratios of 1:1 and 2:1 to being oversaturated at 5:1, and then back to undersaturated at 10:1. This is not entirely consistent with XRD patterns (**Figure 4.22**), that show mackinawite at 1:1 and 2:1; this could be evidence for a nonequilibrium system. Siderite is oversaturated for all biotic ferrihydrite experimental conditions, which is not clear from the XRD spectra at 1:1 and 2:1 Fe:S ratios, but is supported by XPS data. Saturation indices for mackinawite and siderite are plotted versus TCE transformation rate constants in **Figure 4.25**. A higher saturation index of mackinawite is correlated with higher TCE transformation rate constants (slope of 3.76×10^{-5}), but a trend for siderite is not apparent.



4.25. Pseudo first order TCE transformation rate constants for TCE transformation to acetylene plotted against saturation indices for mackinawite and siderite determined from MINTEQA2 for biotic ferrihydrite experimental conditions.

4.5.3 The Redox Potential Correlates to TCE Reactivity in These Complex Mineral Systems

Measured reduction potentials and aqueous phase ferrous iron concentrations for all experiments compared to TCE transformation rates are presented in **Figure 4.26**. Overall, there does not appear to be a correlation between TCE reactivity and aqueous iron concentration (**Figure 4.26A**). Sorbed Fe²⁺ on the iron sulfide surface may be a better indicator of TCE transformation rates than aqueous phase iron, and this will be explored in future work.

In contrast to aqueous phase iron, there is a strong relationship between TCE reactivity and reduction potential (**Figure 4.26B**). More negative redox potentials are correlated with higher TCE transformation rate constants for the biotic ferrihydrite experiments. Abiotic ferrihydrite experiments show less negative redox potentials (open symbols, **Figure 4.26B**), consistent with their inability to reduce TCE to acetylene. The no sulfur controls also show relatively less negative redox potential (black symbols), and no TCE reactivity. The no iron control (X:HS⁻ 0:1) is the lone outlier; it shows a relatively low redox potential but no TCE transformation. Importantly, no mineral precipitates were visible in this experiment, indicating the importance of reactive iron surfaces to catalyze TCE transformation. The dashed linear regression line only includes data points for biotic ferrihydrite and sulfide experiments, and when these points are evaluated on a log scale the relationship between rate constant and redox potential is represented by:

$$\log \left(k_{obs} \left[\frac{1}{\text{hr}} \right] \right) = -10.6 * (-E_H [\text{V}]) - 6.874 \quad (4.9)$$

This relationship in Equation 4.9 is similar to linear free energy relationships (or quantitative structure-activity relationships) presented for TCE and Fe(0) (Arnold and Roberts, 2000), though in this case thermodynamic reduction potentials for elimination reactions have been substituted for a measured experimental redox potential. As presented by Tratnyek et al. (2003), the slope for TCE elimination by Fe(0) to chloroacetylene was -31, which is $\sim 3\times$ greater than the slope determined for biogenic iron sulfides in the present study. This is likely because Fe(0) is a stronger reductant than FeS.

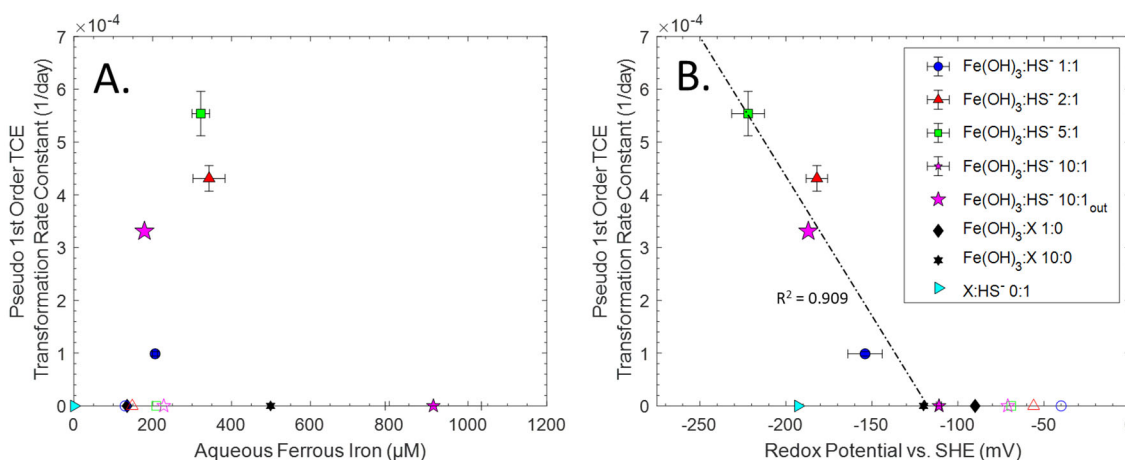


Figure 4.26. Pseudo first order TCE transformation rate constants for TCE transformation to acetylene plotted against (A) aqueous ferrous iron and (B) redox potential (dashed line is a regression of the biotic ferrihydrite experiments with sulfur and has a slope of -5.28×10^{-6} and an intercept at -114 mV). Closed symbols are biotic experiments and open symbols are abiotic experiments.

4.5.4 Discussion

Overall, the results support a mechanism of TCE reduction by mackinawite, and the creation and maintenance of mackinawite by the reducing equivalents provided by *S. oneidensis*. When *S. oneidensis* is not present, the reducing conditions appear insufficient to form mackinawite, and XPS results indicate that Fe(III) species dominate mineral surfaces. Literature results indicate that during mackinawite reaction with an electron acceptor, the Fe(II) is converted to Fe(III), which either forms an oxide or transitions to greigite (Lan et al., 2016; Lan and Butler, 2016). In the presence of water during the three-week aging period, such reactions could result in the iron sulfide surface becoming relatively more oxidized in the abiotic ferrous iron experiments. For example, research has shown that thin oxide shells can form on the surface of mackinawite during reactions with water (Zou et al., 2018). In the presence of iron-reducing bacteria, these iron oxide layers would be “rereduced”, thereby maintaining a reactive mackinawite surface for TCE reaction. The same logic applies to maintaining the reactive surface during TCE transformation. As TCE reacts,

Fe(II) in mackinawite transforms to Fe(III), and iron reducing bacteria reverse this step to maintain iron sulfide.

The mechanism of Fe(III) reduction by *S. oneidensis* is well studied, and can involve direct electron transfer to iron oxides or greigite via surface-bound cytochromes (Richter et al., 2012) or nanowires (Gorby et al., 2006), or indirect electron transfer through soluble extracellular electron shuttles (Canstein et al., 2008; Marsili et al., 2008; Michelson et al., 2019; Newman and Kolter, 2000). Such shuttles, e.g., flavins, can be produced by *S. oneidensis* (Kotloski and Gralnick, 2013; Marsili et al., 2008), or can come from natural organic matter in soil and groundwater systems (Chen et al., 2003; Lovley et al., 1996).

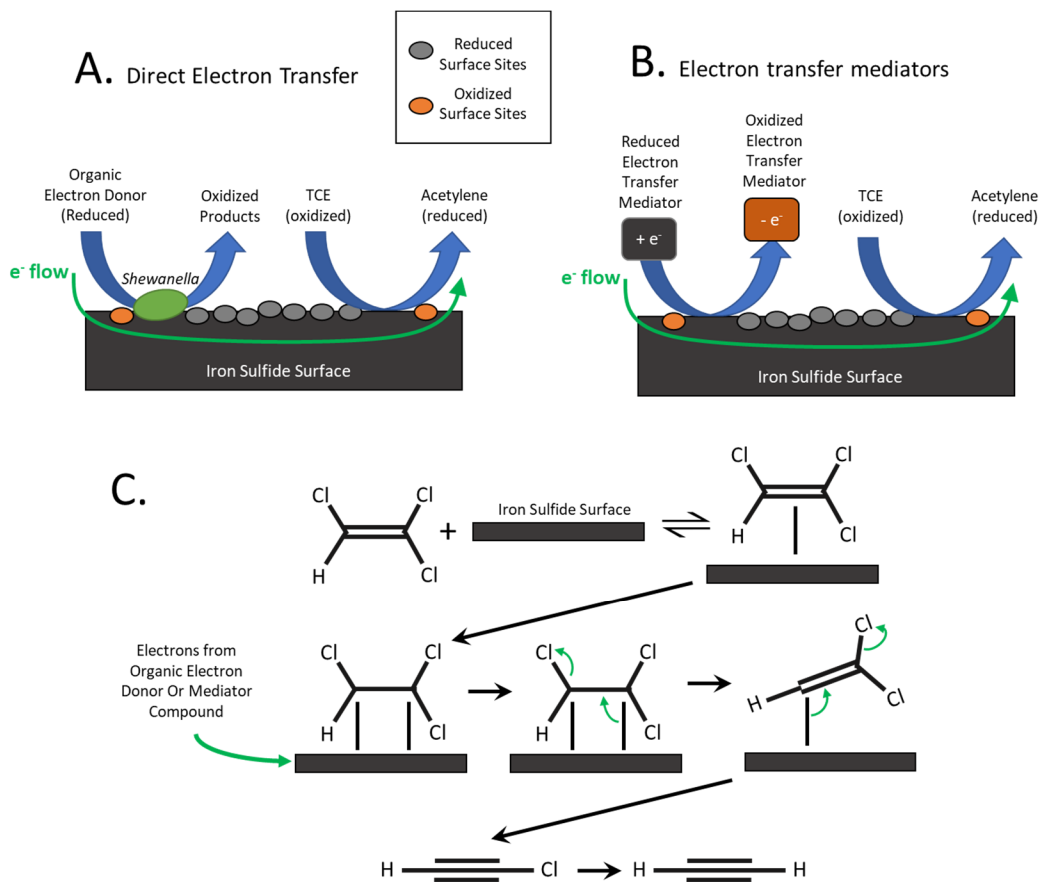


Figure 4.27. Potential electron transfer cycle for *S. oneidensis* in TCE transformation via (A) direct electron transfer at the mineral surface (B) mediator compounds reducing iron at mineral surface. (C) Reaction pathways at iron sulfide mineral surfaces may be mediated by iron-reducing bacteria maintaining a reduced surface (adapted from figure by (Arnold and Roberts, 2000) for Fe(0) transformation of tetrachloroethene, and informed by reaction steps presented by (Butler and Hayes, 2001) for TCE transformation by iron sulfides). Green arrows indicate electron flow.

The aforementioned interpretation leads to the proposed reaction mechanisms in **Figure 4.27**. As illustrated, iron reducing bacteria can reduce iron oxide coatings on the surface of iron sulfides by direct electron transfer (**Figure 4.27A**) or via reduced electron transfer mediators (**Figure 4.27B**). These electrons are then incorporated into the iron-sulfide mineral structure and transferred from the mineral surface to TCE via a β -elimination pathway to produce chloroacetylene, which rapidly undergoes further hydrogenation to acetylene (**Figure 4.27C**). Ultimately, iron-reducing bacteria can promote electron flow to a catalytic mineral surface, that then reductively dechlorinates TCE. This work provides the first direct evidence that iron sulfide formation controlled by metal reducing bacteria is a function of the starting iron amount and type, that aqueous and solid phase iron can promote comparable TCE transformation rates, and that the biogenic mineral reactivity is directly related to reduction potential.

4.5.5 Environmental Significance

The experimental results indicate that TCE transformation to acetylene in iron reducing systems is enhanced by 1) low reduction potentials below -140 mV – which are ultimately influenced by iron reducing bacteria maintaining reducing conditions, 2.) the presence of Fe(II)-S surface species to catalyze TCE β -elimination to acetylene, and 3.) limited precipitation of less reactive mineral species such as siderite and ferrihydrite that may block more reactive Fe(II)-S surface sites. This work lends insight into TCE transformation under iron and sulfur reducing conditions, and the results can be used to inform management of TCE-contaminated sites.

Linear free energy relationships (LFERs) can be used to determine reaction rates from reduction potential (or other equilibrium properties such as pH) (Brezonik, 2002). The relationship identified in this work is a useful basis for comparison to future work, but cannot be used directly because of the limited data set from which it was developed. Robust, statistically relevant LFERs that have been tested across a range of environmental conditions could be used as predictive or design tools for groundwater remediation.

4.6 Flow Cell Results

Effluent data for the flow cell maintained under anaerobic conditions for 180 days is shown in **Figure 4.28**, expressed as mass discharge rate (Mass/Time) of TCE. The TCE decreased to below detection levels in the first 30 days. The *cis*-DCE rapidly increased during this same time, and then reached an approximately steady value of 6 ± 1 $\mu\text{mol/day}$ after 40 days. This value is close to the expected mass discharge rate of TCE (8 $\mu\text{mol/day}$) into the HPZ if it was diffusing without reaction from saturation conditions at the bottom of the clay to an approximately zero concentration boundary at the LPZ/HPZ interface, suggesting that almost all of the TCE was being converted to *cis*-DCE in the LPZ before diffusing into the overlying HPZ.

Near 80 days, there was an increase in VC, and near 110 days there was an increase in ethene. The VC mass discharge rate almost reached the level of *cis*-DCE, but appeared to plateau near 140 days. The ethene increased over 20 days, and then stayed relatively constant and at a low concentration after 130 days. Acetylene production was observed over the entire experiment at a low level of $\sim 2.5 \times 10^{-3}$ $\mu\text{mol/day}$. This represented less than 0.04% of the maximum possible TCE reaction) and indicates abiotic reduction of TCE and/or DCE was sustained but low during the study period. The presence of acetylene throughout the experiment, and potential inhibition (Pon et al., 2003), may explain the persistence of VC and slower ethene production during the last 40 days of the experiment.

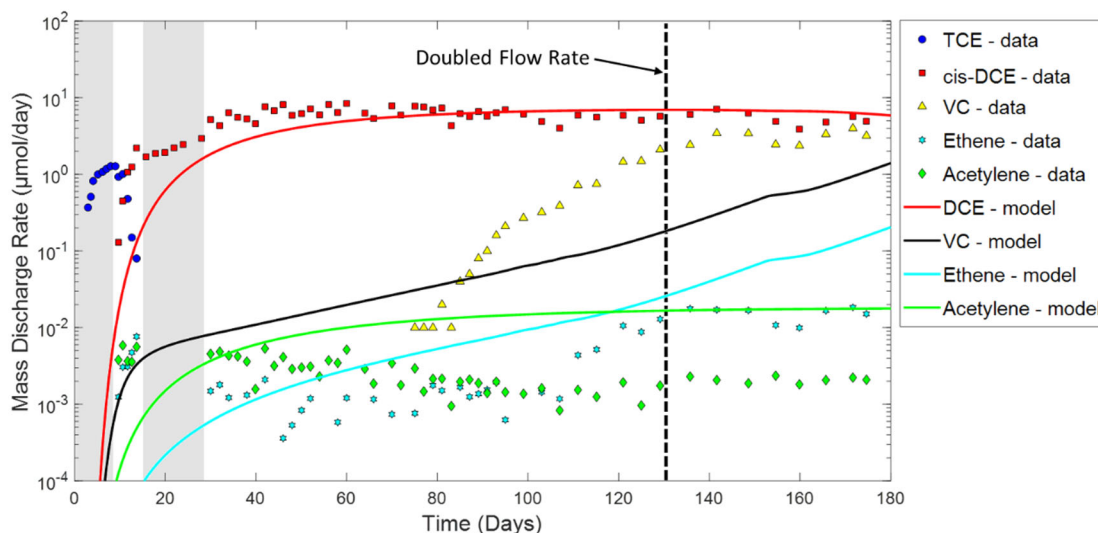


Figure 4.28. Flow cell mass discharge rate, or effluent, data (markers) and 1D diffusion-reaction model (lines). The shaded regions indicate when an octane trap was temporarily used in place of the sample loop for sample collection. Ethene and acetylene measurements were often close to the detection limit.

No lactate was measured in the flow cell effluent after the first week. Instead, propionate and acetate were measured. Lactate is known to be fermented and oxidized to acetate, propionate, and hydrogen. The propionate and acetate levels measured matched those expected for complete lactate fermentation and oxidation to these products.

The flow rate through the HPZ was doubled at approximately 130 days, and there was no significant impact on chlorinated ethene or acetylene mass discharge rate. This indicates that the approximately zero concentration boundary assumed for chlorinated ethenes at the LPZ/HPZ interface is correct, and that the reactions of interest are occurring in the LPZ rather than the HPZ due to the very short residence time in the latter.

The second flow cell experiment performed identical to the first for 70 days, and then switched to groundwater containing oxygen but no lactate, yielded almost identical results for the first 70 days. After oxygenated groundwater was used, however, DCE and VC concentrations decreased and TCE concentrations rebounded until TCE again dominated the flow cell effluent after 140 days. Effluent data was also analyzed for TCE oxidation products, i.e., organic acids, but none were detected.

Depth profiles of the chlorinated ethenes and reduced gases in the first flow cell LPZ at the conclusion of the experiment are shown in **Figure 4.29**, where the clay extends from zero (bottom) to 8 cm (LPZ-HPZ interface), and samples closest to the bottom start at 1 cm to avoid artifacts from Tenax. The TCE concentration is high at the base of the flow cell, and low at the interface. This is expected because the source of TCE is on Tenax beads at the base. *Cis*-DCE increases in concentration close to 7 cm and VC has a peak closer to 7.5 cm; these peaks are likely due to the availability of acetate and hydrogen closer to the interface. Acetylene, like TCE, is higher at the LPZ base, and low near the interface, indicating its transformation does not depend on the electron donor. The results indicate that acetylene production is directly related to the TCE concentration, but not a strong function of *cis*-DCE concentration. This makes sense as acetylene production rates (k_{Norm}) from *cis*-DCE are only $1/10^{\text{th}}$ of those from TCE, and TCE is present at much higher concentrations than *cis*-DCE in the flow cell.

In contrast to chlorinated compounds and acetylene, the ethene concentration profile shows two regions of higher concentration: one near the LPZ/HPZ interface, and another toward the LPZ base. The ethene concentration peak at the LPZ/HPZ interface is expected as a dechlorination product of VC. The ethene concentration increase near the LPZ base is expected if acetylene undergoes hydrogenation to ethene. Hence, ethene production appears to be coming from both VC and acetylene. Depth profiles for four other reduced gases were measured that are strikingly similar to ethene; they are ethane, propylene, propane, propyne. Hence, they too appear to be produced from both VC and acetylene. This finding suggests that we could be underestimating the amount of abiotic TCE reduction in natural systems by ignoring quantification of reduced gas transformation products. Nevertheless, when accounting for the mass of all the reduced gas transformation products, the abiotic products are still orders of magnitude less than the biotic products.

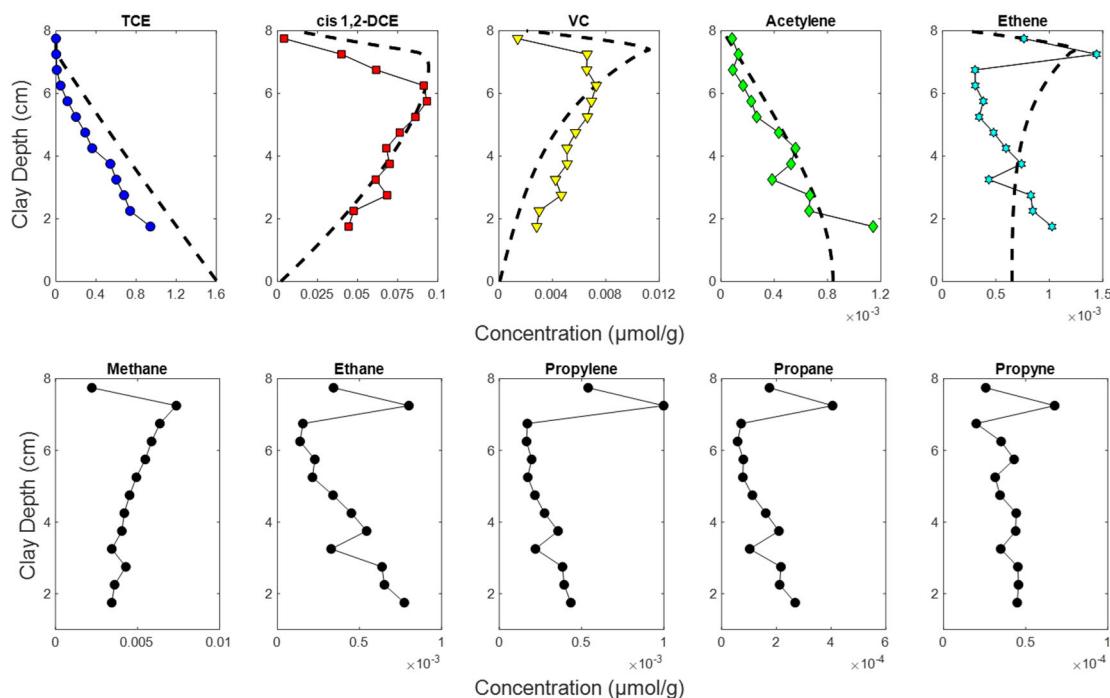


Figure 4.29. Flow cell depth profiles through clay (LPZ). Five species on the top row show a comparison of the numerical model (dashes) to the experimental data (data points). The five species on the bottom were not included in the model but may indicate other important biotic and abiotic reactions. All concentrations are reported as mass of analyte in headspace per mass of clay sample analyzed at each depth, and each data point is an average of three separate measurements at that depth.

The last gas measured was methane, and its depth profile only peaks near the LPZ/HPZ interface. This is likely due to TCE controlling the reducing conditions deeper in the clay profile. Conditions may be more reducing near the interface where TCE does not dominate, allowing for more methanogenesis. Also, the methane concentration profile peak coincides with the highest concentrations of electron donor; this makes sense because lactate is introduced in the HPZ, and its fermentation products diffuse into the top of the clay at the interface (8cm).

Depth profiles were also measured for the 2nd flow cell after 180 days (and after 110 days of being flushed with oxygenated groundwater). The main focus was on identify organic acids created from TCE oxidation. These were not detected, likely because they are easily degraded via biological pathways.

4.6.1 Flow Cell Modeling Results

The 1D diffusion-reaction model was used to simulate results from the first flow cell performed under anaerobic conditions. Parameter values were adjusted to match both the time dependent

effluent data from zero to 180 days, as well as the depth profiles at 180 days. The maximum specific growth rate and biomass decay constant were initially fit to the effluent data, as they were more sensitive to the temporal changes. Further fitting was done using depth profile data for the maximum utilization rates for the two dechlorinating communities as well as the first order rate constant for the elimination reaction of TCE to acetylene. The fitting was optimized for the depth profiles to minimize the sum of the residual squared error (SRSE). The error was not minimized for the effluent (mass discharge rate). A complete list of model parameters is included in **Table 4.11**. Additionally, **Table 4.12** compares Monod kinetic parameters fit in this study with those of previous studies. In most cases, parameter values from this study are in the range of those in the literature.

Table 4.11. 1D diffusion reaction model parameter values.

Parameter (units)	Symbol	Value	Source
Physical			
Porosity (mL_w/mL_T)	n	0.3	This study
Bulk density (g_s/mL)	ρ_b	2.2	This study
Fraction Organic Carbon (g_{oc}/g_s)	f_{oc}	7.5×10^{-4}	Schaefer et al. (2018) and this study
Henry's Constants			
Ethene Henry's Constant (mL_w/mL_g)	H_{Eth}	8.76	Mackay and Shiu (1981)
Acetylene Henry's Constant (mL_w/mL_g)	H_{Acet}	1.005	Wilhelm (1977)
VC Henry's Constant (mL_w/mL_g)	H_{VC}	1.11	EPA (2016)
cis-DCE Henry's Constant (mL_w/mL_g)	H_{DCE}	0.166	EPA (2016)
TCE Henry's Constant (mL_w/mL_g)	H_{TCE}	0.421	EPA (2016)
Hydrogen Henry's Constant (mL_w/mL_g)	H_{H_2}	52.43	Wilhelm (1977)
Organic Carbon Distribution Coefficients			

Ethene k_{oc} (mL _w /g _{oc})	k_{ocEth}	0	Assumed
Acetylene k_{oc} (mL _w /g _{oc})	k_{ocAcet}	0	Assumed
Vinyl Chloride k_{oc} (mL _w /g _{oc})	k_{ocVC}	22.9	EPA EPI Suite™ (2018)
cis-DCE k_{oc} (mL _w /g _{oc})	k_{ocDCE}	43.8	EPA EPI Suite™ (2018)
TCE k_{oc} (mL _w /g _{oc})	k_{ocTCE}	67.6	EPA EPI Suite™ (2018)
Hydrogen k_{oc} (mL _w /g _{oc})	k_{ocH_2}	0	Assumed
Acetate k_{oc} (mL _w /g _{oc})	k_{ocAate}	0	Assumed
Molecular Diffusion Coefficients			
D_{mol} Ethene (cm ² /day)	D_{mol_Eth}	1.30	Huq & Wood (1968)
D_{mol} Acetylene (cm ² /day)	D_{mol_Acet}	1.72	Himmelblau (1964)
D_{mol} Vinyl Chloride (cm ² /day)	D_{mol_VC}	1.20	Chiao et al. (1994)
D_{mol} cis-DCE (cm ² /day)	D_{mol_DCE}	0.95	Payne et al. (2008)
D_{mol} TCE (cm ² /day)	D_{mol_TCE}	0.90	Chiao et al. (1994)
D_{mol} Community 1 (cm ² /day)	D_{mol_Bio1}	0.01	Assumed low
D_{mol} Community 2 (cm ² /day)	D_{mol_Bio2}	0.01	Assumed low
D_{mol} Hydrogen (cm ² /day)	$D_{mol_H_2}$	4.41	Jähne et al. (1987)
D_{mol} Acetate (cm ² /day)	D_{mol_Aate}	1.15	Estimate via Wilke-Chang
Maximum Utilization Rates			
TCE + H ₂ - Community 1 & 2 (μmol/(cells*day))	r_{ut_TCE}	1.0×10^{-8}	* Fit to depth profile
TCE + Acetate - Community 1 (μmol/(cells*day))	r_{ut_TCEac}	1.0×10^{-11}	* Fit to depth profile
DCE + H ₂ - Community 2 (μmol/(cells*day))	r_{ut_DCE}	1.4×10^{-10}	* Fit to depth profile
VC + H ₂ - Community 2 (μmol/(cells*day))	r_{ut_VC}	2.0×10^{-11}	* Fit to depth profile

Half Saturation Constants			
TCE (μmol/L)	k _{TCE}	1.4	Haston and McCarty (1999)
DCE (μmol/L)	k _{DCE}	3.3	Cupples et al. (2004b)
VC (μmol/L)	k _{VC}	2.6	Cupples et al. (2004b)
Hydrogen (μmol/L)	k _{H₂}	0.007	Cupples et al. (2004b)
Acetate (μmol/L)	k _{Acet}	0.11	Chambon et al. (2013) ^a
Biomass Constants			
Maxium Specific Growth Rate - Community 1 & 2 (1/day)	μ	0.03	* Fit to effluent
First Order Biomass Decay - Community 1 & 2 (1/day)	b	0.006	* Fit to effluent
Initial Biomass - Community 1 (cells/L)	B ₁	5x10 ⁹	Assumed from experimental setup
Initial Biomass - Community 2 (cells/L)	B ₂	5x10 ⁸	Assumed from experimental setup
First Order Abiotic Rate Constants			
TCE → Acetylene (1/day)	k _{TCE_abio}	2.50x10 ⁻⁵	* Fit to depth profile
DCE → Acetylene (1/day)	k _{DCE_abio}	0.1*k _{TCE_abio}	Function of k _{TCE_abio}
Acetylene → Ethene (1/day)	k _{acet→eth}	9.3x10 ⁻³	Jeong et al. (2007) ^b

^a From example in review paper

^b Based on Fe hydr(oxide) rate constants for acetylene to ethene

Calculations to estimate effective diffusion coefficients:

Retardation = $1 + (f_{oc} * k_{oc} * \rho_b) / n$

Effective Diffusion = $D_{mol} * n / R$

Table 4.12. Comparison of model parameters to a range of previous literature values

Chosen Reaction Parameters			Comparison	Cupples et al. (2004) ^{a,b}	Holmes et al. (2006) ^b	Schaefer et al. (2009) ^b	Sabalowsky and Semprini (2010) ^b	Chambon et al. (2013) ^c	Rittman and McCarty (2001) ^d
Maximum Utilization Rate TCE + H ₂ - Community 1 & 2 (μmol/(cells*day))	r _{ut_TCE}	1.0x10 ⁻⁸	Relatively High	N/A	2.6x10 ⁻¹⁰	3.1x10 ⁻⁸	4.8x10 ⁻¹¹	3.85x10 ⁻⁹	4.3x10 ⁻¹⁰ - 2.1x10 ⁻⁹
Maximum Utilization Rate DCE + H ₂ - Community 2 (μmol/(cells*day))	r _{ut_DCE}	1.6x10 ⁻¹⁰	Average	7.80E-10	7.6x10 ⁻¹⁰	1.25x10 ⁻⁸	4x10 ⁻¹²	7.3x10 ⁻¹⁰	1.6x10 ⁻¹⁰ - 1.5x10 ⁻⁹
Maximum Utilization Rate VC + H ₂ - Community 2 (μmol/(cells*day))	r _{ut_VC}	2.0x10 ⁻¹¹	Relatively Low	7.80E-10	8.6x10 ⁻¹⁰	3.4x10 ⁻⁸	5x10 ⁻¹²	2.7x10 ⁻¹⁰	1.6x10 ⁻¹⁰ - 1.3x10 ⁻⁹
Maximum Specific Growth Rate (1/day)	μ	0.03	Average	0.39	0.0036-0.01	0.055-0.15	0.0099-0.12	0.15-2.0	0.18 - 0.45

^a parameters from study with a pure culture

^b parameters from batch study

Model-generated mass discharge rates are compared to effluent data in **Figure 4.28**. The model profiles generally capture the experimental data trends, but do not always match their values. The experimental TCE data points at early time are not captured by the model. Based on expected TCE transport rates, TCE flux should not have exceeded $0.1 \mu\text{mol/day}$ until day 10 if the initial TCE concentration in the LPZ is zero. This indicates that TCE in the pore water adjacent to the Tenax beads likely mixed throughout the LPZ depth during flow-cell packing, leading to early-time HPZ breakthrough from the LPZ. The experimental *cis*-DCE trends are well captured by the model. The upward trends of the experimental VC and ethene data points also are captured by the model, but the former are characterized by sharp increases after 80 and 100 days, respectively, while the model profiles increase more gradually. The mass discharge rate of acetylene is over predicted by the model, which may be due to subsequent acetylene degradation that could not be completely accounted for in the model.

Model profiles are compared to depth profiles in **Figure 4.29**, and results show good agreement. The model profiles match the general shapes and magnitudes of most species. These profiles modestly underestimate or overestimate experimental *cis*-DCE and VC data points at various depths, and do not capture the extent of the decrease in ethene near the center. The model appears to represent the acetylene depth profile well, despite failing to adequately describe the acetylene effluent data.

It is important to note that the abiotic rate constants that satisfied the depth profile data are smaller (by more than an order of magnitude) than the rate constants that fit complementary batch experiment data (**Table 4.13**). Some possible reasons for this discrepancy are (1) abiotic processes are diffusion limited in the flow cell, (2) the abiotic processes are limited by biotic competition for TCE, (3) acetylene produced is further transformed to other reduced gases, or (4) the mass loading of TCE has some influence on rate constants, with lower rates observed at higher TCE loading. The fourth option would indicate that uptake of TCE onto reactive mineral surfaces may be described by a Langmuir surface site model. Previous work by (Schaefer et al., 2018) utilizing the same clay at a higher TCE loading in batch experiments showed a TCE to acetylene rate constant of $3.1 \times 10^{-5} \text{ 1/day}$, which compares well with the abiotic rate constant for TCE to acetylene fit in the flow cell. This makes sense, as the TCE concentrations near the TCE source were more similar to the concentrations used in batch in the study by (Schaefer et al., 2018).

The differences between the model and the data could also be influenced by different types of inhibition (ex: redox inhibition, more chlorinated compounds inhibiting transformation of less chlorinated compounds (Yu et al., 2005), or acetylene inhibiting dechlorination (Pon et al., 2003)). The inhibition of more chlorinated compounds on less chlorinated compound transformation was considered but did not significantly improve the model fit, so it was not included in the model fitting and simulations in order to reduce model complexity. Inconsistencies may also exist

because of assumptions made about the use of electron donor: hydrogen produced from propionate was not considered, competition for acetate and hydrogen by non-dechlorinating organisms was not considered in the LPZ, and calculations show that the electron donor utilized by iron-reducing organisms could have been relevant during the first couple weeks of the flow cell experiment. A more complex model including all these processes could potentially fit the experimental data better, but increasing the complexity also increases the number of parameters and unknowns. Capturing all the biological processes in the mixed community – potentially resulting in different donor gradients, or other undetected species was outside of the scope of this work. Future work may focus on further characterizing the microbial community dynamics supported with data from DNA extractions to look at the spatial distribution of the microbial community.

Table 4.13. Comparison of abiotic transformation rate constants (1/day) for batch and model and compared to previous work. (*cis*-DCE rate in model assumed to be 0.1xTCE rate.)

Electron Acceptor	k Model	k _{Norm} Batch	k _{Norm} (Schaefer et al., 2018)
TCE	2.50x10 ⁻⁵	3.23x10 ⁻⁴	3.10x10 ⁻⁵
<i>cis</i> -DCE	2.50x10 ⁻⁶	3.85x10 ⁻⁵	n/a

4.6.2 Field-Scale Implications

The 1D model, with parameter values estimated from flow cell experimental data, was used to simulate back diffusion and reaction from an LPZ at a representative field site. While the model does not appear to account for all reaction mechanisms affecting the fate of TCE and its transformation products, it does approximately capture the dominant reactants and products, as well as the relevant time scales of reaction. To better represent the conditions at a field site that exhibits back diffusion, the initial conditions and boundary conditions for TCE were modified from the conditions used to model the flow cell. The initial TCE profile was determined from boring WCP-70 at an industrial site in Connecticut (**Figure 4.30C**), where samples were taken in the late 1990s (Parker et al., 2004). Based on boring WCP-70, the LPZ thickness is 300 cm. For the simulation, it is assumed that there is no TCE initially in the HPZ, and flow in the HPZ is fast relative to diffusion in the LPZ. (TCE boundary conditions at the LPZ/HPZ interface and base of the domain are zero concentration and zero flux respectively.) Simulations were run for 365 days (1 year) to evaluate biotic and abiotic reaction contributions to chlorinated ethene fluxes from the LPZ.

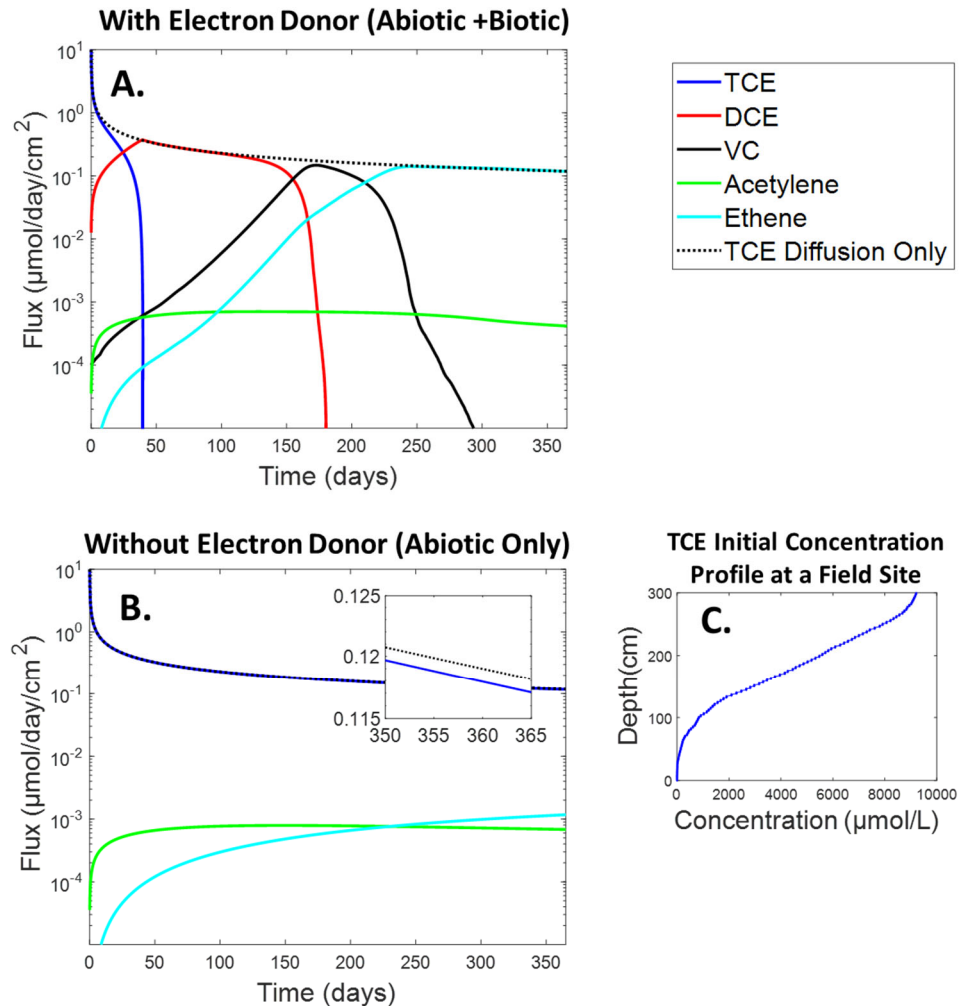


Figure 4.30. Fluxes out of the LPZ into the HPZ over time. Simulation outputs with an electron donor (A) and without an electron donor (B) are shown. Parameters from the experimental flow cell model were used for these simulations. The TCE diffusive flux (black dashed lines) represents a condition with no reaction. TCE initial conditions (C) for these simulations are from WCP-70 presented in Parker et al. (2004), which more accurately represent a field scenario than the initial condition in the flow cell.

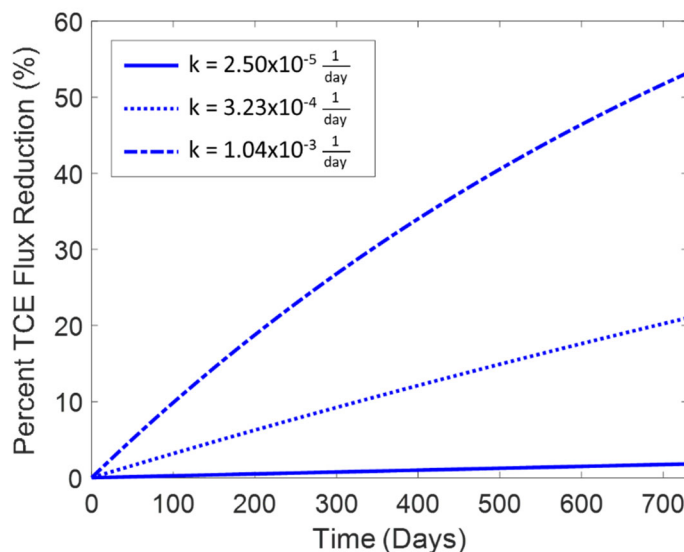


Figure 4.31. Percent TCE flux reduction relative to diffusion only flux with different abiotic rate constants for TCE to acetylene. Rate constant from model in this study (solid line), with rate constant from batch experiments in this study (dotted line), and with rate constant for a pyrite-rich mudstone from, Schaefer et al. (2013) (dashed line).

Model results showing fluxes of TCE, *cis*-1,2-DCE, VC, ethene, and acetylene from the LPZ to the HPZ are presented in **Figure 4.30**. With amended lactate (abiotic + biotic simulation), fluxes of chlorinated ethenes decrease at least five orders of magnitude (to $<10^{-4} \mu\text{mol/day/cm}^2$) after 300 days, and *cis*-DCE and VC are released into the HPZ for a period of approximately 220 days before ethene dominates the fluxes (**Figure 4.30A**). In contrast, without amended lactate, the TCE flux decreases less than two orders of magnitude (to $1.17 \times 10^{-1} \mu\text{mol/day/cm}^2$) after 365 days, and this decrease is mostly due to diffusion. The small inset in **Figure 4.30B** shows the abiotic contribution to decreasing flux near the end of the simulation, which is minimal using the abiotic rate constant from the flow cell experiments. As expected, the flux of acetylene in the presence of lactate at 365 days ($4.16 \times 10^{-4} \mu\text{mol/L/cm}^2$) is less than when lactate is absent ($6.81 \times 10^{-4} \mu\text{mol/L/cm}^2$). This is because abiotic processes are first order with respect to TCE concentration, and TCE concentrations are higher in the absence of lactate. The continued increase in ethene in the absence of electron donor is due to the first order reaction of acetylene to ethene that was included in the model. Further analysis would be necessary to fully understand the fate and transport of the different species released into the HPZ.

In addition to the abiotic simulation with the rate constant determined from the flow cell, two more simulations were conducted to evaluate fluxes from the LPZ in the absence of an electron donor. In one simulation, the abiotic batch rate constant of $3.23 \times 10^{-4} \text{ 1/day}$ was used, and in another simulation a rate constant of $1.04 \times 10^{-3} \text{ 1/day}$ was used, which represents a highly reactive pyrite rich mudstone (Schaefer et al., 2013). Simulations for all three rate constants are shown in **Figure**

4.31 over a two year period (730 days); flux simulation results are presented as a percent reduction in TCE flux from the diffusion only case. After 730 days, TCE fluxes determined using the rate constant of 2.50×10^{-5} 1/day showed a TCE flux decrease of only 2% of the diffusion only case. However, after 730 days, the TCE flux percent decrease using rate constants of 3.23×10^{-4} 1/day (batch fit) and 1.04×10^{-3} 1/day (pyrite rich mudstone) were 21% and 53% of the diffusion only case, respectively. In the presence of highly reactive minerals, abiotic reactions can have a significant impact on TCE flux values from LPZs.

Though most subsurface low permeability environments do not consist of a pyrite rich mudstone, this analysis shows that materials other than the clay used in this study may have a greater potential to abiotically decrease chlorinated ethene flux from LPZs. There may be issues with some assumptions implied in this simulation (such as constant mineral reactivity over time), but it is still useful to consider how more reactive mineral species could maintain faster reaction rates for longer periods of time.

4.7 Computationally Efficient Numerical Model Simulation Using RT3D

Two main quantities are presented in this study to compare performance of the IMZ-RT3D with the fully discretized RT3D (i.e., full-RT3D) model: 1) total mass of solute stored in the LPZ versus time, and 2) flux-averaged outflow concentration versus time. Sum of squared errors normalized with fully discretized model were computed to quantify errors between the two model results. For selected cases, vertical concentration profiles in LPZs are also shown.

4.7.1 Case I: Two Layer System

The results of the tracer simulation in the two-layer case are shown in **Figure 4.32a** and **4.32b**. They indicate good agreement between the IMZ-RT3D and full-RT3D models, for both total mass stored in the LPZ and flux-averaged outflow concentration. In addition, increasing the number of immobile species from 4 to 8 slightly improves estimation of IMZ model for CASE I, while increasing to 12 immobile zones does not seem to improve the results. A depth profile of tracer in the mid-section of the entire domain ($x=55$ cm) is shown in **Figure 4.32c** (22 days) and **Figure 4.32c** (44 days). Four immobile zones accurately capture the LPZ profile at day 22 but result in small errors after 44 days. Based on all comparisons, 8 immobile zones may be needed to accurately simulate tracer back diffusion in CASE I.

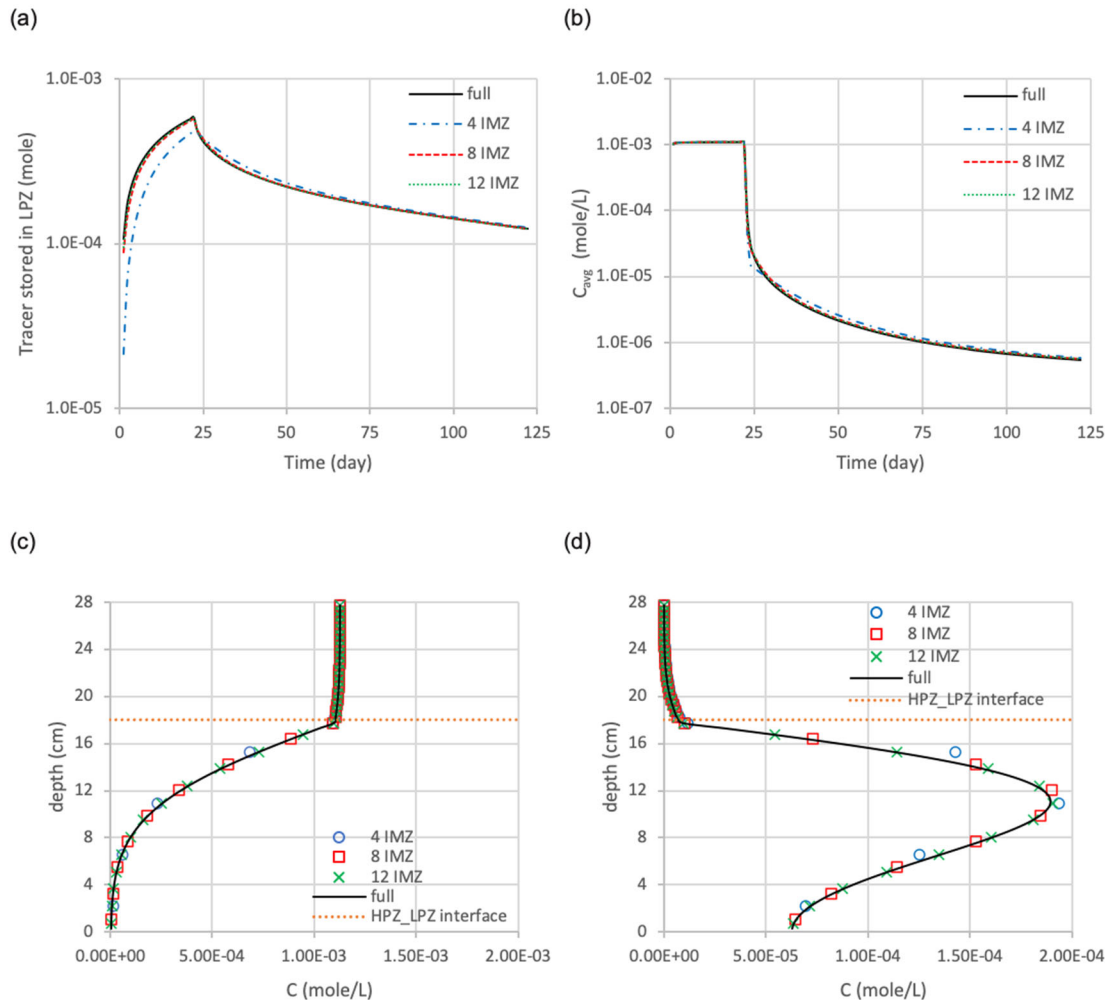


Figure 4.32. Comparison of IMZ-RT3D and full-RT3D results for a tracer in CASE I (two-layer case): (a) Total mass stored versus time in the LPZ, (b) breakthrough curve of the flux-averaged outflow concentration from the entire domain, (c) depth profile of the tracer at day 22 and $x=55$ cm, and (d) depth profile of the tracer at day 44 and $x=55$ cm.

The results of the TCE simulation in CASE I are shown in **Figure 4.33**. Since TCE reacts with hydrogen to form DCE, results for DCE are also shown. The results indicate good agreement between the IMZ-RT3D and full-RT3D models for four immobile zones with respect to both mass remaining in the LPZ and flux-averaged outflow concentration from the entire domain. There is slight improvement when the number of immobile zones is increased to 8 or 12, but less so than with the tracer. The difference between 7IMZ-RT3D and full model is less 10% for reactive scenario. This is especially encouraging because the concentration of DCE is one to four orders of magnitude smaller than that of TCE, making small mass balance errors with reaction of TCE very noticeable.

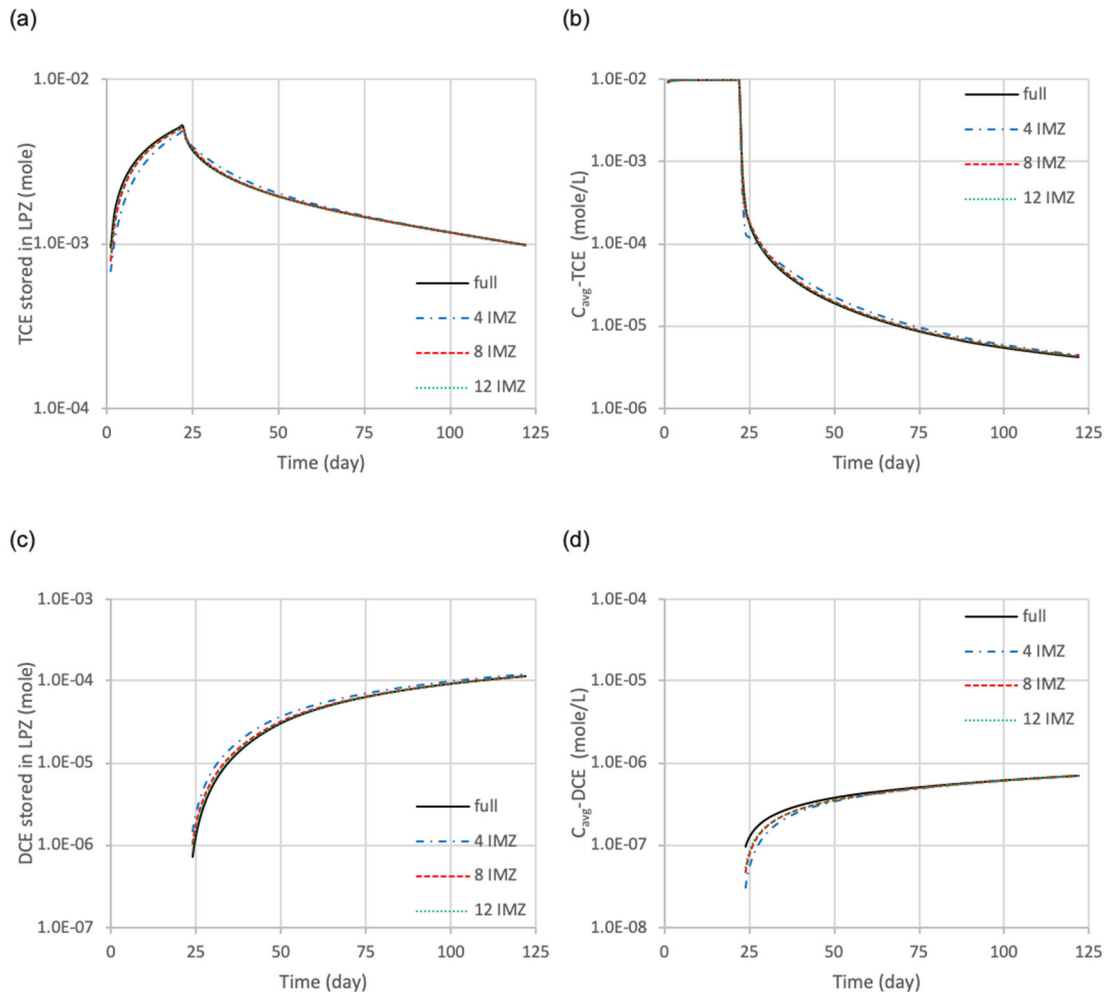


Figure 4.33. Comparison of full-RT3D and IMZ-RT3D model for biotic degradation of TCE and hydrogen into DCE in CASE I, (a, c) TCE and DCE moles stored in the LPZ, (b, d) flux-averaged outflow concentration of TCE and DCE.

4.7.2 Case II and III: Single Low Permeability Lens

The results of applying the IMZ-RT3D model for tracer simulation in CASE II are demonstrated in **Figure 4.34a, 4.34b**. Overall, they indicate good agreement between the IMZ-RT3D and full-RT3D models, for both total mass stored in the LPZ and flux-averaged outflow concentration. The results of IMZ-RT3D for CASE II become more accurate as the number of immobile species increases from 3 to 5, reducing the error in mass stored from 110% to 37%. Total mass stored in the LPZ and flux-averaged outflow concentration are always slightly overestimated by the IMZ-RT3D model. As time progresses, this difference becomes more noticeable.

The results of applying the IMZ-RT3D model for a tracer simulation in CASE III are demonstrated in **Figure 4.34c, 4.34d**. Here too there is good agreement between the IMZ-RT3D and full-RT3D models when 7 IMZ is considered since the error for both total mass stored in the LPZ and flux-averaged outflow concentration is 12% and 8%. However, the results of IMZ-RT3D model with 5 immobile zones are not in good agreement with the full model, especially for tracer mass remaining in the LPZ where the error is around 51%. Assuming 7 IMZs for both CASE II and CASE III, the discrepancy between the IMZ-RT3D and full-RT3D model is higher in CASE II than in CASE III. This could be related to the larger mass transfer rates (k_0 , k_1 , k_2) used for CASE II compared to CASE III, as they result in faster changes in the diffusive flux and possibly larger errors in numerical approximation.

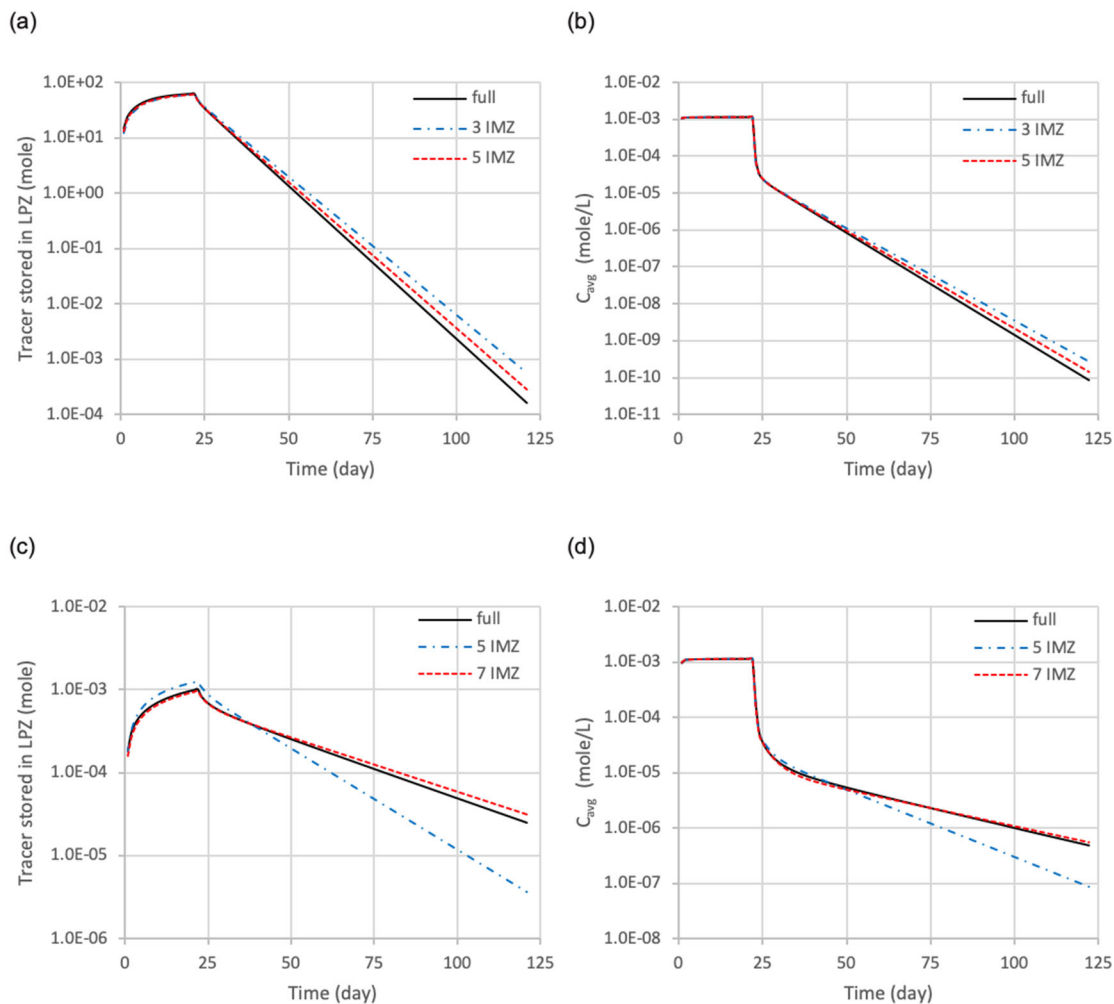


Figure 4.34. Comparison of full-RT3D and IMZ-RT3D model for the tracer test: (a) CASE II; mass stored in the LPZ, (b) flux-averaged outflow concentration, (c) CASE III; mass stored in the LPZ, (d) flux-averaged outflow concentration.

The results of applying IMZ-RT3D for TCE transformation to DCE in Case II are shown in **Figure 4.35**. The IMZ-RT3D results reasonably match the full-RT3D model with respect to both total moles of TCE stored in the LPZ and outflow TCE concentrations (**Figure 4.35a, 4.35b**). The errors decrease from 200% to 55% for total moles of TCE stored in the LPZ and from 56% to 32% for DCE when the number of immobile zones is increased from 3 to 5. For both IMZ numbers, the IMZ-RT3D model slightly overestimates both TCE moles stored in the LPZ and outflow concentration from the domain. Noticeable DCE is produced after enough hydrogen diffuses into the LPZ, by around day 25. For 5 IMZs, total moles of DCE stored in the LPZ from the IMZ-RT3D model are equal to or slightly greater than total moles from the full model (**Figure 4.35c**). However, the average outflow concentration of DCE is initially underestimated by IMZ-RT3D, and later overestimated (**Figure 4.35d**), in both cases by small amounts relative to concentration values.

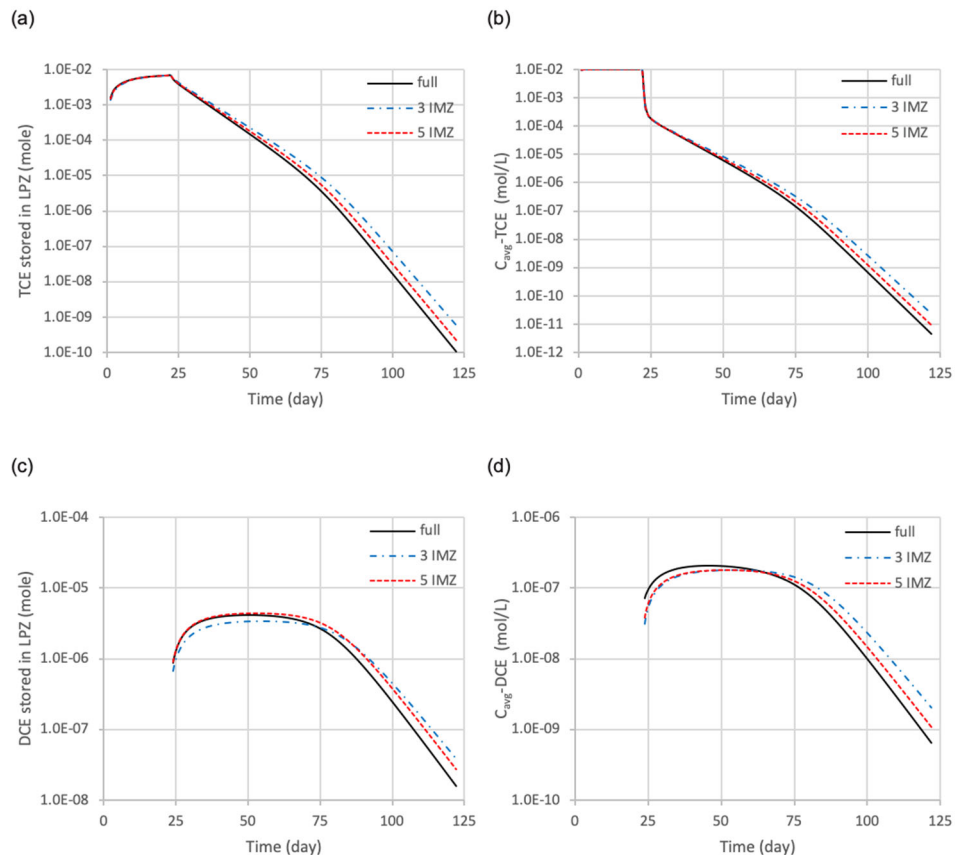


Figure 4.35. Comparison of full-RT3D and IMZ-RT3D model for biotic transformation of TCE and hydrogen into DCE in CASE II; (a, c) TCE and DCE moles stored in the LPZ, (b, d) flux-averaged outflow concentration of TCE and DCE.

The results of IMZ-RT3D for TCE transformation to DCE in CASE III are shown in **Figure 4.36**. The IMZ-RT3D profiles overestimate total moles of solutes stored in the LPZ from the full-RT3D model (**Figure 4.36a, 4.36c**). However, flux-averaged outflow concentration is in good agreement with full-RT3D model (**Figure 4.36b, 4.36d**). For the reactive scenario in the thick lens, increasing the number of immobile zones does not appear to improve the result, which is opposite of the tracer results for this case. A closer look at the results of the tracer scenario in CASE III shows that most of the error in **Figure 4.34c, 4.34d** arises during the flushing phase, when the tracer concentration is declining rapidly in the LPZ. Similar behavior is noticeable for the thin lens (CASE II), in which after 66 days the DCE concentration starts dropping, perhaps due to a decline in TCE concentration. But in CASE III, DCE is still in the phase of production after 122 days (**Figure 4.36c, 4.36d**), which makes the difference between 5IMZ and 7IMZ indistinguishable.

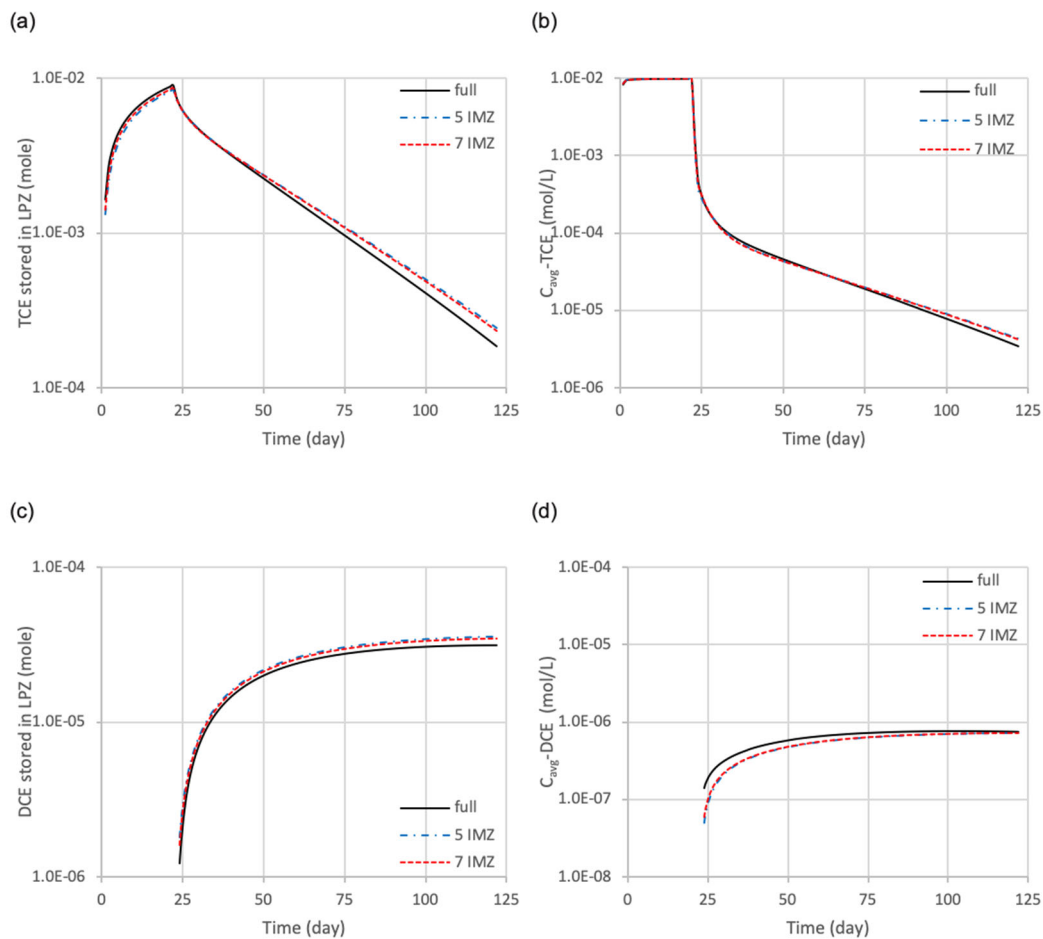


Figure 4.36. Comparison of full-RT3D and IMZ-RT3D model for biotic transformation of TCE and hydrogen into DCE in CASE III; (a, c) TCE and DCE moles stored in the LPZ, (b, d) flux-averaged outflow concentration of TCE and DCE.

4.7.3 Case IV: Multiple Low Permeability Lenses

This setup is inspired from Chapman et al. (2012), with slight modifications to simplify the geometry. This setup is the most complex considered and is more representative of greater complexity encountered in field scenarios. In total, 32 immobile zones are used for 3 lenses in the IMZ-RT3D model, in which the thickness of each lens varies from 1.2-2.4 cm. Simulation results for multiple LPZ lenses (CASE IV) are shown in **Figures 4.37** and **4.38**, for tracer and TCE transformation scenarios, respectively. In **Figure 4.37**, the IMZ-RT3D model overestimates tracer mass retained in the LPZ during back diffusion by 22%. In contrast, the IMZ-RT3D model predicts the outflow concentration throughout the simulation with only 8% error. Evaluating tracer mass stored in individual low permeable lenses reveals that lenses which are thicker and closer to the source contribute more error to tracer mass retained, i.e. lens III and then lens II are mainly responsible for the discrepancy in total mass remaining in the LPZ, since they have more area exposed to the source. Thicker lenses store more solute mass, and therefore contribute more to error.

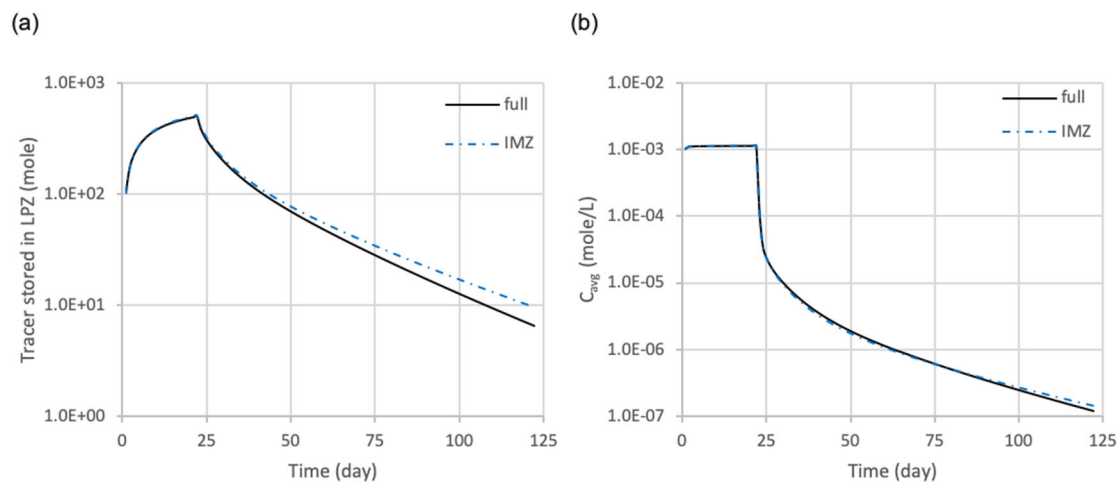


Figure 4.37. Comparison of full-RT3D and IMZ-RT3D model for a tracer test in CASE IV; (a) mass stored in the LPZ, (b) flux-averaged outflow concentration.

In **Figure 4.38**, the IMZ-RT3D model BTCs for TCE are in good agreement with the full-RT3D model, with respect to both mass remaining and average outflow concentration (errors of about 10-14%). This encouraging result suggests the new modeling approach is capable of conserving mass in heterogeneous environments with complex reactions provided a sufficient number of immobile zones is provided. Results for DCE are also good, but 11% and 18% errors are evident in total moles stored in the LPZ and BTC of outflow DCE, respectively. Specifically, there is slight overestimation of DCE mass remaining by the IMZ-RT3D model compared to the full-RT3D model, and there is slight underestimation of DCE outflow concentration. Due to limitations

regarding the maximum number of components in RT3D, we were not able to evaluate finer grids for CASE IV.

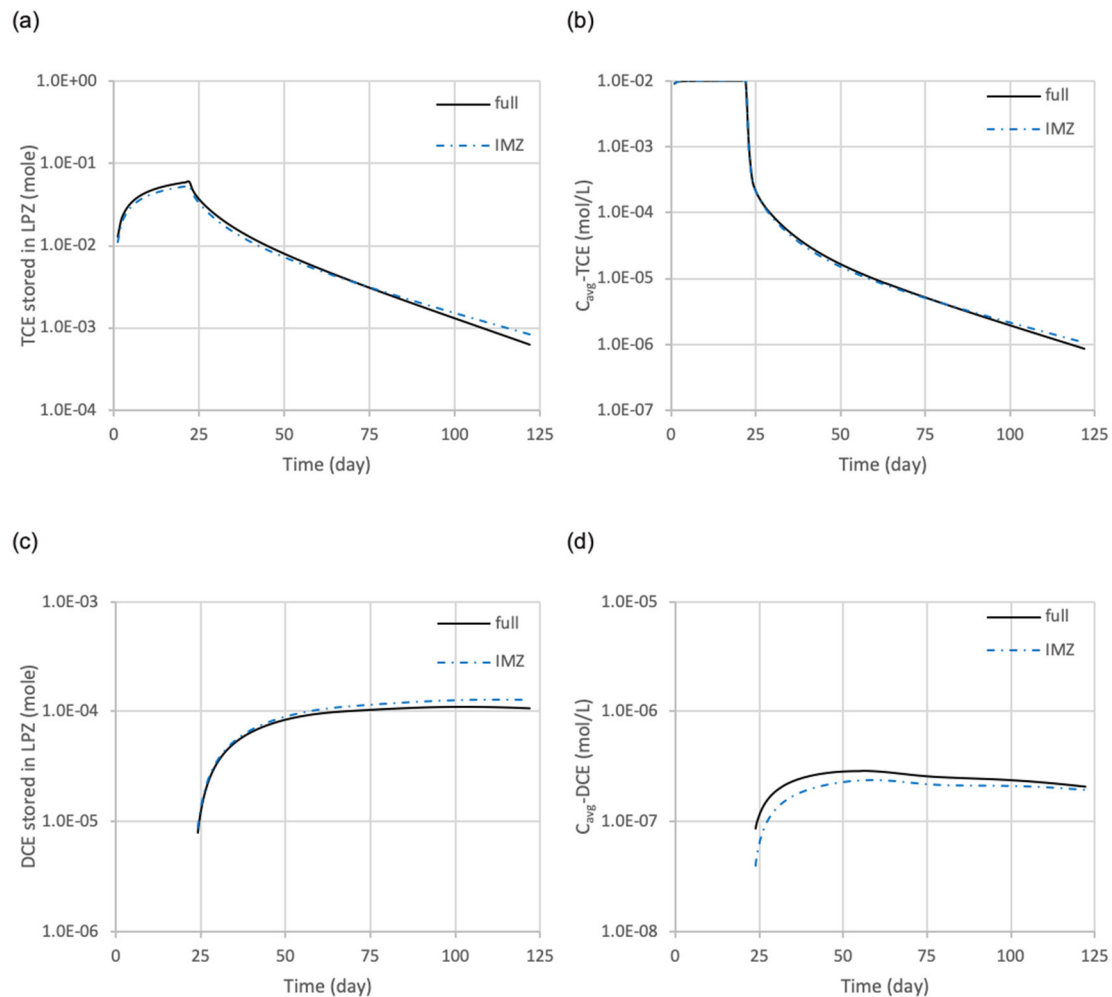


Figure 4.38. Comparison of full-RT3D and IMZ-RT3D model for biotic transformation of TCE and hydrogen into DCE in CASE IV; (a, c) TCE and DCE moles stored in the LPZ, (b, d) flux-averaged outflow concentration of TCE and DCE.

5.0 Guidelines For A Field Screening Protocol For Discerning Abiotic Processes

5.1 Overview of Abiotic Processes Affecting TCE Transformation

The conceptual model that emerges from this work and others for abiotic reactions is illustrated in **Figure 5.1**. Under anaerobic conditions, structural or sorbed ferrous iron donates an electron directly to TCE, promoting its reduction. The ferrous iron is oxidized, possibly coating the ferrous iron with a less reactive iron mineral. When iron or sulfur reducing bacteria are present, the oxidized iron can be reduced back to ferrous iron and possibly regain activity. Other constituents in solution complicate this picture. In bicarbonate buffered media, ferrous carbonate formation might be favored. When sulfide is present, ferrous sulfides may form. Over time, these minerals may transition from less to more crystalline. Also, ferric (hydr)oxides formed from reaction with TCE may coat ferrous mineral surfaces, and the form and crystallinity of these minerals can change with time. All of these factors appear to affect the reactivity of ferrous minerals for TCE reduction in natural systems and have yet to be fully elucidated.

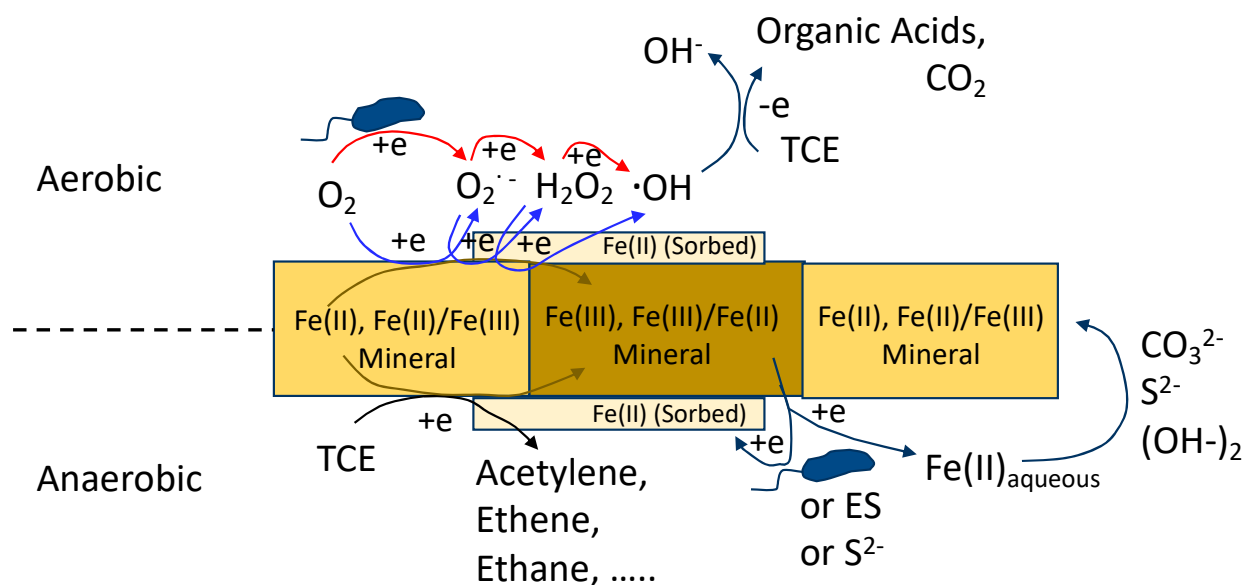


Figure 5.1. Conceptual model of TCE abiotic reactions under aerobic and anaerobic conditions.

The scenario for aerobic conditions is similar to that for anaerobic systems, except that now ferrous iron donates an electron to oxygen instead of TCE. The oxygen transforms by way of superoxide and hydrogen peroxide to a hydroxyl radical, which then oxidizes TCE. If aerobic conditions are maintained, the ferrous iron is depleted via oxidation to ferric (hydr)oxides. However, if conditions become anaerobic, these ferric (hydr)oxides can be reduced back to ferrous iron provided sufficient electron donor is present. As with anaerobic conditions, solution conditions affect the type and amount of different iron minerals that form, and therefore their reactivity for hydroxyl radical production and TCE oxidation.

5.2 Field Screening Measurements That Indicate Potential for Abiotic TCE Reaction

The conceptual model illustrated in **Figure 5.1** provides a framework to propose field screening measurements that indicate potential for abiotic TCE reaction, either reductive or oxidative. In both cases ferrous iron minerals are the driving force, providing electrons directly or via sorbed ferrous iron for either direct TCE reduction or hydroxyl radical formation coupled with TCE oxidation. Also, sufficiently low reducing conditions are required, possibly to provide a sufficient thermodynamic driving force to overcome high activation energy barriers, and/or to prevent the formation of any oxidized iron species that might interfere with the desired reaction. This means that little to no reactivity is expected in groundwater systems that have been aerobic for some time. The exact measurements to be taken depend on the available samples. In some cases, only groundwater might be available. In other cases, both soil cores and groundwater can be obtained. Suggestions for specific measurements are provided in the paragraphs that follow.

Perhaps the most important measure of reaction potential for TCE is the presence of ferrous iron minerals that have been shown to promote reduction or oxidation of this contaminant, and this is only possible when soil cores are available. Preferably these soil cores are relatively fresh, and once sampled were stored in a cold ($\leq 4^{\circ}\text{C}$) and/or oxygen free environment until analysis to prevent mineral transformation/oxidation. A number of ferrous iron minerals have been shown to promote TCE reduction and/or oxidation (**Figure 2.3** and **2.4**), and include several evaluated in this work. These are mackinowite, pyrite, ankerite, siderite, illite, nontronite, and ferrous iron containing clays. It's likely that other ferrous iron minerals also promote TCE reduction and oxidation. Therefore, the presence of ferrous iron minerals can be used as a screening tool to indicate the potential for abiotic TCE reactivity. Two methods for this are X-ray diffraction (XRD) and total structural ferrous iron. Of these two methods, XRD is preferred since it can provide relatively hard evidence of iron minerals known to promote TCE reduction or oxidation. However, XRD sensitivity can be as poor as a few percent in natural sediments, so natural sediments can still be reactive for TCE even if ferrous iron minerals are not detected with this method. Measurements for structural iron are more sensitive, and this method provides an indirect measure of reaction iron minerals. Structural iron can be measured in natural sediments by first extracting sorbed ferrous iron from mineral surfaces with a weak acid, then digesting structural ferrous iron with a strong acid, and then analyzing the strong acid extract using the 1,10-phenanthroline method (e.g., Xie et al., 2020).

A number of studies including our own have provided interesting data suggesting that TCE reactivity via reduction or oxidation is also related to the amount of ferrous iron sorbed to ferrous iron minerals. In one well-controlled study, reduction activity for other pollutants besides TCE was possible via ferrous iron sorbed onto ferric (hydr)oxides (Elsner et al., 2004). In two other

studies, the reactivity of sorbed ferrous iron on natural sediments for hydroxyl radicals was indicated (Tong et al., 2015; Xie et al., 2020). In our own work, sorbed ferrous iron on natural soils and pure minerals was correlated to TCE reduction and oxidation potential, although the number of data points is limited (Schaefer et al., 2017, 2018, 2020). Therefore, it appears that sorbed ferrous iron in reducing environments can be used as a screening method for TCE reactivity. As with measurements of structural ferrous iron, measurements of sorbed ferrous iron also require relatively fresh and preserved soil cores. Measurement approaches vary, but generally require using a mild acid to extract sorbed ferrous iron from natural sediments or minerals, and then analyzing ferrous iron using the 1,10-phenanthroline method. Possible approaches are detailed elsewhere (Schaefer et al., 2017; Xie et al., 2020).

A third indicator not directly illustrated in Figure 5.1 but presented in the results is the reduction potential. We measured this parameter for three different types of experimental systems, all in aqueous suspensions containing ferrous iron minerals, and in all cases using electron shuttle mediators to create an electrical bridge between solid iron minerals and the electrode (Kocur et al., 2020). The first contained pure minerals added to solution to promote TCE or oxygen reduction, and the pure minerals were ankerite, siderite, illite, and biotite. The second was a solution of precipitated iron sulfides in a tris buffer, created abiotically or in the presence of a sulfur reducing bacterium. The third was a solution of precipitated iron sulfides in a carbonate buffer, created abiotically or in the presence of an iron reducing bacterium. In each of these systems, more reducing conditions generally correlate to greater TCE reduction activity, and in one case to greater hydroxyl radical production (reduction potentials were measured before oxygen was added and hydroxyl radicals were measured in solution as an indicator of oxygen reduction activity). However, these correlations varied among the three systems, as did the minimum reduction potential below which TCE reactivity was observed. For example, TCE reactivity was indicated for potentials below ~ 0 mV (v SHE) in the first system, and below -140 mV (v SHE) in the second and third systems. The results suggest that reduced iron minerals require negative reduction potentials to be active for TCE or oxygen reduction (with hydroxyl radical generation from added oxygen), but the exact reduction potential required cannot be determined with the limited data available. Methods for measuring reduction potentials of aqueous-solid suspensions using electron shuttle mediators can be found in Kocur et al. (2020). These measurements can be performed at the bench top using relatively fresh groundwater and soil core material. It also seems possible to perform these measurements *in situ*, but this has not been sufficiently demonstrated.

There are other less direct methods that indicate the potential for TCE reactivity, but they are not as reliable. For example, aqueous ferrous iron is an indicator of structural and sorbed ferrous iron, and as noted above these latter two iron measurements have been related to TCE reactivity. However, aqueous ferrous iron has not been previously correlated to TCE reactivity, indicating it

is not necessarily correlated to concentrations of structural or sorbed ferrous iron that are reactive to TCE. Therefore, this measurement is a weak indicator that TCE reactivity is possible, and can be used when only groundwater but not soil cores are available.

Also possible are measurements of reduction potential using only groundwater, or measurements of only dissolved oxygen. Reduction potential measurements on only groundwater are likely subject to large errors because the true contribution from reduced solid phase minerals may not be reflected from aqueous constituents, and because trace oxygen will have a large effect on measurements when sediments are not present to act as a redox buffer. Dissolved oxygen, on the other hand, is only one species that affects redox potential. While its continued presence over long time periods is a good indicator that reduced iron species which are reactive for TCE are not present, its absence cannot be used as an indicator that such species are present. Therefore, its continued presence can be used to infer a system is likely not reactive for TCE, but its absence cannot be used to infer TCE reactivity.

6.0 Conclusions and Implications for Future Research/Implementation

The relative contributions of biotic and abiotic trichloroethene (TCE) transformation were evaluated, first in batch studies using natural aquifer solids or pure minerals, and then during back diffusion from a low permeability zone (LPZs). Prior to this project, no study had quantified the relative contributions of biotic and abiotic TCE transformation during back diffusion from low permeability zones. It is also the first time the rates and mechanisms of abiotic TCE reactions are compared for pure minerals under anaerobic and aerobic conditions, and for iron sulfide minerals under sulfate or iron reducing conditions. A summary of key findings are presented below.

6.1 Conclusions for Batch Experiments Evaluating Abiotic Reduction and Oxidation with Natural Materials

Bench-scale experiments were performed on natural sediments to assess abiotic dechlorination of trichloroethene (TCE) under both aerobic and anaerobic conditions. In the absence of oxygen ($<26 \mu\text{M}$), TCE dechlorination proceeded via a reductive pathway generating acetylene and/or ethene. Reductive dechlorination rate constants up to $3.1 \times 10^{-5} \text{ d}^{-1}$ were measured, after scaling to in situ solid water ratios. In the presence of oxygen greater than $120 \mu\text{M}$, TCE dechlorination proceeded via an oxidative pathway generating formic/glyoxylic and glycolic/acetic acids, and oxidative dechlorination rate constants (again scaled to in situ conditions) up to $7.4 \times 10^{-3} \text{ d}^{-1}$ were measured. These rates correspond to half-lives of 60 and 0.25 years for abiotic TCE dechlorination under anaerobic and aerobic conditions, respectively, indicating the potentially large impact of aerobic TCE oxidation in the field. For both reductive and oxidative TCE dechlorination pathways, measured first-order rate constants increased with increasing ferrous iron content, suggesting the role of iron oxidation. Hydroxyl radical formation was measured and increased with increasing oxygen and ferrous iron content. Rate constants associated with TCE oxidation products increased with increasing hydroxyl radical generation rates, and are zero in the presence of a hydroxyl radical scavenger, suggesting that oxidative TCE dechlorination is a hydroxyl radical driven process.

6.2 Conclusions for Batch Experiments Evaluating Iron Sulfide Mineral Reactivity for TCE in The Presence of Sulfate Reducing Bacteria

Experiments were conducted to explore the mechanisms controlling enhanced TCE transformation kinetics of iron sulfide minerals in the presence of biological sulfate reduction. Abiotic and biotic systems were evaluated over a range of ferrous iron loading. *Desulfovibrio vulgaris* was used as a model sulfate reducing bacteria for biotic experiments. Controls on TCE transformation kinetics were determined using aqueous phase measurements of ferrous iron, total iron, redox potential, and pH as well as XPS data for the solid precipitates and MINTEQ calculations that determined

which mineral species were favored to precipitate under different experimental conditions. The results indicate that low redox potentials and iron-sulfide precipitates are necessary to promote efficient TCE transformation in iron sulfide mineral systems maintained by sulfate reducing bacteria. They indicate that Pseudo first order rate constants for TCE transformation to acetylene in systems with biological sulfate reduction increase with increasing ferrous iron loading from 2 mM (3.63×10^{-5} 1/day) to 50 mM (1.13×10^{-4} 1/day), and then decrease sharply at 100 mM (3.13×10^{-6} 1/day). Lower redox potentials are generally correlated with higher TCE transformation rates, with the lowest redox potentials observed in biotic experiments with elevated ferrous iron concentrations. The Results provide compelling evidence that soluble species produced during biological sulfate reduction can maintain a more reduced environment and allow for more faster TCE transformation rates to acetylene relative to abiotic systems. Lastly, geochemical modeling indicated that lower reducing conditions promoted by the soluble redox-active species favored mackinawite precipitation, but not ferrihydrite, supporting the conclusion that mackinawite was necessary for TCE transformation.

6.3 Conclusions for Batch Experiments Evaluating Iron Sulfide Mineral Reactivity for TCE in The Presence of Iron Reducing Bacteria

Biological iron reduction contributes to formation of TCE-reactive reduced iron minerals. During biological iron reduction, the terminal electron acceptor is often a ferric iron mineral, but there is limited understanding of how iron reducing bacteria and iron source – either ferric mineral or aqueous ferrous iron – influences TCE transformation rates in iron-sulfur systems. Experiments were conducted with both ferrihydrite and Fe^{2+} as a source of iron, over a range of iron concentrations, both with and without iron reducing bacteria present. *Shewanella oneidensis* was used as model iron reducing bacteria in the biotic experiments. Aqueous phase measurements of ferrous iron, total iron, pH and redox potential were conducted, and XRD and XPS were used to evaluate the mineralogy and the redox state of the solid precipitate surfaces. MINTEQA2 calculations were used to interpret the XRD and XPS data in the context of mineral saturation. Major findings from this work include that TCE transformation rates during biological ferrihydrite reduction are strongly correlated with reduction potential. Pseudo first order rate constants for TCE transformation to acetylene increase with lower reduction potentials from 1 mM to 5 mM ferrihydrite loading, and at 10 mM ferrihydrite when the reduction potential increases, TCE transformation rates decrease. XPS data indicate that all experiments that promoted TCE transformation had reduced iron and sulfur as Fe(II)-S bonds on the mineral precipitate surface, but that low reduction potentials in addition to Fe(II)-S surface species were necessary to promote TCE transformation. Abiotic ferrihydrite experiments with Fe(II)-S surface species, but relatively higher reduction potentials did not promote TCE transformations to acetylene. TCE transformation rates were likely hindered by precipitation of iron carbonate mineral precipitates at higher iron loading. The decrease in TCE transformation rates from 5 mM to 10 mM is associated with

increased precipitation of siderite (iron carbonate), which has previously been shown to facilitate slower TCE transformation than iron sulfide minerals. Precipitation of siderite may limit access to Fe(II)-S surface species, resulting in a decrease in TCE transformation rates under conditions that preferentially promote siderite precipitation over mackinawite. Lastly, TCE enhancement due to biological iron reduction does not depend on the source of iron. Both solid phase ferrihydrite and aqueous ferrous iron showed comparable TCE transformation rates to acetylene.

6.4 Conclusions for Flow Cell Experiment and Associated 1D Modeling

A flow cell was designed and fabricated to experimentally test transformation processes controlling TCE flux from LPZs into adjacent HPZs during back diffusion. The LPZ material was pre-tested in batch experiments for abiotic dechlorination potential and showed TCE transformation to acetylene could be promoted by natural, low permeability clays with a pseudo first order TCE transformation rate constant of 3.23×10^{-4} 1/day upon normalizing by in situ solids to water ratios. During flow cell experiments, the LPZ material was seeded with a dechlorinating enrichment community to simultaneously promote both biotic and abiotic TCE transformation. The findings show that both biotic and abiotic TCE transformation can occur simultaneously for extended periods of time (at least 6 months) during back diffusion. In the presence of electron donor and a dechlorinating microbial community, biotic TCE transformation promotes significantly more transformation of TCE through cis-DCE, VC, and finally to ethene than abiotic transformation to acetylene. Biotic TCE transformation will be constrained to spatial locations near the electron donor source (in this case, near the LPZ/HPZ interface where donors were available via diffusion into the LPZ from the HPZ). In contrast, more abiotic acetylene production will be associated with higher TCE concentrations – in the experimental system, abiotic processes were more active deeper in the LPZ profile (further from the LPZ/HPZ interface) where the TCE concentrations were greatest.

A 1D diffusion-reaction model was developed to simulate diffusive transport and both biotic and abiotic TCE transformation during back diffusion from LPZs. The model was coded in MATLAB and operator splitting was used to separately solve for transport and reactions. The model was developed assuming an infinite source of TCE in the LPZ, and that advection in the adjacent HPZ was fast relative to diffusion out of the LPZ, allowing for a zero TCE concentration boundary at the LPZ/HPZ interface. Dual Monod kinetics were used for biological reductive dechlorination to account for both electron donor and electron acceptor availability; and pseudo 1st order TCE transformation was used for abiotic TCE transformation to acetylene. Unknown parameters were fit using effluent and depth profile data from the flow cell experiment, and simulations were conducted for a thicker LPZ (300 cm) that may be more representative of some field sites. The model fit and simulations demonstrated that the 1D diffusion-reaction model can adequately capture the concentration depth profiles of TCE and its biotic and abiotic daughter products but

cannot fully capture the magnitudes of the mass discharge rates (flow cell effluent data). Simulations indicate that TCE flux from LPZs could decrease by up to 2% after two years via reactions promoted by reduced clay minerals, and the flux could decrease by up to 53% after two years when TCE transformation is promoted by pyrite-rich mudstone. These results indicate that abiotic TCE transformations could be very relevant and significantly reduce TCE flux to adjacent HPZs in some contaminated aquifers.

6.5 Conclusions for Computationally Efficient Numerical Simulation Using RT3D

A new model, called IMZ-RT3D, was developed based on the mathematical equivalence between finite volume discretization of a 1D diffusion-dominated zone and an approximation using mass transfer between multiple immobile species in sequence, and incorporation of Monod kinetic expressions into the RT3D user defined reaction module. The IMZ-RT3D model was evaluated by comparing simulation results with those from the fully discretized model for several LPZ geometrical set ups, including two-layers, a single thin LPZ lens surrounded by HPZ, single thick LPZ lens surrounded by HPZ, and multiple LPZ lenses surrounded by HPZ. As long as each LPZ is divided into a sufficient number of immobile species, the IMZ-RT3D model profiles are in good agreement with the full-RT3D model, with respect to predicting mass of solute remaining in the model domain and average outflow concentration, for all cases. However, one obvious discrepancy is the consistent and small overestimation of total mass of solutes stored in the LPZ by the IMZ-RT3D compared to the full-RT3D model. Another discrepancy is larger errors between the IMZ and full model for the thin lens compared to the thick lens. This could be attributed to larger gradients of concentration in the thin relative to the thick lens, which can cause higher mass transfer rates and consequently larger errors in numerical approximation. However, considering that IMZ-RT3D model is estimating the full model, the discrepancies are slight as long as enough immobile zones are used for IMZ-RT3D model.

The IMZ-RT3D model is limited to cases in which the transport of solutes in the LPZ can be presented by Eq. (3.1). This means any significant advection or dispersion in the LPZ which deviates transport from merely vertical diffusion contradicts the assumption of IMZ-RT3D and causes large errors in estimation. An inherent weakness of the new IMZ-RT3D model is that it is constrained by the existing capabilities of RT3D, which is no longer under active development even though it is still widely used in practice. For example, the maximum number of immobile zones is limited because only 100 total species are allowed in RT3D. Since the current implementation of IMZ-RT3D requires a separate species for each immobile zone, this limits application of RT3D to one or a few LPZs with having reactions involving a small number of species (e.g., a few sequential dual Monod reactions). It cannot be applied to more complicated domains or multi-species reactions without further modification of the underling RT3D code.

Since Chapman et al. (2012) declared that accurate modeling of back diffusion depends on high resolution grids for low permeable cells, different techniques have been suggested to resolve this computational inefficiency. Although some techniques run faster, prior approaches have all been limited to either equilibrium sorption or simple first order reactions (Carey et al., 2015; Falta and Wang, 2017; Muskus and Falta, 2018). The IMZ-RT3D model presented here, on the other hand, is available for users of MODFLOW/RT3D, and is capable of modeling a flow field with complex heterogeneity LPZ structures, as well as arbitrary reaction kinetics like dual-Monod. It also has strong RT3D solvers available for solving system of reactions. Consequently, the IMZ-RT3D model runs faster than the full model for the more complex structures (like CASE IV in this study) that involve nonlinear reactions. However, despite considerable decrease in the number of grid blocks by IMZ-RT3D, this model runs slower than full model for simpler cases. There are constraints when running RT3D within GMS which do not allow the user to take advantage of options within RT3D for more computationally efficient solution of reaction ODEs. To enhance the computational efficiency of IMZ-RT3D, simpler ODE solvers can be used instead of the more general stiff solvers implemented in RT3D. Other approaches to increase computational efficiency such as using a smaller number of mobile and immobile zones will be studied in our future work.

6.6 Future Work

The insights provided by this research raise new questions that motivate future work.

The first and most important set of questions relates to screening measurements that indicate TCE reactivity in the field. We proposed measuring mineralogy along with structural and sorbed ferrous iron. We also proposed complementing this with reduction potential measurements. However, these are generally on and off indicators of activity, or at most relatively indicators of activity when comparing one site to another. More important is how do these measurements relate to the amount of reactivity for TCE. Hence, a critical research gap is to advance these measurements so that a correlation relating them to TCE reactivity is possible. This might involve normalizing mineralogical measurements by ferrous iron and/or reduction potential measurements, or possibly augmenting these measurements with other yet to be identified parameters. Filling this critical research gap will allow characterization of TCE reactivity at DoD field sites.

The remaining sets of questions focus on reaction mechanisms, and addressing them will provide insights into developing improved screening methods. A key question is why are some ferrous iron minerals more reactive than others? Are there electronic properties of minerals that others have quantified which can be used to determine their ability to donate an electron to TCE or oxygen? Is this reactivity or the electronic properties affected by sorbed reactive species such as ferrous iron or dissolved organic matter. These questions motivate future research on fundamental measures of mineral electron donating capacity and relating this to mineral structure and solution conditions.

Another key question is how do nonreactive species affect ferrous iron mineral reactivity. For example, do ferric (hydr)oxides coat and inactivate ferrous iron minerals when they form, and can they promote TCE reaction when they are coated with sorbed ferrous iron? Also, do other ions or mineral colloids in solution coat and inactivate ferrous iron minerals? These questions motivate future research on the role of nonreacting species on ferrous iron mineral activity in natural and model systems.

Another important question is how do bacteria regenerate ferrous iron minerals in natural systems. Is it through direct iron reduction, or possibly through generated sulfide and reaction of this intermediate with ferric iron. Does natural organic matter play a role as an electron shuttle? How is the type of ferrous iron mineral formed affected by these conditions? What naturally present electron donors drive this process, and how does the microbial community structure contribute to their availability? These questions motivate future research on the role of pure and mixed microbial communities on the type and reactivity of ferrous iron minerals for TCE.

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APPENDIX A: Supporting Data

Flow Cell Electron Donor Availability Calculation

Experimental System

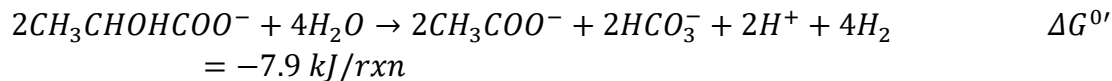
Influent: 1 mM lactate

0.37 mM SO_4^{2-}

Sulfate considered significant competition for donors.

Evaluated representative boundary conditions.

FIRST: Lactate consumed by sulfate reducers



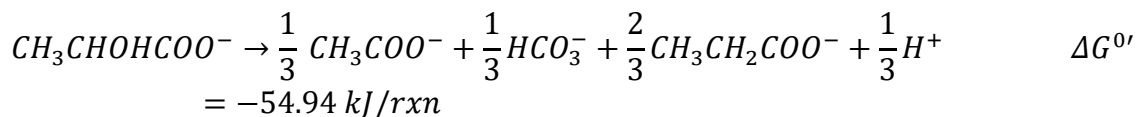
Free energies of formation taken from Brock Biology of Microorganisms (Madigan et al., 2012).

0.37 mM sulfate reacts with 0.74 mM lactate to produce 0.74mM acetate

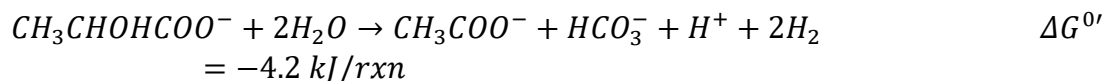
0.26 mM lactate remaining

SECOND: Lactate fermenters compete with lactate oxidation for remaining lactate

Fermenters (assume 2/3 of lactate):



Lactate Oxidation (assume 1/3 of lactate):



0.26 mMx(2/3) lactate from fermenters produces 0.058mM acetate and 0.115mM propionate

0.26 mMx(1/3) lactate from oxidation produces 0.087mM acetate and 0.173 mM hydrogen

OVERALL: Lactate consumption and resulting boundary conditions

1mM lactate $\rightarrow$ 0.885mM acetate + 0.115mM propionate + 0.173mM hydrogen

Assume: Propionate reactions are very slow and insignificant kinetically relative to reactions that utilize acetate over the experiment timescale.

Flow Cell Supplementary Modeling

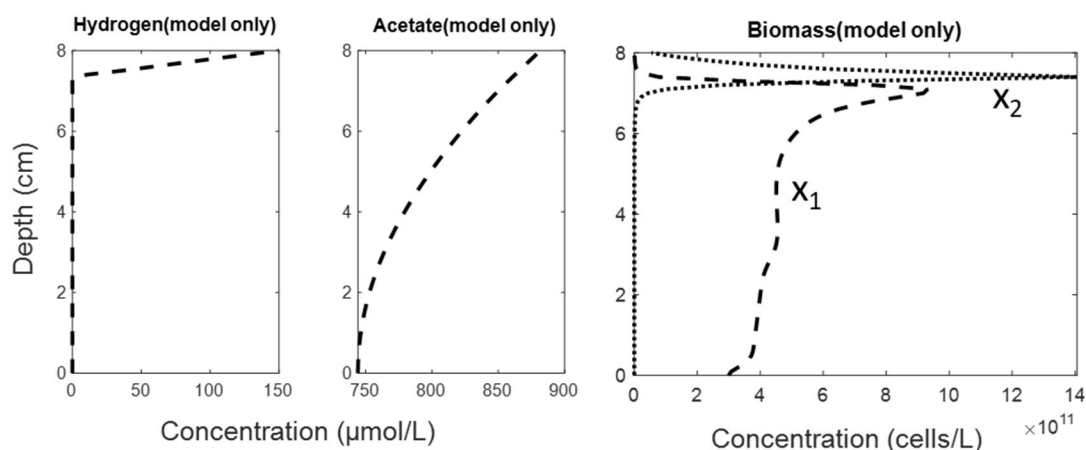


Figure A1. Depth profiles for hydrogen, acetate, and biomass concentrations from model associated with flow cell fitting profiles (Figure 2.6 in main article).

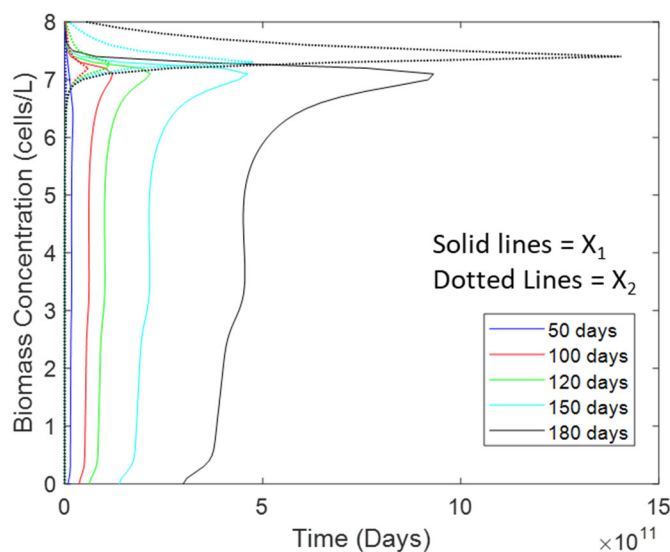


Figure A2. Biomass depth profiles over time. The data at 180 days (black series) is the same as the biomass data in Figure A9.1. At approximately 150 days, community X_2 (utilizes hydrogen to promote all three dechlorination steps) bypasses community X_1 for peak biomass concentration.

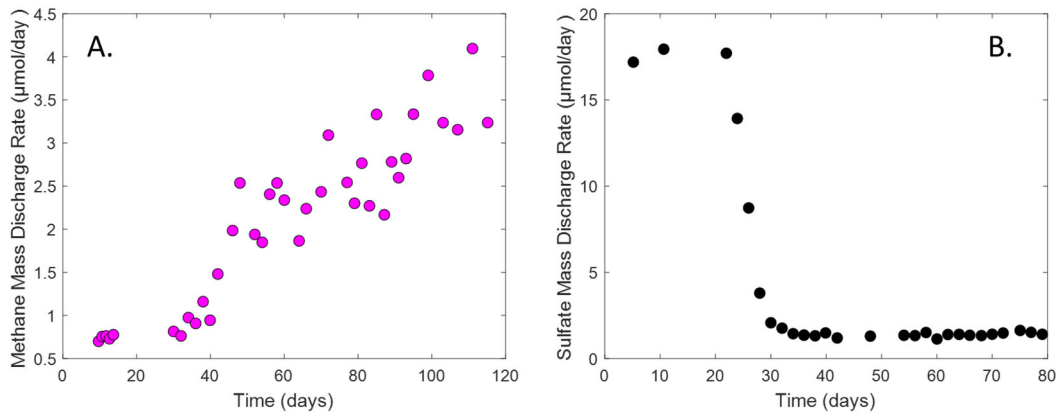


Figure A3. Flow cell effluent data as mass discharge rate (Mass/Time) for (A) methane and (B) sulfate in the flow cell experiment.

Batch Iron Sulfide w/ Sulfur Reducing Bacteria: Geochemical Equilibrium Modeling

Table A1. Saturation indices for selected mineral phases in biological sulfate, abiotic sulfide, abiotic sulfate/sulfide, and microbial product experiments.

Mineral	Biotic Sulfate $\text{Fe}:\text{SO}_4^{2-}$					Abiotic Sulfide $\text{Fe}:\text{HS}^-$			Abiotic Sulfate/Sulfide $\text{Fe}:\text{SO}_4^{2-}:\text{HS}^-$		Microbial Product $\text{Fe}:\text{HS}^-$	
	2:1	20:1	35:1	50:1	100:1	2:1	20:1	50:1	50:1:0	50:0.5:0.5	Filtered 50:1	Autoclaved 50:1
CaS(s)	-22.278	-18.812	-15.932	-14.676	-17.669	-19.82	-19.889	-17.249	-18.543	-17.107	-14.938	-14.898
Epsomite	-4.856	-5.072	-5.216	-7.136	-5.498	-4.845	-5.073	-5.295	-5.292	-5.294	-10.91	-9.198
Fe(OH)2 (am)	-1.794	-1.266	-1.224	-0.971	-1.064	-2.03	-1.167	-1.047	-1.027	-0.948	-1.509	-1.429
Fe(OH)2 (c)	-1.194	-0.666	-0.624	-0.371	-0.464	-1.43	-0.567	-0.447	-0.427	-0.348	-0.909	-0.829
Fe(OH)2.7Cl.3(s)	3.841	3.978	3.667	3.503	4.04	3.336	4.196	3.99	4.169	4.057	2.608	2.884
Fe2(OH)5(s)	56.231	57.047	57.041	57.605	57.238	55.64	57.295	57.413	57.463	57.662	56.26	56.46
Fe2(SO4)3(s)	-44.568	-43.58	-44.076	-50.431	-43.33	-44.917	-43.411	-43.709	-43.396	-43.84	-62.103	-56.628
Fe3(OH)7(s)	70.727	72.071	72.107	72.924	72.464	69.9	72.418	72.656	72.725	73.004	71.042	71.321
Fe3(OH)8(s)	-1.797	-1.133	-1.762	-1.798	-0.918	-3.116	-0.567	-0.924	-0.54	-0.661	-4.289	-3.632
Fe3(OH)8.2(s)	75.366	76.469	76.415	77.292	76.65	74.419	76.866	76.983	77.063	77.381	75.139	75.458
Fe4(OH)8Cl(s)	54.97	55.495	55.535	55.786	55.69	54.734	55.594	55.71	55.73	55.81	55.249	55.329
Fe6(OH)12SO4(s)	127.07	130.042	130.173	129.785	130.908	125.661	130.637	131.172	131.294	131.77	122.78	124.971
Ferrihydrite	0.164	0.232	-0.103	-0.248	0.239	-0.377	0.466	0.228	0.41	0.31	-1.224	-0.935
Ferrihydrite (aged)	0.674	0.742	0.407	0.262	0.749	0.133	0.976	0.738	0.92	0.82	-0.714	-0.425
FeS (ppt)	-7.644	-3.152	-0.031	1.372	-1.317	-5.188	-4.229	-1.193	-2.488	-1.051	1.107	1.147
Goethite	2.874	2.943	2.608	2.464	2.952	2.333	3.176	2.939	3.121	3.021	1.487	1.776
Greigite	-25.702	-9.182	2.504	7.072	-2.436	-16.252	-13.318	-2.011	-6.884	-1.578	5.671	6.169
Gypsum	-2.543	-2.758	-2.9	-4.817	-3.174	-2.533	-2.759	-2.977	-2.974	-2.977	-8.592	-6.88
Hematite	8.15	8.287	7.618	7.33	8.307	7.067	8.755	8.281	8.645	8.445	5.377	5.955
Lepidocrocite	1.994	2.063	1.728	1.584	2.072	1.453	2.296	2.059	2.241	2.141	0.607	0.896
Mackinawite	-6.994	-2.502	0.619	2.022	-0.667	-4.538	-3.579	-0.543	-1.838	-0.401	1.757	1.797
Maghemite	0.346	0.483	-0.186	-0.474	0.503	-0.737	0.951	0.477	0.841	0.641	-2.427	-1.849
Magnesianferite	1.243	0.884	0.016	-0.166	0.418	-0.074	1.449	0.702	1.086	0.965	-2.654	-1.997
Magnetite	15.027	15.692	15.065	15.031	15.917	13.708	16.259	15.906	16.29	16.169	12.54	13.197
Melanterite	-4.447	-3.638	-3.541	-5.313	-3.373	-4.438	-3.638	-3.466	-3.463	-3.465	-9.09	-7.378
Pyrite	-4.976	2.559	8.004	9.765	5.636	-0.439	0.577	5.813	3.529	5.962	8.894	9.312
Sulfur	-10.745	-7.702	-5.378	-5.021	-6.459	-8.665	-8.608	-6.407	-7.397	-6.4	-5.626	-5.248
Thenardite	-7.484	-7.695	-7.829	-9.741	-8.079	-7.476	-7.695	-7.898	-7.895	-7.897	-13.519	-11.806

Table A2. Input parameters for MINTEQ calculations for biological sulfate, abiotic sulfide, abiotic sulfate/sulfide, and microbial product experiments.

Parameter	Abiotic Sulfide			Abiotic Sulfate/Sulfide	
	Fe:HS ⁻ 2:1	Fe:HS ⁻ 20:1	Fe:HS ⁻ 50:1	Fe:HS ⁻ :SO ₄ ²⁻ 50:0.5:0.5	Fe:SO ₄ ²⁻ 50:1
pH	7.34	7.27	7.1	7.18	7.14
Eh (mV)	-173	-170	-184	-188	-175
Concentration (mM)	Fe+2	2	20	50	50
	HS-1	1	1	0.5	0
	Lactate-1	10	10	10	10
	Fe+3	0	0	0	0
	SO4-2	0	0	0.5	1

Parameter		Biotic Sulfate					Microbial Product	
		Fe:SO ₄ ²⁻ 2:1	Fe:SO ₄ ²⁻ 20:1	Fe:SO ₄ ²⁻ 35:1	Fe:SO ₄ ²⁻ 50:1	Fe:SO ₄ ²⁻ 100:1	Fe:HS ⁻ 50:1 Autoclave	Fe:HS ⁻ 50:1 Filter
pH		7.44	7.35	7.15	7.16	7.02	6.94	6.9
Eh (mV)		-120	-175	-192	-219	-90	-219	-229
Concentration (mM)	Fe+2	2	20	35	50	100	50	50
	HS-1	0	0	0	0	0	1	1
	Lactate-1	10	10	10	10	10	10	10
	Fe+3	0	0	0	0	0	0	0
	SO4-2	1	1	1	1	1	0	0

Batch Iron Sulfide w/ Iron Reducing Bacteria: Geochemical Equilibrium Modeling

Table A3. Saturation indices for selected mineral phases in biological ferrihydrite experiments.

Mineral	Fe(OH)3:HS- 1:1	Fe(OH)3:HS- 2:1	Fe(OH)3:HS- 5:1	Fe(OH)3:HS- 10:1	Fe(OH)3:HS- 10:1_out	Fe(OH)3:HS- 10:0	Fe(OH)3:HS- 1:0
CaS(s)	-22.488	-18.63	-13.634	-28.141	-17.94	-40.176	-43.506
Epsomite	-5	-5.009	-5.504	-4.998	-5.077	-17.691	-17.684
Fe(OH)2 (am)	-2.529	-2.241	-1.911	-2.67	-1.546	-2.588	-3.005
Fe(OH)2 (c)	-1.929	-1.641	-1.311	-2.07	-0.946	-1.988	-2.405
Fe(OH)2.7Cl.3(s)	3.147	2.954	2.601	3.719	3.557	3.698	3.725
Fe2(OH)5(s)	54.63	55.195	55.846	54.329	56.576	54.563	53.638
Fe2(SO4)3(s)	-45.295	-45.653	-47.804	-44.037	-44.592	-82.549	-81.999
Fe3(OH)7(s)	68.391	69.244	70.225	67.949	71.32	68.265	66.924
Fe3(OH)8(s)	-3.993	-4.097	-4.478	-3.002	-2.2	-2.92	-3.337
Fe3(OH)8_2(s)	72.9	73.742	74.713	72.437	75.808	72.823	71.392
Fe4(OH)8Cl(s)	54.232	54.52	54.85	54.091	55.214	54.174	53.757
Fe6(OH)12SO4(s)	122.643	124.36	125.842	121.8	128.465	109.596	107.096
Ferrihydrite	-0.566	-0.762	-1.118	0	-0.161	0	0
Ferrihydrite (aged)	-0.056	-0.252	-0.608	0.51	0.349	0.51	0.51
FeS (ppt)	-8.175	-4.01	1.331	-13.929	-2.604	-26.027	-29.598
Goethite	2.145	1.949	1.593	2.711	2.55	2.711	2.711
Greigite	-27.08	-11.672	7.986	-48.542	-6.935	-97.18	-110.213
Gypsum	-2.715	-2.723	-3.217	-2.712	-2.791	-15.405	-15.397
Hematite	6.691	6.3	5.588	7.823	7.501	7.823	7.823
Lepidocrocite	1.265	1.069	0.713	1.831	1.67	1.831	1.831
Mackinawite	-7.525	-3.36	1.981	-13.279	-1.954	-25.377	-28.948
Maghemite	-1.113	-1.504	-2.216	0.019	-0.303	0.019	0.019
Magnesianoferrite	-0.601	-1.011	-1.738	0.492	0.17	0.636	0.459
Magnesite	-0.344	-0.355	-0.364	-0.364	-0.378	-0.285	-0.378
Magnetite	12.833	12.729	12.348	13.824	14.627	13.906	13.489
Melanterite	-4.941	-4.643	-4.792	-5.04	-3.995	-17.795	-18.029
Pyrite	-5.293	1.784	10.762	-15.246	3.71	-39.688	-45.579
Siderite	2.763	3.058	3.395	2.641	3.752	2.658	2.325
Sulfur	-10.531	-7.62	-3.982	-14.731	-7.099	-27.075	-29.394
Thenardite	-7.538	-7.547	-8.046	-7.536	-7.617	-20.231	-20.227

Table A4. Input parameters for MINTEQA2 calculations for biological ferrihydrite experiments.

Parameter	Biotic Ferrihydrite				
	$\text{Fe}(\text{OH})_3:\text{HS}^-$ 1:1	$\text{Fe}(\text{OH})_3:\text{HS}^-$ 2:1	$\text{Fe}(\text{OH})_3:\text{HS}^-$ 5:1	$\text{Fe}(\text{OH})_3:\text{HS}^-$ 10:1	$\text{Fe}(\text{OH})_3:\text{HS}^-$ 10:1 _{out}
pH	7.31	7.30	7.29	7.29	7.30
Eh (mV)	-154	-182	-222	-111	-187
Concentration (mM)	$\text{Fe}(\text{OH})_3$ (s) _{initial}	1	2	5	10
	Fe+2	0	0	0	0
	HS-1	1	1	1	1
	Lactate-1	10	10	10	10
	Fe+3	0	0	0	0
	SO4-2	0	0	0	0

Parameter	Biotic Ferrihydrite (no sulfide)	
	$\text{Fe}(\text{OH})_3:\text{X}$ 10:0	$\text{Fe}(\text{OH})_3:\text{X}$ 1:0
pH	7.36	7.27
Eh (mV)	-120	-90
Concentration (mM)	$\text{Fe}(\text{OH})_3$ (s) _{initial}	10
	Fe+2	0
	HS-1	0
	Lactate-1	10
	Fe+3	0
	SO4-2	0

Appendix B: List of Publications

Published

Esfahani, S.G., A.J. Valocchi, and C.J. Werth. 2020. Using MODFLOW and RT3D to Simulate Diffusion and Reaction without Discretizing Low Permeability Zones, *Journal of Contaminant Hydrology*, Accepted Pending Minor Revisions.

Shuo, Y. and A.J. Valocchi. 2020. Flux-corrected Transport with MT3DMS for Positive Solution of Transport with Full-tensor Dispersion. *Groundwater*, 58(3):338-348.

Berns, E.C., R.A. Sanford, A.J. Valocchi, T.J. Strathmann, C.E. Schaefer, and C.J. Werth. 2019. Contributions of Biotic and Abiotic Pathways to Anaerobic Trichloroethene Transformation in Low Permeability Source Zones. *Journal of Contaminant Hydrology*, 224, 103480, 2019.

Schaeffer, C.E., P. Ho, E. Berns, and C.J. Werth. 2018. Mechanisms For Abiotic Dechlorination Of TCE By Ferrous Minerals Under Oxidic And Anoxic Conditions In Natural Sediments. *Environmental Science & Technology*, 52(23):13747-13755.

Schaefer, C.E., P. Ho, C. Gurr, E. Berns, and C. Werth. 2017. Abiotic Dechlorination Of Chlorinated Ethenes In Natural Clayey Soils: Impacts Of Mineralogy And Temperature. *Journal of Contaminant Hydrology*, 206:10-17.

In Review

Schaefer, C.E., P. Ho, E. Berns, C.J. Werth, Abiotic Dechlorination in the presence of ferrous minerals, *J. Contam. Hydrology*, In Review, 2020.

In Final Stages of Preparation

Berns, E.C., R.A. Sanford, A.J. Valocchi, T.J. Strathmann, C.E. Schaefer, C.J. Werth, The effect of sulfate reducing bacteria on trichloroethene reduction by iron sulfide minerals, In Preparation, 2020.

Berns, E.C., R.A. Sanford, A.J. Valocchi, T.J. Strathmann, C.E. Schaefer, C.J. Werth, Evaluation reduction potential as an indicator of reactivity for trichloroethene reduction, In Preparation, 2020.