5-2017

Comparing the Effect of Carbon Sources, Lactate and Whey, on Biological Reductive Dechlorination of TCE in Laboratory Flow Through Columns

Sarah M. Kissell
Utah State University

COMPARING THE EFFECT OF CARBON SOURCES, LACTATE AND WHEY, ON BIOLOGICAL REDUCTIVE DECHLORINATION OF TCE IN LABORATORY FLOW THROUGH COLUMNS

by

Sarah M. Kissell

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Environmental Engineering

Approved:

R. Ryan Dupont Major Professor	Joan E. McLean Committee Member
William J. Doucette Committee Member	Mark R. McLellan Vice President for Research and Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2016

ABSTRACT

Comparing the Effect of Carbon Sources, Lactate and Whey, on Biological Reductive Dechlorination of TCE in Laboratory Flow Through Columns

By

Sarah M. Kissell, Master of Science

Utah State University, 2016

Major Professor: Dr. R. Ryan Dupont
Department: Civil and Environmental Engineering

Trichloroethylene (TCE) is one of the most prevalent groundwater contaminants in the U.S., and is classified as a Group 1 carcinogen by the International Agency for Research on Cancer. Anaerobic reductive dechlorination is an effective bioremediation technique when biogeochemical and energy requirements are met. In this study, the impacts of applying of a simple versus complex substrate (lactate versus whey) during biostimulation and bioaugmentation of aquifer material, were compared in order to determine which form of carbon would support the biogeochemistry and energy production necessary to achieve dechlorination of TCE.

Glass columns were packed with aquifer material collected from Hill Air Force Base Operable Unit 5, Utah, received a continuous flow of groundwater containing TCE and carbon in the form of whey, lactate, or no carbon (control), and were inoculated with a culture containing *Dehalococcoides mccartyi* (*Dhc*). Changes in carbon metabolites, redox conditions, and TCE degradation byproducts were measured weekly. Soils were analyzed at the point of iron reduction, and TCE reduction to each sequential degradation byproduct; cis-dichloroethene (DCE), vinyl chloride (VC), and ethene for iron mineralogy, sulfides, and microbiology.

Sulfate reducing conditions were met in both carbon treatments. With both carbon sources, TCE was being reduced to ethene by the end of the study, although there was a significantly greater amount of VC accumulation in the lactate treatment than in the whey. Concentrations of butyrate, hydrogen, and reduced iron (aqueous) were significantly greater in the whey than the lactate treatment, which may have facilitated the high rates of VC reduction. Propionate concentrations were greater in the lactate treatment than in the whey, along with acetate during ethene production. During DCE and VC reduction, the difference in metabolites among the lactate and whey treatments did not lead to a difference in the concentration of the genes *vcrA*, *tceA*, or *Dhc*. The addition of whey supported the biogeochemical conditions and energy production required to achieve full dechlorination of TCE with high rates of VC reduction. When compared to lactate, the use of whey during TCE bioremediation could reduce the risk of human exposure to VC, a carcinogenic TCE degradation byproduct. (137 pages)

PUBLIC ABSTRACT

Comparing the Effect of Carbon Sources, Lactate and Whey, on Biological Reductive Dechlorination of TCE in Laboratory Flow Through Columns

Sarah M. Kissell

Trichloroethylene (TCE) is a chlorinated solvent most commonly used as an industrial degreaser for cleaning mechanical equipment. Historic improper management and disposal of TCE has resulted in contaminated soil and groundwater across the United States, including Hill Air Force Base in Utah. The abundance of TCE in the environment presents a public health risk because it is categorized as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC).

The purpose of this study was to improve the bioremediation techniques of biostimulation and bioaugmentation. A continuous flow-through column study was performed where columns packed with aquifer material received a continuous flow of groundwater collected from Hill AFB. The groundwater contained TCE and a carbon source, lactate or whey, a waste product of the cheese industry to stimulate the aquifer microbial community, create anaerobic conditions, and facilitate the use of TCE as a terminal electron acceptor during respiration.

Both carbon treatments reduced TCE to the final product of ethene gas, but unlike the lactate treatment, whey provided the energy required to fully reduce TCE, without accumulating the harmful degradation byproduct, vinyl chloride. The substrate, whey, provides an effective carbon and energy source for the bioremediation of TCE, and is also more economical than highly refined chemicals, such as lactate.

ACKNOWLEDGMENTS

I would like to first acknowledge my advisor, Dr. Dupont, and my committee members, Professors Joan McLean and Bill Doucette for welcoming me to Utah State University, providing me with a great research opportunity, and supporting me throughout my Master's degree. I am thankful for the opportunity they provided for me to study and earn my Master's degree at USU, and now I feel prepared to begin my professional career as an environmental engineer.

I would also like to thank my colleague, Suzy Smith, who was a great teammate throughout the journey of our Master's research. The project would not have been feasible without such a patient and supportive friend. Also, the project would not have been possible without all of the laboratory support we had from Tessa Guy and Joe Stewart. I'd like to thank Joe Stewart for training and assisting me in the lab and also being a great mentor and friend. I'd also like to thank all of the students that helped us throughout the project, including Jared Richens, Kaisa forsyth, Will Fullmer, Darianne Willey, Jeremey Jensen, and Rashelle Wegelin.

Lastly, I'd like the thank all of my friends and family, especially my parents Catherine and Victor Kissell, and my partner, Zachary Magdol for providing me with so much mental and emotional support and strength throughout all of the excitement and challenges of a completing my Master's degree. I am so thankful for the opportunity I had to further my education at Utah State University, and for all of the people who were there to assist me while earning my Master's degree.

Sarah M. Kissell

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

INTRODUCTION

Trichloroethylene (TCE, C_2HCl_3) is a chlorinated solvent most often used as an industrial degreaser for cleaning mechanical equipment, and one of the most common man-made organic chemical released into the environment. Due to the widespread use, mishandling, and improper disposal, TCE is a source of aquifer contamination across the country. The solvent has been recently classified as carcinogenic to humans (International Agency for Research on Cancer 2014). Techniques for remediating aquifers and soil contaminated with TCE are continually being investigated and optimized by engineers and scientist. Many chemical and biological pathways of TCE degradation have been established, but optimizing these techniques to avoid toxic degradation byproducts presents a challenge. It is important that during the process of remediation, full dechlorination is achieved, without resulting in an accumulation of environmentally harmful byproducts such as vinyl chloride (VC). Many of these remediation techniques are only effective under specific biogeochemical conditions, so it is important to evaluate and characterize a contaminated site before implementing a plan for remediation.

During bioremediation, the TCE is degraded through microbial metabolism. *Dehalococcoides* (*Dhc*) is the only known species of bacteria capable of fully dechlorinating TCE through reductive dechlorination, when TCE is used as the electron acceptor, and hydrogen as the electron donor. Full dechlorination of TCE by *Dhc* occurs under highly reduced conditions, during sulfate reduction and methane production. With the necessary supply of hydrogen, reductive processes can occur simultaneously. Iron reduction has been found to hinder TCE reduction by presenting competition for hydrogen, and has also been found to promote TCE reduction through abiotic processes depending on the mineralogy (Darlington

and Rectanus 2015; Paul et al. 2013; USEPA 2009). During the process of biostimulation a carbon substrate is supplied, and through fermentation enough hydrogen may be supplied to support the growth of a diverse microbial community, and simultaneous reducing processes.

Dhc has been found in many contaminated aquifers, but the community may not be concentrated or diverse enough to support the metabolic requirements of *Dhc* to carry out complete reductive dechlorination. Through bioaugmentation, aquifers can be inoculated with a diverse community that includes *Dhc* as well as other organisms that will support the metabolism of *Dhc* and full TCE reduction, including fermentation of carbon substrates for hydrogen production, synthesis of vitamin B, and highly reduced conditions. Often, full dechlorination of TCE does not occur without the combination of bioaugmentation and biostimulation.

The purpose of this study was to improve bioremediation of TCE contaminated aquifers through an analysis of the effect of two vastly different carbon sources on the rate and extent of TCE reductive dechlorination. The degradation pathways of anaerobic reductive dechlorination were analyzed during the process of biostimulation and bioaugmentation of aquifer material. During the process of biostimulation, carbon sources, lactate and whey were applied. The impact of the different carbon sources on soil biogeochemistry, microbial community composition, and resulting degradation of TCE were compared by performing a continuous flow-through column study. This study was based on a long-term column study performed by McLean et al (2015), which also analyzed the impact of different carbon sources applied during biostimulation and bioaugmentation of aquifer material. Similarly, to the study performed by McLean et al. (2015), glass columns were packed with soil from a TCE contaminated aquifer located at Hill Air Force Base, Utah and

received a continuous flow of groundwater, which was collected from the site and spiked with TCE. Unlike the previous study which used large, 2 m long 15 cm diameter columns, this study used small diameter, small length columns to focus analysis on the most active region (the upper 15 cm) of the large columns used by McLean et al. (2015). This column study consisted of the two treatments, receiving different carbon sources (lactate and whey), and a control, receiving no carbon addition. All columns were bioaugmented with a dechlorinating culture, the derived Bachman Road (DBR) culture, known to contain the bacteria genus, *Dehalococcoides* (*Dhc*), as well as a diversity of microorganisms with other supporting metabolic capabilities.

The changes in biogeochemistry and dechlorination were monitored in column effluent twice a week throughout the study. At different redox stages, including the reduction of iron and TCE to the sequential degradation byproducts (dichloroethene, vinyl chloride, and ethene) the characteristics of the soil geochemistry and the microbial community composition were evaluated and the rates of TCE degradation were calculated. The progress of TCE dechlorination was monitored by assessing the Chlorine Number (N_{Cl}) for each treatment throughout the study, which is defined as $N_{Cl} = w_i\ Cl_i / Cl_i$, where w_i is the number of chlorine atoms in molecule i, and Cl_i is the molar concentration of each chlorinated ethene species (McLean et al. 2015). The simple or complex carbon source (lactate or whey) that provides the ideal biogeochemistry for full dechlorination of TCE, based on the extent and rate of dechlorination, was determined.

Hypothesis and Objectives

During the process of biostimulation and bioaugmentation, the carbon source added to the system facilitates highly reduced conditions, where TCE is used as a terminal electron acceptor (TEA) by a select type of bacteria during respiration. During this study, the effects of a simple (lactate) and complex carbon source (whey) were analyzed to determine their influence on the rate and extent of TCE degradation, along with the change in biogeochemistry of the aquifer material and groundwater. In order to analyze the impact of the different carbon types on the biogeochemistry, such as the changes in chemical, physical, and biological characteristics resulting from biostimulation, carbon metabolites such as low molecular weight organic acids and hydrogen were analyzed, along with TEAs (SO_4^{2-}, NO_3^-, Fe(III)) and their effect on soil mineralogy, inorganic carbon, and the microbial community based on genes supporting TCE reduction. The study was conducted to test the following hypothesis and complete the following objectives.

Hypothesis: The carbon type used during biostimulation and bioaugmentation does not have an effect on the biogeochemistry, microbial community composition, and the resulting rate and extent of trichloroethylene degradation.

- *Objective 1*: In a continuous flow through column study, compare the rate and extent of dechlorination of TCE among treatments receiving a complex carbon source, whey, versus a simple carbon source, lactate, along with controls, receiving no added carbon.

- *Objective 2*: Compare metabolites that are associated with TCE and carbon degradation, such as low molecular weight organic acids, inorganic carbon and hydrogen produced during fermentation, among carbon treatments and controls.
- *Objective 3*: Characterize and compare the biogeochemistry among treatments, by describing how the added carbon and nutrients affect the surrounding soil chemistry and redox conditions associated with the increase in microbial activity. Also, describe the changes in microbial community composition, characterized by the reductive dehalogenase genes present at each stage of TCE dechlorination. Stages include initial reduced conditions (Fe reduction) and partial to complete reductive dechlorination resulting in Chlorine Numbers 2, 1, and 0.

LITERATURE REVIEW

Trichloroethylene

Trichloroethylene (1,1,2-trichloroethene) is a solvent primarily used for degreasing machinery. Other uses include chemical extractions of grease and oils, manufacturing of other chemicals, and dry-cleaning. Historically, TCE was used as a disinfectant, anesthetic, pet food additive, and during the process of decaffeinating coffee (ATSDR 2012). The solvent can behave as a dense non-aqueous phase liquid (DNAPL) in the environment. Compared to other DNAPLs commonly found in the environment, such as polychlorinated biphenyls (solubility <1 mg/L) and petroleum additives including naphthalene (solubility of 32 mg/L at 25°C), TCE is relatively water soluble (Table 1. When released into the environment at amounts greater than its aqueous solubility most of the TCE migrates through the soil, and its high density causes it to accumulate along confining layers that may be below the water table. The release of TCE into water bodies could pose a threat to human health, as the maximum contaminant level for public drinking water is 5 μg/L. TCE also has a high vapor pressure, indicating that inhalation is the primary route of exposure when working with TCE. The 8-hour time weighted average (TWA) for TCE exposure is 10 ppm, set by the American Conference of Governmental Industrial Hygienists (2007). Physical and chemical properties of TCE listed in Table 1, which were retrieved from Stroo and Ward (2010) and USEPA (2014), where most values were based on experimental and estimated values summarized in Yaws' Handbook of Thermodynamics and Physical Properties of Chemical Compounds by Carl L. Yaws (2003).

Table 1. Physical and Chemical Properties of Trichloroethylene (Stroo and Ward 2010; United States Protection Agency 2014)

Melting Point	-84.7°C
Boiling Point	87.2°C
Density	1.46 g/cm^3 (@20°C)
Solubility	1,100 mg/L (@25°C)
Henry's Law Constant	0.012 atm·m^3/mol (@25°C)
Vapor Pressure	0.097 atm (@25°C)
Log Kow	2.53

Prior to the Resource Conservation and Recovery Act (RCRA) and Occupational Safety and Health Act (OSH Act), the wide use, along with improper handling and disposal of TCE resulted in its release and subsequent contamination of soil and aquifers across the United States (Figure 1). Many sites were eventually placed on the National Priorities List (NPL) to become Superfund Sites. Approximately 40% of the sites that are currently, or have historically been on to the NPL are contaminated with TCE (U.S. Department of Health and Human Services 2015). Remediation of TCE contaminated soils and aquifers is estimated to eventually cost site owners billions of dollars (USEPA 2000).

Air Force Bases around the country, such as Hill Air Force Base (AFB) located in Ogden UT, used TCE to degrease equipment and aircraft. Throughout the early 1950s until 1980 it is estimated that between 100,000 to 1,000,000 gallons of TCE were disposed of in pits located around Hill AFB (USEPA 1991). From years of disposing TCE in trenches and landfills on site, the aquifer beneath Hill AFB contains a number of TCE plumes, some of which have migrated to residential areas that surround the base. High aqueous concentrations have caused detectable levels of TCE in residents' homes (ASTDR 2009).

The contamination of the shallow aquifers does not pose a threat to drinking water quality, but water used for agriculture may be impacted by this shallow contamination.

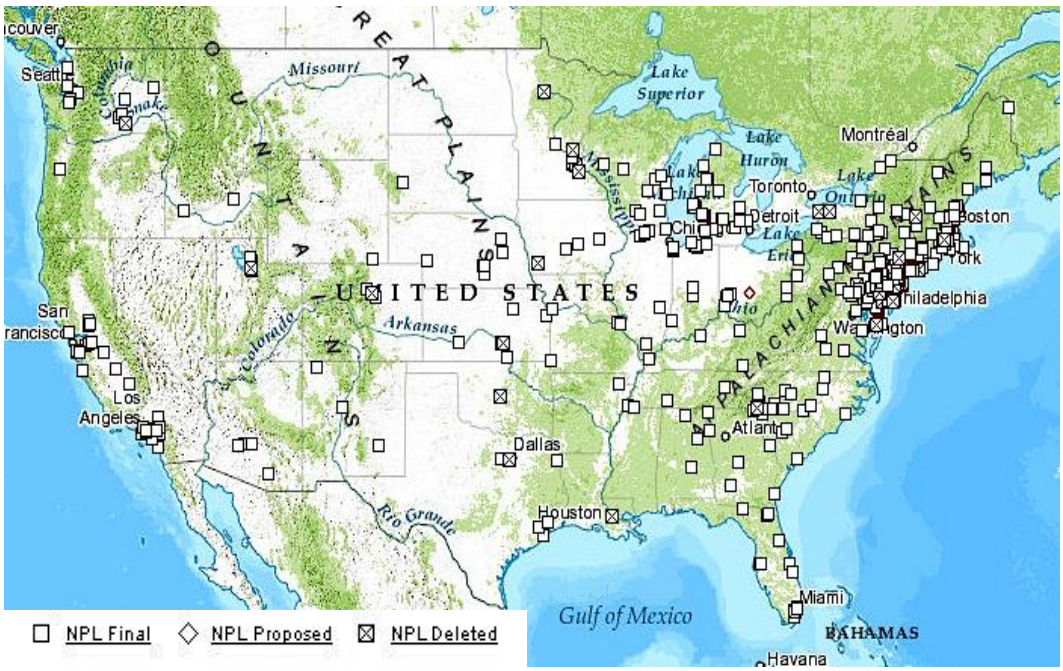


Figure 1. National Priority List sites contaminated with TCE (U.S. Department of Health and Human Services 2015)

The release of TCE poses an environmental and health concern. As of 2014, it has been classified as a Group 1 carcinogen with supporting evidence showing TCE causes severe health issues including cancer (IARC 2014). Prior to occupational health and safety guidelines being instituted, many manufacturing and industrial workers were exposed to TCE. Human exposure to TCE has resulted in adverse health effects, including cancer of the kidney, liver, and pulmonary system, along with non-Hodgkin lymphoma and aplastic anemia (IARC 2014; Klassan 1996). The metabolites of TCE are particularly harmful in the kidneys, where the most prevalent cases of cancer have occurred.

Historically, workers at Hill AFB developed chronic symptoms of TCE exposure. A study completed by Blair et al. (1998) analyzed the impact of TCE exposure on the health of 14,457 aircraft maintenance workers who worked at Hill AFB for at least a year between 1951 and 1956. During this period, about half the workers were exposed to TCE. When comparing deaths within the general population of Utah and those who worked at Hill AFB, an increase occurred among aircraft workers due to multiple myeloma, non-Hodgkin's lymphoma among women, and cancer found in areas such as the biliary passages, bone marrow, lymph node tissue, and the liver (Spirtas et al. 1991).

The abundance of TCE contamination around the country has created a health and environmental risk. It is important to evaluate and characterize contaminated sites to determine the fate of TCE, the impact on the surrounding ecosystem, and the risk of human exposure. Each site where TCE contamination has occurred consists of different physical and biogeochemical characteristics that should be considered when determining the best remediation approach to eliminate exposure to humans and to restore the quality of the environment.

Site characteristics must be evaluated to determine their impact on TCE mobility and transformation. Considering the physical-chemical characteristics of TCE, when released into the environment TCE would potentially partition to all phases, i.e., solid, liquid, and vapor. When in the DNAPL phase, TCE is often found at the confining layer of aquifers, where it can take years to solubilize and remain at residual saturation (ITRC and RTDF 1999). TCE can be detected in the vapor phase above shallow aquifers due to the high Henry's law constant. Degradation in organic soils and consequent release of gases, may also facilitate the release of TCE in the vapor phase. TCE comes into equilibrium among the

surrounding soil, groundwater, and vapor phase, and may remain as a non-aqueous phase liquid. When determining the best remediation technique for a site, it is important to consider the chemical and physical changes of TCE, along with the disruption of the biogeochemical equilibrium in the environment that will occur in response to active remediation.

Trichloroethylene Remediation

Chemical, Physical and Thermal Treatments

Depending on iron mineralogy present in the aquifer, or the type of iron added to the aquifer, iron can abiotically reduce TCE. For example, an aquifer contaminated with TCE can be treated by injecting ZVI nanoparticles, or installing a ZVI permeable reactive barrier. During this process electrons are transferred from the ZVI to the TCE. The primary degradation pathway for TCE via ZVI is through beta-elimination, where TCE is degraded directly to acetylene, followed by ethylene (Ibrahem et al. 2012). Under ideal conditions, ZVI can efficiently reduce TCE without the accumulation of harmful degradation byproducts such as VC, but the efficiency of ZVI can be hindered due to competition with other oxidized chemicals such as NO_3^- and iron oxides (Kaifas et al. 2014), and changes is geochemistry can result in corrosion and precipitates, preventing TCE from reacting with the ZVI (Ulsamer 2011).

Naturally occurring iron minerals have been shown to inhibit as well as promote TCE reduction depending on reaction conditions within the contaminated aquifer. During the process of microbially-mediated iron reduction and formation of biogenic minerals, competition for the electron donor, hydrogen, can occur between iron and TCE reduction

bacteria and inhibit TCE reduction (Paul et al. 2013). A number of studies have shown, however, that with available hydrogen, iron and TCE reduction can occur simultaneously (Badin et al. 2016; Dupont et al. 2003; Wei and Finneran 2011). Under iron and sulfate reducing conditions, reactive compounds capable of reducing TCE abiotically can be formed. Iron minerals such as iron sulfides, iron oxides, and iron hydroxides can abiotically degrade TCE to products such as acetylene and cis-DCE (He et al. 2015). Sorption of Fe(II) to other iron minerals, such as hematite or pyrite, can increase abiotic degradation of TCE (Badin et al. 2016; Brown et al. 2009; Darlington and Rectanus 2015).

The primary abiotic degradation pathway of TCE is through beta-elimination by FeS (He et al. 2015). Microbial reduction of $Fe^{3+}$and SO_4^{2-} will release Fe^{2+} and HS^-, and these products can precipitate as FeS (NAVFAC 2014); Kennedy et al. 2006). Reduction of TCE by FeS is most efficient at an elevated pH of 9.3 when compared with pH levels 7.3 and 8.3 (Butler and Hayes 2001). During recent studies acetylene was not detected, but due to that lack of intermediate byproducts associated with biotic pathways such as vinyl chloride, it was assumed the TCE reduction occurred through abiotic reactions (Lojkasek-Lima et al. 2011, Ibrahem et al. 2012).

Remediation techniques for physically treating and removing TCE include pump and treat methods or excavation. During pump and treat processes, the groundwater is pumped from the aquifer, and can be treated through air stripping, passed through activated carbon where VOCs sorb to solid phase carbon, or VOCs can be combusted in a catalytic incinerator. Trichloroethylene extraction can be enhanced with thermal methods such as steam enhanced extraction, electrical resistance heating, or thermal conductive heating (USEPA 2004). During these processes, the aquifer material is heated, causing an increase in

TCE aqueous solubility and reduction in viscosity and density, facilitating TCE migration, along with an increase in the vapor pressure and Henry's constant of TCE (USEPA 2004). The vapor is extracted from the aquifer material through vacuum extraction wells, and VOCs are removed with activated carbon or combusted in a catalytic incinerator. Thermal treatments can be efficient and may only require a year or less for full removal of VOCs when site contamination is not extensive (CES 2012).

Aeration curtains are also used for treating groundwater contaminated with VOCs, where the contaminant plume flows through a trench containing gravel, and a perforated pipe located at the bottom the trench pumps air through the groundwater. The vapor produced during the air sparging process is collected via vacuum extraction wells and is treated to remove the contaminants (Environmental Management Directorate 1998).

Phytoremediation is another technique for remediating contaminated groundwater. During phytodegradation, contaminants such as TCE are taken up through plant roots, and are then degraded by enzymes within the plant. During phytovolatilization, the compound is released into the atmosphere through plant respiration (Lewis et al. 2015). When released into the atmosphere, the half-life of TCE is about 7 days (Doucette et al. 2013). Significant TCE removal through phytoremediation was accomplished at Fairchild Air Force Base, Washington, and Travis Air Force Base, California (Doucette et al. 2013). It was estimated that between 2004 and 2009 TCE was being removed at a rate of 1.7 and 0.02 kg/yr from aquifers at Travis and Fairchild, respectively (Doucette et al. 2013). After measuring the TCE emissions through the leaves and trunks of trees being used for phytoremediation and the surrounding soil at each site, it was estimated that emissions through leaves accounted for

about 20% and 50% of TCE removal at Travis and Fairchild sites, respectively (Doucette et al. 2013).

Remediation of TCE through chemical and physical properties can remove TCE without accumulating harmful degradation byproducts, but over time can lose effectiveness and have increasing operation and maintenance costs. Methods including air sparging, thermal treatment, and vacuum extraction are energy intensive. Chemical, physical, and thermal methods are intrusive and require installation of equipment and manipulation of the soil and surrounding environment.

Bioremediation

Basic concepts

Bioremediation is an effective technique for restoring aquifers contaminated with various pollutants, including TCE. During the process of bioremediation, the indigenous microbial community, or organisms added to the aquifer via bioaugmentation, degrade the contaminant using various metabolic processes. Bioremediation may take more time to remove a contaminant, but generally requires less energy and produces less waste than physical, chemical or thermal methods. It can be carried out as an in situ method, causing less site disturbance and release of contaminants than ex situ methods. The flow of the groundwater is essential for facilitating the movement and contact between the microbes and contaminants of concern. Challenges that are presented with bioremediation include ensuring the contaminant is available to the microbes, limited permeability which inhibits delivery and recovery of reactants and end products, and proper biogeochemical conditions for the microbial community to function and fully degrade the contaminant(s) of concern.

Chlorinated solvents can be degraded through aerobic cometabolic processes, and directly or cometabolically through anaerobic reductive dechlorination processes (USEPA 2000). TCE is primarily degraded through anaerobic metabolism. During anaerobic degradation, a carbon source, such as lactate, is metabolized into hydrogen and low molecular weight organic acids (LMWOAs), such as acetate, during fermentation (Parsons Corporation 2004). Hydrogen is the electron donor during dehalorespiration, and the chlorinated solvent serves as the terminal electron acceptor. Often in contaminated aquifers, the system is anaerobic due to the consumption of available carbon and oxygen during microbial degradation. In aquifers, reduced conditions and degradation through anaerobic reduction is more easily achieved and maintained than managing the processes involved with aerobic degradation, such as oxygen supply and the volatilization of TCE under aerobic conditions.

In aerobic metabolism, the chlorinated solvent can serve as the growth substrate, and energy is gained as the compound is oxidized and CO_2 is produced, but this process occurs with less chlorinated compounds such as dichloroethene or vinyl chloride (USEPA 2000). Studies have shown the presence of methane, phenol, and toluene as growth substrates, aerobic co-metabolism of TCE is more efficient (Li et al. 2014). Active enzymes that degrade other contaminants, such as methane monooxygenase, toluene dioxygenase, and toluene monooxygenase can support the metabolism of TCE (Li et al. 2014). During the process of oxidizing other compounds, chlorinated solvents can be oxidized to form epoxides, which are rapidly broken down into alcohols or fatty acids (USEPA 2000). Literature shows that through cometabolic processes, TCE can be oxidized and serve as carbon substrate under aerobic conditions (Ensley 1991; Li et al. 2014; Pant and Pant 2010; USEPA 2014). Without

the presence of the microorganisms capable of degrading other compounds such as toluene and phenol, TCE is essentially non-biodegradable under aerobic conditions.

Bioremediation techniques can result in the accumulation of potentially harmful degradation byproducts. During fermentation a carbon and energy source is oxidized, and the hydrogen produced serves as the electron donor. With the addition of a carbon and energy source under highly reducing conditions, through reductive dechlorination (dehalorespiration), TCE serves as the terminal electron acceptor, and is sequentially reduced to dichloroethene (cis-DCE), and then degraded to vinyl chloride (VC), and finally ethylene (ethene) (Figure 2).

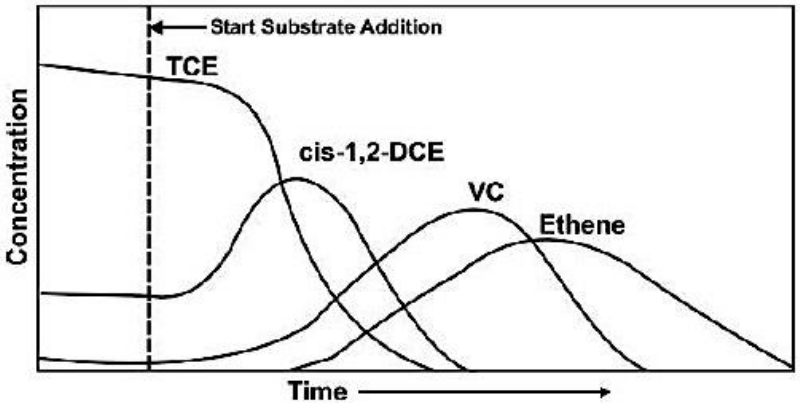


Figure 2. TCE degradation sequence (Solutions IES Inc. et al. 2007)

With the loss of chlorine atoms, DCE and VC are produced which have a higher solubility than the parent compound TCE (Table 2). cis-DCE has only a slightly higher vapor pressure than TCE, resulting in a cis-DCE's lower Henry's Law Constant. As cis-DCE is reduced to VC, VC's increased vapor pressure and lower solubility results in its higher Henry's Law Constant than cis-DCE. Anaerobic reductive dechlorination is more rapid with

highly chlorinated compounds because they are more oxidized. As TCE is reduced, degradation byproducts may degrade more readily through aerobic pathways. Due to the instability of the reduced VOCs in aerobic environments they are not as persistent during aerobic remediation processes as they are under anaerobic conditions in the environment.

Table 2. Chemical Characteristics of TCE and its Degradation Byproducts (Stroo and Ward 2010)

Compound	Solubility (25°C)	Henry's Law Constant atm·m^3/mol (25°C)	Log Kow
Trichloroethylene	1,100 mg/L	0.0117	2.53
cis-Dichloroethene	3,500 mg/L	0.0074	1.86
Vinyl Chloride	2,670 mg/L	0.0792	1.38

Background on Dechlorinating Bacteria

Through reductive dechlorination TCE is used as the terminal electron acceptor during respiration. As TCE becomes reduced, further degradation through reduction becomes less preferable. As the oxidation state decreases, reductive metabolism often stalls at VC. The accumulation of VC poses a greater risk to human health than TCE, with a carcinogenic risk factor (slope factor) from oral exposure of 7.2 x10^{-1} per mg/kg-day and from inhalation of 4.4 x10^{-6} per μg/m^3, while the oral and inhalation carcinogenic risk factors for TCE are 4.6 x10^{-2} per mg/kg-day and 4.1 x10^{-6} per μg/m^3, respectively (USEPA 2000a, 2011). Full dechlorination of TCE is required to prevent the accumulation of VC, and can be achieved with stimulation of the bacteria capable of complete reductive dechlorination.

Evidence of reductive dechlorination through microbial metabolism was first observed in the early 1980s (Environmental Security Technology Certification Program

2005). Originally, only mixed cultures studied could perform dechlorination of tetrachloroethylene (PCE) to VC (Freedman DL 1989). Isolation of specific dechlorinating bacteria began in the mid-1990s (ITRC and RTDF 1999). The first dehalogenating bacteria, was classified around 1984 when a microcosm study was performed on a mixed culture containing the *Desulfmonoile tiedjei* strain DCB-1, and the partial pressure of hydrogen in the headspace was correlated with an increase in cell count and dechlorination rates of 3-chlorobenzoate (El Fantroussi et al. 1998). An increase in dechlorination occurred when providing dechlorinating cultures with hydrogen (Deweerd et al. 1991). In 1997, Professors Gossett, Zinder, and Maymo-Gatell, of Cornell University, were the first to isolate a single strain of bacteria capable of dechlorination of chlorinated solvents such as TCE and PCE (Segelken 1997). The bacterium was isolated from a sludge sample taken from a wastewater treatment plant in Ithaca, NY, when chlorinated solvent waste was treated in the same manner as municipal sewage. The dehalorespiring coccoid bacteria, which functions under anaerobic conditions, was named *Dehalococcoides ethenogenes* strain 195, now known as *Dehalococcoides mccartyi* (Maymó-Gatell et al. 1997). The culture was isolated from a partially purified enriched culture containing no methanogens nor acetogens, which was dechlorinating PCE to ethene with the use of H_2 as the electron donor (Maymó-Gatell et al. 1997).

As of now, *Dehalococcoides mccartyi (Dhc)* is the only bacterial species known to be capable of complete dechlorination through dehalorespiration, reducing TCE to ethene (Lee et al. 2013). Each strain has reductive dehalogenase (RDase) genes enabling it to use chlorinated solvents as terminal electron acceptors during respiration. Each strain of *D. mccartyi* contains at least one of the RDase genes (Table 3), and the type of gene dictates

which chlorinated compound the bacteria can dehalogenate. The RDase genes include *pceA, tceA, mbrA, bvcA*, and *vcrA*. With *tceA,* strains can only degrade the byproduct DCE cometabolically to VC, and to the final product ethene (Lee et al. 2013). Strains containing the vinyl chloride reductase gene (*vcrA*) such as GT and VS, along with the strain BAV1 containing *bvcA*, can continue to dehalogenate from DCE to VC, and VC to ethene gas (Mészáros et al. 2013).

Table 3. Reductive Dehalogenase Functional Genes (Löffler et al. 2013)

Functional Gene	Dechlorinating Process
pceA	PCE → TCE
tceA	TCE → VC, TCE → DCE, limited amount of DCE → VC and VC → Ethene via cometabolism
bvcA	DCE → VC → Ethene
vcrA	DCE → VC → Ethene

Biochemistry

Fermentation of a carbon source creates low molecular weight organic acids and provides an electron donor, hydrogen, and the simple carbon, acetate. As TCE dechlorination occurs, and hydrogen replaces chloride, the system has the potential to become more acidic with the release of chloride and formation of hydrochloric acid. Early observations of reductive dehalogenation of chlorinated solvents found that the moles of hydrogen consumed are equivalent to the number of moles of chlorine released (Maymó-Gatell et al. 1997). The pH can be buffered by inorganic carbon produced during

metabolism of the carbon source, but the alkalinity may need to be stabilized with addition of bicarbonate (McCarty et al. 2007). TCE dechlorination is most efficient between pH 6.5 and 7.5 (Lacroix et al. 2014). Studies have shown that during the process of fermentation and dechlorination, with the production and accumulation of acetic acid and hydrochloric acid the pH can drop significantly, hindering the rate of dechlorination (Lacroix et al. 2014, McCarty et al. 2007). Equations 1 to 4 show the HCl production potential from TCE reductive dechlorination.

$CHCl=CCl_2 + H_2 \rightarrow CHCl=CHCl + HCl$ (1)

$CHCl=CHCl + H_2 \rightarrow CH_2=CHCl + HCl$ (2)

$CH_2=CHCl + H_2 \rightarrow CH_2=CH_2 + HCl$ (3)

Net Reaction: $CCl=CCl_2 + 3H_2 \rightarrow CH_2=CH_2 + 3HCl$ (4)

The use of simple forms of carbon, such as acetate, may prevent the system from accumulating acid (McCarty et al. 2007). Changes in alkalinity have been compared between treatments containing different electron donor sources. The buffering capacity of electron donors including formate, lactate, hydrogen, ethanol, triolein, and glucose were compared by McCarty et al. (2007). After dechlorinating 10 mM of TCE in a system containing an initial concentration of bicarbonate of 800 mg/L, accumulation of acetic acid occurred in treatments containing lactate, ethanol, triolein, and glucose (McCarty et al. 2007). The only treatment able to maintain the initial bicarbonate concentration was formate, with about half of the bicarbonate content consumed in the lactate treatment (McCarty et al. 2007). A carbon source, such as formate or lactate, will provide hydrogen as the electron donor and

buffering capacity to counteract acid production by producing sodium bicarbonate when metabolized (McCarty et al. 2007). The chloride released into the system can be tracked to determine the number of moles of chlorinated solvent being consumed. It is also important to monitor and maintain a neutral pH to promote dechlorination.

Different carbon sources also produce different amounts of hydrogen, and can result in changes in the microbial community, which would affect the rate and extent of dechlorination. In the following example, Equations 5 to 7, lactate is fermented to produce acetate and hydrogen, which can be easily consumed by *D. mccartyi* (McCarty et al. 2007). With lactate, 2 moles of hydrogen gas are produced, which are then used by *D. mccartyi* as an electron donor.

$C_3H_5O_3^-$ (lactate) + $2H_2O \rightarrow C_2H_3O_2^-$ (acetate) + HCO_3^- + H^+ + $2H_2$ (5)

$HCO_3^- + H^+ \rightarrow CO_2 + H_2O$ (6)

Net Reaction: $C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + CO_2 + H_2O + 2H_2$ (7)

Dhc has complex growth requirements. These bacteria require acetate as the carbon source, hydrogen as the electron donor, nutrients, and cobalamins (vitamin B12) (Men et al. 2012). The availability of synthesized cobalamins has been observed as a limiting growth factor, as *Dhc* is unable to biosynthesize the corrin ring of cobalamin (Men et al. 2013). Cobalamin is necessary for reductive dehalogenase genes, and must be met through biosynthesis completed by other bacteria. *Dhc* growth is more robust when it is present in mixed cultures since other fermenting bacteria can break down carbon source providing it with hydrogen and cobalamins (Men et al. 2012). Although *Dhc* growth increases when in

mixed cultures, competition with methanogens can inhibit dechlorination as hydrogen is also consumed during methane production (Men et al. 2013). Dechlorination can occur at hydrogen levels up to ten times lower than required by the methanogens (USEPA 2000) (Table 4). A slow release of hydrogen may inhibit the growth of methanogens, but a large supply may allow for simultaneous consumption of different electron acceptors. A carbon source that can support a diverse community, providing a continuous supply of hydrogen and provide the vitamins and nutrients necessary for efficient dechlorination, may reduce the amount of competition for the electron donor.

Table 4. Hydrogen concentrations required for redox processes (Parsons Corporation 2004)

Terminal Electron Accepting Process	**Dissolved Hydrogen Concentration**	
	nmol/L	**atm**
Denitrification and Manganese Reduction	<0.1	$< 1.3 \times 10^{-7}$
Iron (III) Reduction	0.2 - 0.8	$0.26 - 1.0 \times 10^{-3}$
Sulfate Reduction	1.0 - 4.0	$1.3 - 5.0 \times 10^{-3}$
Methanogenesis	5.0 -20.0	$63 - 250 \times 10^{-2}$
Reductive Dechlorination	2.0 - 11.0	$2.6 - 125 \times 10^{-2}$

Although, only small concentrations of hydrogen are required for reductive dechlorination, column studies performed by Ballapragada et al. (1997) have reported high concentrations of hydrogen with the addition of lactate. When adding lactate at a concentration of 5.5 mmol/L (16.5 mmol-C/L), the partial pressure of hydrogen peaked at levels greater than 1,000 ppm, which equates to liquid concentrations greater than 743 nmol/L (K_H=0.000743 mol L^{-1} atm^{-1} @ 35°C). With the addition of 650 mg-C/L (54.17 mmol-C/L) hydrogen concentrations in columns could reach an estimated 3,200 ppm, or

2,723.3 nmol/L. Another study performed by Men et al. (2014), analyzing TCE reduction in a defined lactate-fermenting culture in serum bottles, measured hydrogen being produced at concentrations as high as approximately 0.8 mmol of H_2/100 mL of medium with the addition of 0.7 mmol of lactate (252 mg-C/L). As indicated above, by providing such high concentrations of carbon substrate, it is expected that enough hydrogen is produced to support simultaneous redox processes.

Biogeochemistry

Dechlorination most often occurs when the system has reached a redox state below the point of sulfate reduction. A redox potential of -360 mV has been observed to be efficient for dechlorination (Ahsanul Islam et al. 2010). Studies have shown that a redox potential above -110 mV will inhibit dechlorination (Löffler et al. 2013). When a continuous dose of carbon is supplied to a microbial community, biogeochemical changes are expected to follow the theoretical sequence shown in Figure 3. Based on the theoretical redox sequence, a depletion in oxygen will occur as the carbon is aerobically degraded. The following terminal electron acceptors (TEAs) are then consumed, nitrate and iron, resulting in the production of N_2 gas and an increase in dissolved Fe (II). More energetically preferred TEAs are consumed and the microbial community will shift towards the use of sulfate and CO_2 during methanogenesis. The use of TCE as a terminal electron acceptor begins to occur under sulfate reducing and methanogenic conditions. The availability of other electron acceptors inhibits TCE reduction, but with enriched cultures containing a diverse community, reduction of compounds such as Fe(III) and TCE may occur simultaneously (McLean et al. 2015). As stated previously, depending on the iron mineralogy, iron

reduction can promote or inhibit TCE reduction. Iron reduction may present completion for hydrogen, but the formation of compounds with reduced iron, such as FeS have been shown to facilitate abiotic TCE reduction.

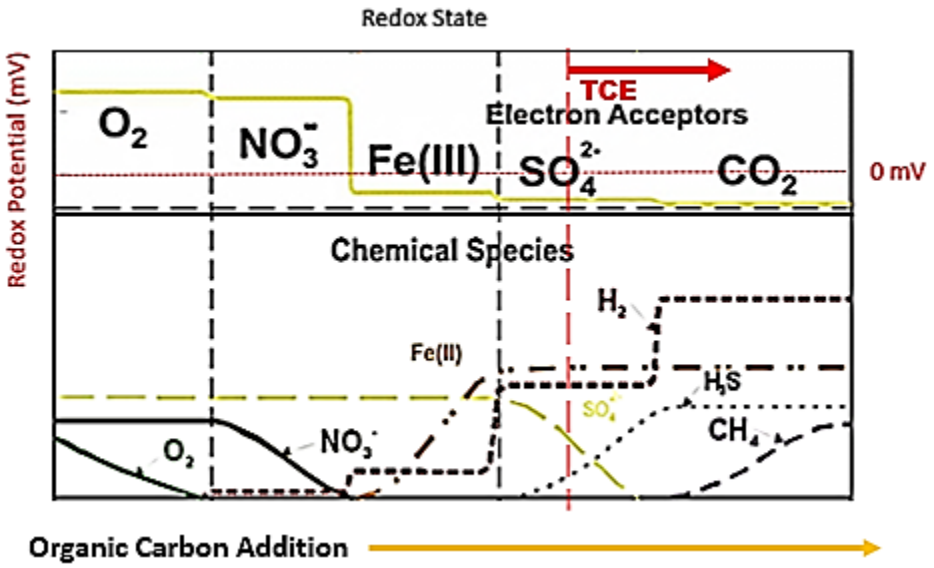


Figure 3. Theoretical redox sequence (Parsons Corporation 2004)

Biostimulation

Bacteria capable of reductive dechlorination can be found at 90% of the sites contaminated with chlorinated solvents, and 75% of these sites have bacteria capable of complete dehalogenation (ITRC 1999). Even though many sites do host dechlorinating bacteria, TCE degradation may not be achieved because proper energy and geochemical conditions are not provided for required metabolic processes to occur. The redox state within an aquifer drives the composition and overall activity of the indigenous microbial community, and whether or not reductive dechlorination is possible.

During biostimulation a carbon and energy source is added to the system in order to stimulate microbial growth, the consumption of terminal electron acceptors, and to shift the redox state to provide the required geochemical conditions to metabolize TCE. By creating a highly reduced environment more energetically preferable terminal electron acceptors are depleted, enabling the bacteria to use TCE during anaerobic respiration. The addition of a carbon source stimulates a diverse community that supports bacteria capable of TCE degradation.

The type of carbon source used for biostimulation will drive what type of microbial community will grow. Simple carbon sources, such as lactate, are easily fermentable, and will provide electron donor (hydrogen) and acetate. Acetate is a form of carbon that is required for the growth of dechlorinating bacteria (Men et al. 2012). Other studies have shown successful dechlorination with the addition of emulsified oil, a slowly degradable carbon source. Instead of a continuous feed of carbon, the hydrophobic phase of TCE partitions into the emulsified oil and provides a slow release of TCE, energy and the electron donor, hydrogen (Harkness et al. 2012). The type of remediation method also dictates the type of carbon used. Bioreactive barriers require slow degrading carbon sources such as oil and mulch, while easily degradable carbon sources are used in recirculating systems (Stroo et al. 2010).

The initial objective of biostimulation for TCE remediation is to add sufficient carbon to reach a low redox state, where TCE can be used as an electron acceptor (Stroo et al. 2010). Once a low redox state is reached, the carbon and energy source will maintain microbial growth and metabolism for dechlorination to occur if TCE reducing organisms exist at the site. Finding the carbon source that will improve the efficiency of biological

reductive dechlorination by providing enough electron donor, and supporting a diverse microbial community capable of full dechlorination, has presented a challenge (Schneidewind et al. 2014).

Bioaugmentation

In order to achieve full dechlorination in a short timeframe, sites can be augmented with dechlorinating bacteria. Bioaugmentation is the process of introducing a microbial community, which has the ability to metabolize a specified contaminant, to a site to achieve more rapid and complete degradation and remediation than can be provided by the native microbial community. Typically, microorganisms are collected from a site where degradation of the contaminant is occurring, and the microbes in the collected sample are grown in the lab to produce an enriched culture, which is then used for bioaugmentation. Successful enrichment cultures, now used for bioaugmentation at chlorinated solvent sites have been collected from sites such as the Department of Energy site Pinellas (Largo, Florida), an industrial site located in Victoria, Texas, a wastewater treatment plant located in Ithaca, NY, and at a contaminated aquifer at the Bachman Road site located in Oscoda, Michigan (Parsons Corporation 2004).

During the process of bioaugmentation for TCE remediation, a mixed culture is added to a contaminated aquifer to increase and support the growth of dechlorinating bacteria. Biostimulation alone can take a long period of time to develop a dechlorinating community and achieve full dechlorination. As shown in field and laboratory studies, dechlorination through biostimulation can take years to be achieved. During a continuous flow through column study performed by McLean et al. (2015), full dechlorination of TCE

was not observed until 5.5 years after biostimulation. Biostimulation without bioaugmentation under some site conditions can also result in the accumulation of cis-DCE or VC when microorganisms required for cis-DCE and VC transformation are not active at a site (Ellis et al. 2000; Major et al. 2002; McLean et al. 2015). Through bioaugmentation a diverse community is added to assist in the transformation of TCE by breaking down carbon and nutrients to simple forms of energy and vitamins that can be used by dechlorinating bacteria, and by increasing the population of *Dhc* available for TCE transformation.

Field Studies

A study performed at Kelly Air Force Base, Texas, observed TCE reduction only to the extent of cis-DCE during a period of 6 months after biostimulation, the aquifer was then bioaugmented (Major et al. 2002). Once the site was bioaugmented, dechlorination to ethene was detected within 72 days after augmentation, and full dechlorination occurred 142 days after inoculation (Major et al. 2002).

A field study reported by Fowler and Reinauer (2013) was completed in 2006 at an aquifer located by a manufacturing facility in Oregon which contained 150 μg/L of TCE and small amounts of PCE and DCE. During this study sodium lactate was added in two injections. During the period of the first injection, approximately 4 months, the aquifer contained a total organic carbon (TOC) concentration of 4,790 mg/L, and a decrease in the concentration of TCE and degradation products was observed, but recovered to the original concentrations 3 months after the injection. Sulfate concentrations were only slightly lower than initial values, and only a small amount of methane was produced. There also was not a significant increase in sulfate reducing or methane producing bacteria. The second injection

of sodium lactate contained the same TOC concentration, but was injected with the addition of nitrogen, phosphate, and yeast extract. In the period of 1 month following the lactate and nutrient addition, TCE and PCE had been fully dechlorinated to ethene, and within 2 months the microbial population had increased from the value of 5,000 colony forming units per milliliter (cfu/mL), observed after the first injection, to 100,000 cfu/mL. Based on this study, it is important that the microbial community is supplied nutrients, along with a carbon and energy source, to achieve full dechlorination.

At a site contaminated with TCE located at Fort Lewis in WA, the aquifer was biostimulated with monthly injections of whey (10-13% protein and 70-75% lactose) at a dose of 10 g/L and 100 g/L at two plots (Lee et al. 2011). After biostimulation an increase in DCE production was observed, but VC and ethene production did not occur until after bioaugmentation (Lee et al. 2008). After bioaugmentation, rRNA genes associated with dechlorinating bacteria increased by three orders of magnitude in 1 year, and rRNA genes associated with dechlorination of DCE and VC to ethene increased between 2 and 19 orders of magnitude at the site receiving 10 g/L and 100 g/L of whey, respectively (Lee et al. 2008). Through bioaugmentation and biostimulation with a carbon, energy, and nutrient source, successful bioremediation of TCE can be achieved, but the necessary biogeochemical conditions that allow for reductive dechlorination must be met.

Laboratory Studies

Whey has proven to be an effective carbon source for biostimulation. Due to its complexity compared to other simple carbon sources, it is able to provide proteins, electron donors, and nutrients that support a diverse community and the consumption of many

terminal electron acceptors (Lee et al. 2011). The column study performed and investigated by McLean et al. (2015) and Mirza et al. (2015) evaluated the impact of biostimulation and the combination of biostimulation with bioaugmentation using different carbon sources, including whey (concentration of 1,000 mg /L on a continuous basis) and two formulations of Newman Zone® (standard surfactant emulsified oil (EOL) and nonionic surfactant emulsified oil (EOLN), on TCE degradation and the microbial community. Both of the Newman Zone® emulsions consisted of 4% lactate and 46% soybean oil, and provided a slow release of electron donor to simulate a reactive barrier treatment scenario. With the combination of lactate and oil, a quick release of carbon from lactate would stimulate the microbial community, and the oil would provide a slow release of carbon allowing for an extended supply of electron donor to maintain the community.

Biostimulation of contaminated aquifer soil was carried out by providing a carbon source to continuous flow-through columns (15 cm diameter by 200 cm length), which were packed with soil from Hill AFB site OU5, and were continuously fed groundwater from OU5. The groundwater contained TCE (10 mg/L) and a carbon source, which was added continuously in the form of whey, or in increments as the emulsified oils. The effect of biostimulation with bioaugmentation was determined by inoculating a set of columns receiving each carbon type with the Bachman Road culture, where a bioaugmented control received inoculation but no carbon. Bioaugmentation was performed with an enriched culture containing *Dehalococcoides (Dhc)*, the derived Bachman Road culture (DBR), which was a subculture of an established dechlorinating microbial community that originated from an aquifer where complete PCE reduction was occurring (He et al. 2003). Carbon sources along with a control column, receiving groundwater without carbon addition, were compared to

determine which electron donor addition would result in the most optimal biogeochemical conditions and microbial community for efficient reductive dehalogenation of TCE.

These columns operated for 7.5 years. At approximately 4.5 years into the study, columns that were biostimulated with whey and bioaugmented were fully dechlorinating TCE to ethene. Columns that were only biostimulated with whey were fully dechlorinating at about 5.5 years into the study, and at the end of the 7.5-year period, the columns receiving the emulsified oils were dechlorinating only to the point of cis-DCE or VC. Whey was found to be a more effective electron donor than the emulsified oil treatments, which may have been a result of the proteins and nutrients it contained. The 16S gene for *Dhc* was present in all treatments except for the control, non-bioaugmented columns. The concentrations of *Dhc* and the reductase genes *tceA* and *bvcA* did not correlate with the extent of TCE dechlorination, but the concentration of vcrA was associated with full TCE dechlorination.

Field Site History and Characteristics

The aquifer material used in the study conducted by McLean et al. (2015) and the current study was collected from Hill AFB, located in Ogden, UT. The Hill AFB site is divided into 12 different plumes that are characterized as operational units (OU). In 1987 Hill AFB was listed on the USEPA's National Priorities List (NPL). The NPL includes sites that are contaminated and pose a threat to human health or the environment. In 1991 a Federal Facility Agreement (FFA) was established with EPA Region 8 and the Utah Department of Environmental Quality for implementing response procedures, which are described by CERCLA (CH2M Hill 2008). Before the Superfund Record of Decision (ROD) was decided upon, remediation plans for OU5 included three phases consisting of the

implementation of an Aeration Curtain, Groundwater Extraction System, which proved to be inefficient, and a Groundwater Containment System (USEPA 2008). In 2006 an ROD was signed and the Aeration Curtain and Groundwater Containment System continued operation. The OU5 plume extends under the cities of Sunset and Clinton and contained TCE concentrations up to 1,000 µg/L (Figure 4). The goal set by the 2008 Five-Year review was to reduce this TCE concentration to meet the drinking water maximum contaminant level (MCL) of 5 µg/L. Groundwater monitoring confirmed that the Groundwater Extraction System was reducing TCE to concentrations below 10 µg/L downgradient of the system, but still did not meet the goal of 5 µg/L (USEPA 2008). The next CERCLA 5-year report was to be completed in 2013, but remains unpublished.

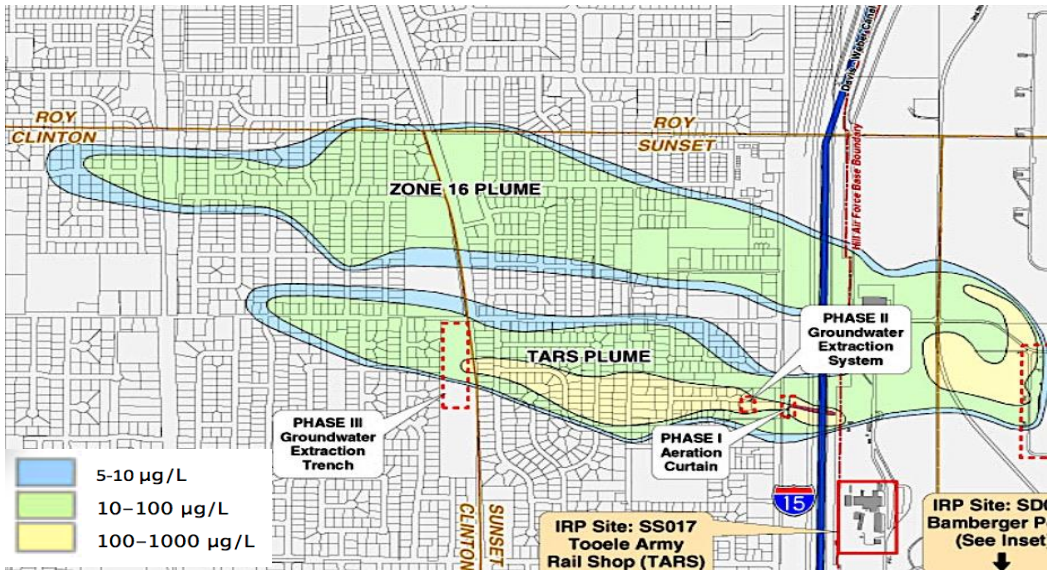


Figure 4. Site features for OU-5 TCE plume in Weber and Davis County from Hill AFB. (URS Corporation 2003)

Summary

Bioremediation can be an effective technique for remediating aquifer material contaminated with TCE. Once the feasibility of biostimulation and bioaugmentation is confirmed by characterizing the biogeochemical conditions, including redox state and investigating the potential change in surrounding mineralogy, the following processes associated with the technique have little operation and maintenance requirements. Also, compared with chemical or physical treatment methods, bioremediation is less energy intensive and produces less waste.

The purpose of this study is to improve the process of bioremediation by investigating the effect of different carbon types applied during biostimulation and bioaugmentation in order to determine which energy source would result in rapid and complete TCE dechlorination. This study was based on findings of the long-term column study performed by McLean et al (2015), and analyzed the changes in biogeochemistry and the microbial community over time during TCE dechlorination. The comparison of biogeochemical characteristics included evaluating constituents associated with changes in redox state, such as the depletion of terminal electron acceptors and redox potential, along with changes in iron mineralogy, which could result from biostimulation. Monitoring the production of metabolites, included analyzing for low molecular weight organic acids (LMWOAs), hydrogen and inorganic carbon, and the transformation of TCE and its degradation byproducts. The information collected through these analyses assist in determining the characteristics that may have supported or hindered *Dhc* metabolism, such as acetate and hydrogen production, or competition for the electron donor with other terminal electron acceptors, such as iron. The impact of carbon source on the concentration

of *Dhc* (16S) and the genes associated with TCE reduction (*tceA, vcrA, and bvcA*) were also evaluated throughout the study. Based on the data collected while monitoring the changes in biogeochemistry, microbial community, and TCE degradation, the impact of each carbon source on efficient TCE degradation was determined.

MATERIALS AND METHODS

Experimental Design

A continuous flow-through column study was performed to analyze the effect of different carbon sources on biogeochemistry, microbial community composition, and the resulting rate and extent of TCE degradation during the process of biostimulation and bioaugmentation of aquifer material. During this study, glass columns were packed with aquifer soil and operated in a closed system under anaerobic conditions. In total there were 36 columns, which were divided into three treatments. Within each treatment, columns were sampled in triplicate. One treatment received a complex carbon source, whey, another treatment received a simple carbon source, lactate, and the third treatment served as the control, and received no carbon. All columns received a continuous flow of groundwater containing TCE, nutrients, and the carbon type associated with each treatment. The column effluent was analyzed twice a week, and as TCE was reduced to the sequential degradation byproducts, DCE, VC, and ethene, columns were sacrificed from each treatment in triplicate.

The experiment was designed to operate under conditions close to those in the field. Columns were packed with aquifer soil collected from OU5 located in Clinton, UT. All columns received a constant flow of groundwater that was collected from OU5. The groundwater was spiked to a nominal TCE concentration of 10 mg/L. Groundwater was pumped through each column at the same rate of 9 cm/day (0.086 mL/min ≈ 125 mL/d) to match the groundwater flow at OU5, as described in McLean et al. (2015).

Once the columns were assembled, groundwater (without carbon or TCE) was pumped through the system until flows stabilized and effluent volumes were 125 mL/day. A bromide tracer study (Appendix A) was performed to ensure all columns were operating at the same flow rate with minimal short circuiting, channeling, or accumulation. The process of stabilizing the flow rates took approximately 4 months, and during this time the columns received a continuous flow of only groundwater. If flow issues did occur, the columns were repacked. Once the flow rates were established, the carbon feed solutions associated with each treatment were applied, and monitoring of electron acceptors began. Upon feed application the theoretical sequential consumption of terminal electron acceptors occurs. Once the dissolved oxygen (DO) was depleted the Fe (III) was converted to Fe (II). Once SO_4 was reduced, the redox state is at a level that TCE can be used as a terminal electron acceptor. At the stage of iron reduction, the first sacrifice took place to determine the biogeochemical conditions prior to inoculation, and to gain information for a concurrent experiment.

When the redox state reached sulfate reducing conditions, the remaining columns of each treatment were inoculated by injection with the mixed culture containing *Dhc*. The following three column sacrifices occurred when TCE was being reduced to DCE, VC, and finally ethene. Sacrifices occurred when all columns from at least one treatment were operating at the desired chlorine number by producing the desired degradation byproduct.

Twice a week metabolites, as well as hydrogen production, carbon use and transformation, gas production, terminal electron concentrations, and TCE degradation byproducts were monitored from triplicate samples of each treatment. Upon each sacrifice, the biogeochemistry, along with the microbial community stimulated by each treatment,

were determined to develop a more complete understanding of the conditions created by the application of these two carbon donor types.

Column Construction

Column Materials

The glass columns (Prism Glass, PRG-8000-71) were 7.62 cm high with 7.62 cm diameters. The bottom and top caps of the columns were made of glass, and had 0.32 cm (1/8") diameter openings for the influent and effluent flow. The caps held approximately 50 mL, and were filled with clean, dry sand to hold the soil column in place. Any sorption that occurs between TCE and the sand was assumed to reach a maximum and no longer be a sink by the time of inoculation. Steel couplings with rubber gaskets and Teflon seals were used to hold the glass caps and columns together.

Soil Preparation and Packing

Soil cores were taken from the aquifer located at OU 5 on December 2, 2013. By using a Geoprobe® Direct Push Subsurface Sampling Technology, 1.52 m long by 5.08 cm cores were collected. Cores were taken from approximately 0.76 m above to 0.76 m below the water table in order to capture the water table-aquifer interface profile. Using an electronic water level meter, the water table depth was measured from surrounding sample wells. The water table was at a depth of 3.66 m below ground surface. At each coring location, the first two 1.52 m cores were discarded and the third was collected in order to reach the water table. When removing the cores from each location, the first two were

visibly dry, and the third was moist. All cores were collected from 3.05 to 4.57 m below the surface. The 1.52 m long cores were contained in plastic sleeves. A total of 11 cores were collected, enough volume to fill all 36 columns at a density of 1.6 g/cm^3 and to perform the required sample analyses. The cores were capped, sealed, and transported on ice back the Utah Water Research Laboratory. The cores were then refrigerated at 4°C in order to limit changes in the soil biogeochemistry from occurring while the cores were enclosed in the plastic sleeve without circulation or air awaiting further processing. The cores were removed from the fridge, cut open, mixed and laid out to dry at room temperature (~23°C)

Once the soil was dried, all cores were mixed, and sieved through a 2 mm sieve. The soil was then homogenized and samples were collected for analysis. Soil samples were sent to the Utah State University Analytical Laboratory for analysis including a complete farmer's panel (pH, EC, plant available PO4-P, K, NO_3-N, NH4-N, Zn, Fe, Cu, Mn, SO_4-S, and organic matter), inorganic carbon ($CaCO_3$ Equivalent), cation exchange capacity (includes cation exchange capacity, NH_4OAc extractable cations, water-soluble cations, water saturation percent), particle size distribution by hydrometer, and total elemental composition (EPA 3050 digestion and ICP emission analysis). The soil had a sandy loam texture, with 0.24% organic carbon (Table 5). Select elements that make up nutrients found in the soil are presented in Table 6. The concentration or iron and sulfur present in the soil could influence rate and extent of TCE degradation if present in the form of terminal electron acceptors.

Table 5. Composition of OU5 Soil

Parameter	Value
pH (Standard Units)	8.3
EC (dS/m)	1.46
Organic Carbon (%)	0.24
Sand (%)	61
Silt (%)	26
Clay (%)	13
CEC (cmol/kg)	19.1
$CaCO_3$ (%)	8.7

Table 6. Select Soil Elemental Constituents (mg/kg)

Iron	Phosphorus	Sulfur
9,800	700	100

Column Assembly and Operation

The columns were assembled first with a bottom cap and coupling. The inlets of the caps were packed with glass wool. Washed and dried sand, sifted to a diameter of between 0.8 to 0.2 mm, was used to fill the bottom cap. In order to pack the soil at a density of 1.6 g/cm^3, 556 g of soil were mixed with 111.2 g of water (20% moisture content on a dry basis). The wet soil was packed using a plunger made of a wooden rod and a plastic base with a diameter of 7.62 cm. The columns were then packed in 1 cm intervals to avoid air pockets forming during construction. Once the entire column was packed, the top cap was assembled with a coupling, sand was funneled in through the top port, and glass wool was

used to fill the outlet tube, preventing sand from escaping the cap during operation. The columns received groundwater for approximately 4 months prior to carbon addition.

The feed solutions were contained in 3 L SKC FlexFoil bags (SKC Fullerton, CA), suitable for use with TCE, which have stainless steel fittings. Each feed solution (whey, lactate, control) was fed through a separate manifold system connected by 76.2 cm (2') long 3.175 mm (1/8") diameter Viton tubing. The solution was supplied at a rate of ≈ 125 mL/day (0.086 mL/min) to each column, which mimicked the flow of the aquifer. The flow from each bag (initially 1,500 mL/day) was split 12 ways (≈ 125 mL/day) through a stainless steel manifold constructed of 1.588 mm (1/16 ") tees and cross fittings. From the manifold, 12 sets of Viton tubing (1.588 mm (1/16") ID, Cole Parmer) carried the solution through a multichannel peristaltic pump using Size 13 Viton Masterflex peristaltic pump tubing, and flowed upward to the 12 columns.

As shown in Figure 5, the columns were fed bottom up to facilitate gas movement through the soil during filling and flow through operation. The column effluent flowed out the glass top through Swagelok reducers containing a septa and needle, leading to Viton tubing, which carried the flow into a 250 mL effluent jar via an effluent needle that passed through a 58 mm septa lid (Fisher Scientific, Santa Clara, California). Another needle exited through the septa and led to a gas collection bag via more Viton tubing. Gas produced from microbial activity occurring within the columns was collected in 1 L SKC Tedlar gas collection bags prior to volume measurement and component analysis.

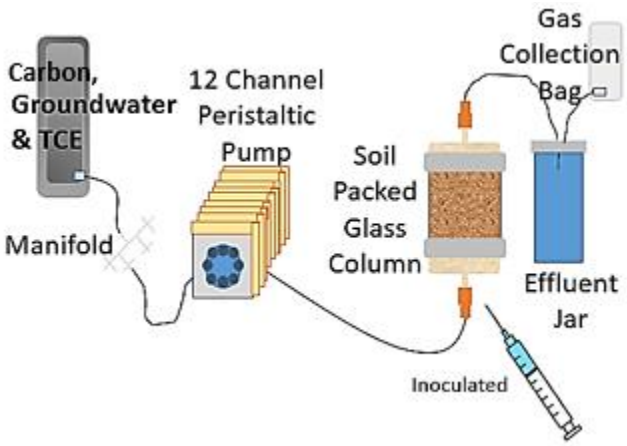


Figure 5. Experimental Setup

Figure 6 displays the column setup with collection jars and gas collection bags. The jars were exchanged daily and effluent mass was recorded in order to calculate flow rates. In Figure 6, sulfate reduction had occurred and sulfides had discolored the effluent, creating a dark tinge to the collected effluent liquid as well as portions of the soil columns.

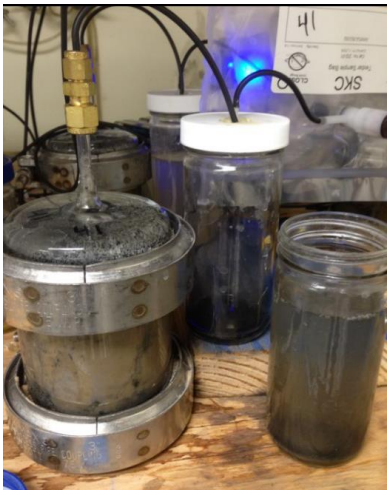


Figure 6. Glass column with collection vials and gas bag.

Feed Solutions

Groundwater

The groundwater used in the study was collected from Hill Air Force Base at OU5. The constituent concentrations were measured to determine background levels of pH and EC, anions, organic and inorganic carbon (Table 7). The groundwater was stored in the fridge (≈4°C) until use. Each carboy of groundwater was purged with nitrogen under a hood for 20 minutes to lower the oxygen concentrations and remove any volatile compounds (TCE and degradation products) prior to use, and groundwater characteristics were determined. When preparing the feed solution, the water was funneled into a feed bag and sealed while avoiding any headspace from forming in the bag.

Table 7. OU5 Groundwater Characteristics

Parameter	Value
pH	7.64
EC (mS/cm)	1.4
Eh (mv)	197
Chloride (mg/L)	178
Nitrate-N (mg/L)	7.7
Sulfate (mg/L)	75.0
Alkalinity (mg $CaCO_3$/L)	987
Dissolved Organic Carbon (mg C/L)	6.8

Carbon Sources

All 36 columns received a continuous flow of groundwater containing ≈ 10 mg/L of TCE, which was the concentration initially measured in the plume at OU5 and used in the prior column study performed by McLean et al. 2015, along with nutrients (nitrogen and phosphorus). Of the 36 columns, 12 received 650 ± 60 mg-C/L from whey, while one set of 12 columns received 650 ± 60 mg-C/L of calcium lactate pentahydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$), which is a simple, easily fermentable carbon source (Schneidewind et al. 2014) The remaining 12 columns were operated as control columns, receiving only groundwater with TCE and nutrients. Whey powder was purchased from Barry Farm located in Wapakoneta, OH, and was analyzed for available phosphorus (Olsen $NaHCO_3$ Method), Ammonia-N and Nitrate-N (2M KCl extraction), total nitrogen by combustion (LECO Instrument) at the Utah State Analytical Lab, and total organic carbon (combustion with CO_2 analyzer) performed at Utah State University Soil Chemistry and Biogeochemistry Laboratory.

The whey used in this study contains 1.7% nitrogen, 0.026 % as NH_4-N, and 0.002 % NO_3-N, 41.67% organic carbon, and 434 mg-P/kg (Table 8). In order to eliminate the possibility that nutrients drive the difference in the rates and extent of dehalogenation, nutrients were added to all non- whey columns. All columns receiving calcium lactate, along with the controls, received phosphorus and nitrogen at a continuous dose that matched the calculated concentrations in the whey treatments, which was based on the 1.7% nitrogen and 434 mg-P/kg. The control and calcium lactate columns received 40.8 mg-N/L as NH_4Cl and 4.57 mg-P/L as KH_2PO_4. The nitrogen source (NH_4Cl) was chosen to avoid adding additional electron acceptors, such as nitrate, which could reduce the rate of dehalogenation. The added phosphorus (KH_2PO_4) and nitrogen source (NH_4Cl) were added because they

were also used in the media to prepare the DBR culture. It was assumed that the nutrients bound in the proteins of whey are easily degradable, and the nutrients provided in the whey or with the lactate was not a limiting factor to the progress of reductive dechlorination taking place within the reactors.

Table 8. Whey Composition (USU Analytical Laboratory, Logan UT and USU Soil Chemistry and Biochemistry Lab)

Phosphorus (mg/kg)	Ammonium-N (mg/kg)	Nitrate-N (mg/kg)	Organic Matter (%)	Total N (%)	TOC (%)
434	261	20.2	92.2	1.71	41.67

Calcium lactate penta-hydrate ($C_6H_{10}CaO_6 \cdot 5H_2O$) was purchased from Fischer Scientific. It is made up of 23.4% organic carbon, based on its molecular formula.

Bioaugmentation

The culture containing *Dhc* was originally from the Bachman Road site in Michigan, and has been grown at the Utah Water Research Lab for the past 10 years. The original DBR contained bacteria species *Anaeromyxobacter dehalogenans, Desulfobulbus sp., Desulfitobacterium sp., Geobacter sp.* and bacteria related to *Chlorobium sp.* as well as the dechlorinators, *Dehalococcoides mccartyi* strains (Zhou 2008). The culture has been fed lactic acid and TCE and has been monitored throughout numerous dehalogenation cycles to ensure the culture's continued viability. The DBR culture maintained prior to the study in a batch system, and was performing full dechlorination prior to inoculating the columns (Figure 7).

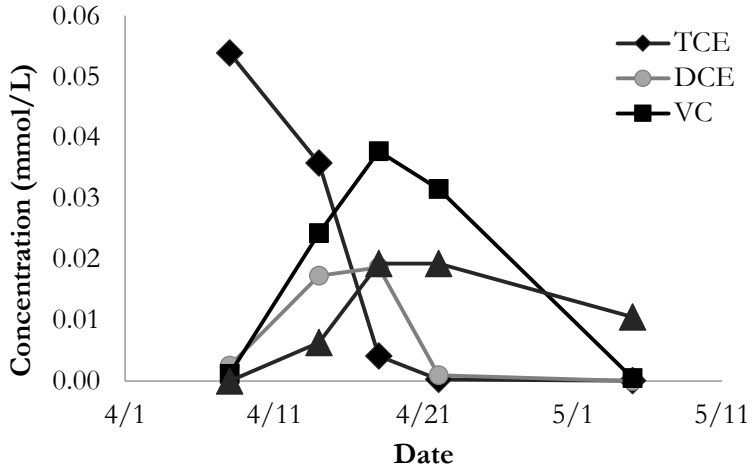


Figure 7. TCE degradation sequence of derived Bachman Road culture measured 4/6 to 5/6/2014.

The culture was injected at a volume of 10% of the pore space within each column. About 500 mL were taken from the original culture and placed into an anaerobic glove bag. In the glove bag, volumes of 10 mL were injected into individual Thermo Scientific™ Gas chromatography headspace vials and sealed to prevent oxygen exposure. Outside of the glove bag, the 10 mL were taken from the vial using a sterile 10 mL syringe and injected into the bottom of each column through the influent septum, as shown in (Figure 5.)

Effluent and Sacrifice Sampling

Effluent Sampling Plan

The mass of the effluent was recorded every day to ensure that each column was operating at a rate of 125 mL/day. The samples collected in the effluent jars were analyzed for As (III), Fe (II), low molecular weight organic acids (LMWOAs), DOC, DIC, and NH_4-N and metals, along with pH, EC, ORP, DO, and chloride, nitrate and sulfate. The effluent was also analyzed for hydrogen, TCE and degradation byproducts (VC and DCE) directly

from the effluent needle. Originally, gas samples were collected from Tedlar bags and measured for methane, CO_2, and ethene. Due to insufficient gas collection in the Tedlar bags, later in the study, dissolved gas samples were taken directly from the column effluent. All sampling techniques and analyses are described in detail in Section Sampling and Analytical Methods. Each treatment was sampled twice a week, randomly in triplicates following the order described in Table 9. With this sampling scheme, over the course of 2 weeks all 36 columns were sampled.

Table 9. Sampling Schedule

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Weigh Effluent	Weigh Effluent pH DO Fe(III) As(II) EC Eh (ORP) Metals	Weigh Effluent Anions LMWOAs TCE DOC/DIC Gasses Hydrogen	Weigh Effluent	Weigh Effluent pH DO Fe(III) As(III) EC Eh (ORP) Metals	Weigh Effluent Anions LMWOAs TCE DOC/DIC Gasses Hydrogen	Weigh Effluent

Sacrifice Sampling Plan

The first sacrifice occurred prior to inoculation, during the stage of iron reduction, where Fe(II) was measured in the effluent of the columns. The subsequent sacrifice events occurred based on the chlorine number (N_{Cl}). The second sacrifice occurred when TCE was degraded to DCE in all the columns of one treatment, i.e., when the chlorine number was between 2.5 and 2. The third sacrifice occurred when a chlorine number between 1.5 and 1.0

existed (cis-DCE to VC transformation) for every column of one treatment. The final sacrifice occurred at a chlorine number of 0.5-0, when TCE was converted to ethene within at least one full set of columns.

During each column sacrifice, three randomly selected columns of each treatment were taken down at and their solids and porewater were analyzed. Soil cores were taken from each column and analyzed for genetic material, including DNA for 16S of *Dhc* and functional genes *bvcA*, *tceA*, and *vcrA*. One core was taken and analyzed for TCE and degradation products. Soil porewater was analyzed for total metals, As (III)/As(V), Fe(II), and sulfide, along with anions, low molecular weight organic acids, and ammonia. Porewater characteristics, such as pH, EC, and ORP, were also measured. Soil metals and mineralogy were analyzed at each sacrifice.

Sampling and Analytical Methods

Water

An Ion Chromatograph, Dionex ICS-3000 System (Dionex Corp., Sunnyvale, CA), was used to measure anions and LMWOAs. Procedures from Dionex Application Note 123 (2006) were used. A Dionex IonPac AS11 analytical column, with a Dionex ASRS-ULTRA Anion Self-Regenerating Suppressor, and a Dionex CD20 EC detector were used to determine anion (NO_2-N, NO_3-N, SO_4, PO_4-P) and LMWOA concentrations. Soluble Fe (II) concentrations were determined using the ferrozine procedure of Lovley and Phillips (1986) and colorimetrically measured with Genesys 6 UV-Vis Scanning Spectrophotometer (Thermo Scientific).

ORP and pH were measured using an Accumet Excel XL25 Dual Channel pH/Ion Meter with Standard Method 4500 (APHA et al. 2012). Dissolved oxygen was measured with an Orion Star A223 DO Meter (Thermo Scientific) with Standard Method 4500-O G (APHA 2012). Electrical conductivity was measured using an Accumet Model 30 Conductivity Meter and Accumet 13-620-160 Conductivity Cell with Standard Method 2520 B (APHA et al. 2012). Dissolved inorganic and organic carbon were measured from filtered effluent (0.2 μm) using a Combustion Total Organic Carbon Analyzer (Appollo 9000, Teledyne Tekmar). Standard Method 5310 B (High-Temperature Combustion Method) was used to measure organic carbon. During the process of organic carbon analyses, inorganic carbon is removed by sparging the sample with phosphoric acid addition. The sample was then combusted, and CO_2 is released. The CO_2 produced during combustion or sparging after acidification (inorganic carbon analysis) was detected by a non-dispersive infrared (NDIR) detector. Metals (trace elements) were analyzed using an Agilent 7000x Inductively Coupled Mass Spectrometer (ICP-MS) with USEPA Method 6020 (USEPA 1994).

TCE Sampling

Influent and effluent TCE concentrations were measured using a Hewlett-Packard 6890 GC/5973 MS using a Tekmar 7000HT Headspace Analyzer/Autosampler and procedures from USEPA Methods SW-846 Methods 5021 (1996) and 8260 (1996). Standards from Ultra Scientific® were made from a haloethene mixture including VC, trans-DCE, cis-DCE, and TCE at concentrations of 200 μg/mL and 2,500 μg/mL (Products DWM-520 and CUS-16209), respectively. Standards were diluted with methanol and used to

make a standard curve ranging from concentrations of 0.2 to 2,500 μg/L for VC, trans-DCE, cis-DCE, and TCE.

Standards and samples were prepared in glass Thermo Scientific™ Gas chromatography headspace vials (21 mL). A matrix modifier solution was used for standards and samples, which is a saturated salt solution (350 mg/L NaCl) with a pH of approximately 2 (~ 3 drops of concentrated phosphoric acid). The saturated salt solution reduces the solubility of the gas, and the volatile organic compounds (TCE, DCE, and VC) partition into the headspace of the vials. Calibration and Continuing Calibration Verification (CCV) standards were prepared in GC vials by adding 9 mL of matrix modifier with 1 mL of water and sealed (crimped Thermo Scientific™ black molded butyl septa). Headspace vials used for sampling had 9 mL of matrix modifier added and were sealed, and 1 mL of headspace was removed prior to sampling. Column effluent was sampled by inserting the effluent needle from the column through the septa on the sample vial to collect ~1mL of effluent. Influent samples were taken from 1/16" tees intersecting the influent tubes on three columns of each treatment to obtain an average influent concentration. The collection needle on the tees were pinch closed until influent samples were taken. When sampling the influent, the column influent flow was blocked, allowing the flow to exit through the tee, where a needle was inserted from the line into the sample vial.

Hydrogen Sampling

Hydrogen sample vials were prepared using 10 mL Thermo Scientific™ glass vials, which were filled with 4 mL of matrix modifier and sealed with butyl rubber septa. The sample vials were evacuated (~2 mL) and effluent samples were collected when the vial was

inserted onto the effluent needle. The samples were analyzed using a Shimadzu Gas Chromatograph 14 A with a 300 m x 0.32 ID mol sieve 5A column and a hydrogen ion detector (GC-HID). The GC operated isothermally at 35°C. A Scott™ Gas hydrogen cylinder (Lot # 216PLU4SPC02L) with a concentration of 100 ppm was used to generate standards and a calibration curve. The hydrogen added to vials partitions between the gas and liquid phases, but the analytical response (peak area) is associated with the total amount of hydrogen added to the vial. The samples were collected in a closed system, directly from the effluent tube of the column. The hydrogen from the liquid sample reaches equilibrium in the same manner as the standards, and the hydrogen that partitions from the liquid to the gas phase is measured. Based on the analytical response, the total amount of hydrogen in the vial was determined, which all originated as dissolved hydrogen in the liquid sample and can be described with units of nmol/L.

Gases

Originally, the CO_2, methane, and ethene gasses were sampled from the 1 L Tedlar bag using a 5 mL gas tight syringe, and the volume of gas produced was measured using a 1 L gas tight syringe. Evacuated and sealed (butyl septa) 22 mL GC vials were used to contain 2 mL gas samples. At the end of the study, the gas sampling method was adjusted to analyze dissolved gasses. Dissolved gasses were sampled using the same sampling procedures for TCE and degradation by products. Approximately 1 mL was collected into a 22 mL GC vials containing 9 mL of matrix modifier. The mass of the effluent was measured to determine the dilution factor, and the headspace was analyzed for gasses. The gas samples were analyzed using a Tekmar 7000 Headspace Autosampler and a Schimadzu GC-14 with dual

flame ionization and thermal conductivity detectors. Gas standards of 100% Carbon Dioxide from Air Liquide (UN 1013), 100% Methane (Compressed) (UN 1971) from Praxair Inc. Danbury, CT, and 1% Ethylene (C_2H_2) from Airgas® (Lot# MAO-62N-1%-1) were used to form a calibration curve ranging from 0.045-4.5% CO_2 and CH_4, and 0.002-0.08% Ethene. Gas concentrations were measured twice a week in triplicates from each treatment. Procedures for gas analyses were derived from Bradley and Chapelle (1999) and Smatlak et al. (1996). Gas standards were converted from volume percent to molar concentrations by using the ideal gas law, multiplying percent by the headspace volume, then dividing by the moles of gas which occupies 1 L of volume at the operating temperature and pressure. At standard temperature and pressure 1 mol of gas occupies 22.4 L. At an elevation of 4500 ft and lab temperature of 20°C, pressure is 0.85 atm, resulting in 1 mole occupying 28.37 L.

Solids Processing

Upon sacrifice, columns were disassembled in an anaerobic glove bag. Three cores were taken with autoclaved, sterilized steel borers (0.95 cm ID x 7.65 cm) for DNA extraction. The soil was preserved with LifeGuard™ (Mo Bio Laboratories Inc.) and stored at -70°C until DNA extraction. A core was taken from each column and quickly transferred to a GC vial containing 9 mL of matrix modifier and sealed. The weight of each core was determined based on the initial and final weight of the GC vial with matrix modifier after adding the core. The cores were analyzed with the same method on the GC-MS as used for influent and effluent TCE analysis, and the calibration curve was adjusted to account for the mass of TCE, DCE, and VC in each vial rather than concentration.

The remaining soil was put into a Ziploc bag and homogenized. Homogenized soil was collected for determining the moisture content and for pore water extraction. Soil samples placed on pre-weighed aluminum boats were placed in a 100°C oven for 24 hours, and the change in mass from before and after drying was used to calculate the moisture content. Moisture content was calculated on a dry weight basis, the ratio of mass of water per mass of dry soil. Approximately 40 g of the homogenized soil were placed in 50 mL capacity 0.2μm Ultra Clean® Maxi Plasmid Spin Filters (Mo Bio Laboratories, CA). The samples were spun for 20 minutes at 10,000 rpm to extract the pore water from the soil. The pore water was analyzed for the same constituents as the column effluent as was described in the effluent sampling section, which includes LMWOAs, anions, total elemental metals, Fe (II), ORP, and EC.

After the pore water was extracted from the soil, the centrifuged, homogenized soil was also analyzed for pH and EC, and sampled for total metals, iron minerals, Fe (II), and sulfide. As sulfate is reduced, sulfide will precipitate with metals such as iron sulfide (FeS). Acid volatile sulfides (AVS) were extracted from soil following USEPA Method 821-R-91-100 (1991) and Van Griethuysen et al. (2002). The extraction was done by adding the soil (2-5 g) to a 125 mL glass jar with a Teflon lid, which contained a 25 mL glass jar attached to the top inner wall. Ten mL of sulfide antioxidant buffer (SAOB) was added to the 25 mL jar, and 20 mL of degassed 1 M HCL was added to the soil at the bottom of the jar. The jar was sealed and the soil and acid were mixed with a Teflon stir bar for 3 hours. Hydrogen sulfide was released from the acid and soil mixture and partitioned to the SOAB in the small jar. The sulfide contained in the SAOB was measured using an Orion selective ion probe, Model 9616, following USEPA Method 821-R-91-100 (1991) The 1 M HCl was analyzed for total

elemental metal on an ICP-MS with USEPA Method 6020 (1994). A 0.5 M HCl extraction was performed to determine Fe(II) (Lovley and Phillips 1986), which was compared to the results from the total elemental metals determined by ICP-MS to calculate the amount of Fe(III) present. The iron mineralogy was determined through a sequential extraction following methods from Huang and Kretzschmar (2010), where a series of extractions were performed to determine ligand exchangeable iron, and iron bound to carbonates, Mn oxide minerals, sulfides, amorphous iron, crystalline sulfides, and crystalline oxides. The remaining soil was processed through a microwave acid digestion with nitric acid to release any remaining iron (Modified USEPA Method 3052/3050B). All extracts were analyzed by ICPMS. The soil was also tested for EC and pH (Sparks et al. 1996).

The DNA was extracted from the soil using MoBio PowerLyzer, PowerSoil DNA Isolation Kit. The quantitative real-time PCR was performed using plasmid standards with TaqMan probes containing the genes *vcrA*, *tceA*, and *Dhc* (16S). The qPCR program runs for 10 min at 95˚C and then repeats the following sequence 45 times with 15 sec at 95˚C, 45 sec at 55˚C, and 45 sec at 72˚C, which is then followed by 10 min at 72˚C and the melting curve of 10 min at 57˚C and increases at increments of 0.5˚C, until 97˚C. The gene sequences and further information on the extraction and qPCR procedure can be found in the study performed by Mirza et al. (2015). The method detection limits for the reductase genes *vcrA*, *bvcA*, and *tceA*, are 2.41, 2.25, and 1.56 log copies/g of dry soil, which was determined based on the MDL for the qPCR and applied to the average amount of soil used for DNA extraction.

Mass Balance

Each treatment received 10 mg/L of TCE, which is approximately 0.076 mmol/L. A mass balance was performed to track the molar concentration of chlorinated solvent entering the system, and the degradation byproducts exiting the system. As the molar concentration of TCE decreases, an increase in DCE should occur. As molar concentrations of degradation byproducts decrease, an increase of the same magnitude should occur for the proceeding products, VC and ethene. The total number of moles of TCE and its degradation products should remain constant if there is no loss of mass in the system. Chloride ion was not monitored because the nutrients added to the lactate treatment contained high concentrations of chloride masking the release of chloride into the solution phase due to chlorinated solvent degradation.

Data Analysis

The metabolite data collected were converted to units of moles, to allow each analyte entering the system to be related to the metabolites produced. The gasses CH_4, CO_2, and H_2, originally measured in parts per million, and low molecular weight organic acids, along with TCE and degradation byproducts, which were originally measured in mg/L, were converted to moles/L so constituents entering exiting the system could be compared.

From the data collected on TCE degradation for each treatment, rates were determined for TCE transformation through ethene production. The order of the rate of TCE degradation was initially evaluated by linearizing the average TCE measurements, taken at each sampling period, with respect to time. The transformation that produced the best linear fit for the relationship between concentration and time, during the period when TCE

was degraded, determined the rate order. In the previous column study performed by McLean et al. (2015) and the data analysis performed by Murch (2003), a sequential first order degradation model was used, and first order was found to best fit the degradation of TCE.

A first order degradation rate for TCE was determined based on the hydraulic residence time (HRT) of the column and the effluent TCE and degradation byproduct concentrations. The HRT of each column was adjusted from the original HRT determined during the bromide tracer study to account for changes in flow that occurred throughout the study. The HRT_{Br} determined during the bromide tracer study was multiplied by the flow measured at that time (Q_{Br}), which equates to the pore volume of the column. This product was then divided by the average flow (Q_{avg}) measured prior to the sacrifices of the column ($HRT=(HRT_{Br}*Q_{Br})/Q_{avg}$). A retardation factor ($R=1+ (K_{oc}f_{oc}\ \rho_b)/n$; ($K_{oc}$) = compound organic carbon partition coefficient; f_{oc} = organic carbon content of the soil; n = soil porosity; and ρ_b = the bulk density of the soil) was applied the HRT to account for a reduction in compound flow velocity due to their partitioning to the soil-organic carbon. The sand only made up a small fraction of the column soil surface area, and it was assumed that no net sorption was occurring by the time TCE reduction took place. The soil contained 0.4 % organic matter and a factor of 0.58 was applied to calculate the soil organic carbon content, as described by USDA Natural Resource Conservation Service. The organic carbon partitioning coefficient for TCE was determined by averaging coefficients reported by Chiao et al. 1994, GSI Environmental 2009, Lawrence 2006, and Stroo and Ward 2010, and coefficients for cis-DCE, VC, and ethene were reported by Murch 2003.

Table 10. Organic Carbon Partitioning Coefficient for a Soil Organic Carbon Content of 0.2%

Compound	K_{oc}
TCE	61.45
DCE	43.00
VC	29.50
Ethene	26.90

The degradation of DCE, VC, and the production of ethene were dependent on the changing concentrations of the sequential byproducts and reactants, and the first order degradation rate constants were adjusted until the final estimated concentrations were equal to the measured effluent concentrations at the time of sacrifice. The rate constants were averaged and compared among treatments and sacrifices (Appendix F).

Statistical Analysis

Statistical analyses were performed to evaluate differences among treatments, including dechlorination rates, metabolite concentrations, and biogeochemical characteristics. The statistical software R was used to perform an initial redundancy analysis on data generated from each of the sacrifice events to determine which constituents contributed to the differences in chlorinated solvent concentrations observed in each treatment. Based on significance variables at each sacrifice, correlations between the significant variables and chlorinated solvent concentrations were analyzed. Significant variables were analyzed through an analysis of variance (ANOVA) in order to determine if there was a difference among treatments using a Tukey's honest significant difference (Tukey HSD) test.

RESULTS

Column Effluent Results

Columns operated with flowing groundwater, no TCE or carbon source, for approximately 4 months prior to the start of the study. During this period flows were stabilized, and a bromide tracer study was performed. Based on the bromide tracer study (Appendix A), the hydraulic retention times (mean ± 95% CI) of the control, lactate, and whey treatments were initially 30.7 ± 1.1 hr, 30.4 ± 4.0 hr, and 25.2 ± 1.1 hr, respectively. The HRT for columns at each sacrifice was determined based on changes in flow. Over the course of the study, the lactate treatment had an average flow of 113 ± 4.1 mL/day, whey operated at an average of 114.5 ± 5.1 mL/day, and the control columns had an average of 102.9 ± 5.8 mL/day (Figure 8a). Once the flow rates were established, the respective feed solutions were added to the groundwater of each treatment. On Day 0, the carbon sources were added and data collection began. Lactate and whey treatments received an average carbon concentration of 650 ± 54 mg-C/L. All treatments received an average TCE concentration of 0.076 ± 0.003 mmol/L (10.03 ± 0.42 mg/L) (Figure 8b). Fluctuations in the flow and HRT of each treatment occurred throughout the study due worn tubing, tubing replacements, and biomass buildup.

After the carbon sources were added to the groundwater feed solutions, the oxidation/reduction potential (ORP) dropped, and reducing conditions were observed in the lactate and whey treatments by Day 20 (Figure 9). During the inoculation process the ORP of the control columns dropped below 0 mV for only one sampling event, at the point of inoculation.

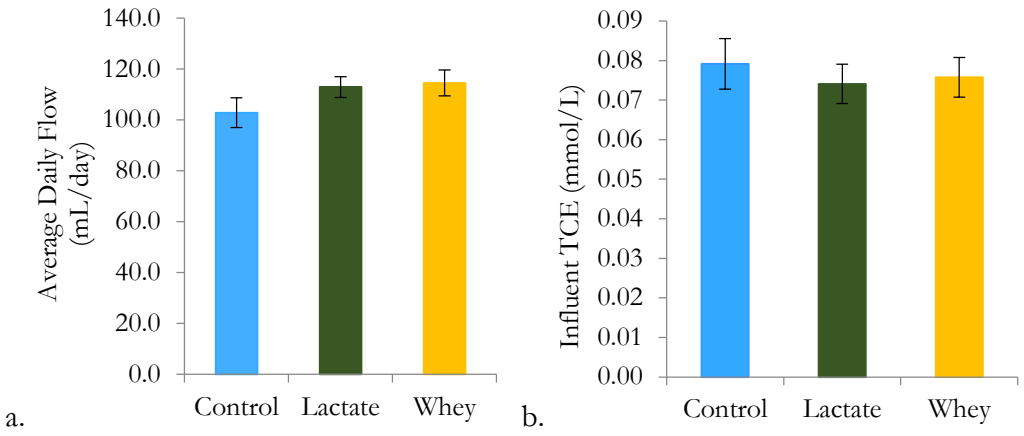


Figure 8. Average daily flows of each treatment. Error bars represent 95% confidence intervals of replicate measurements. (b) Average Influent TCE concentrations (mmol/L) for each treatment. Error bars represent 95% confidence intervals of replicate measurements.

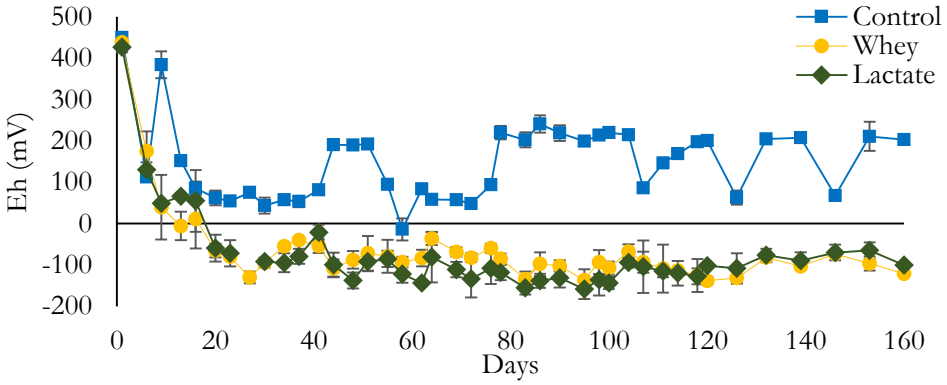


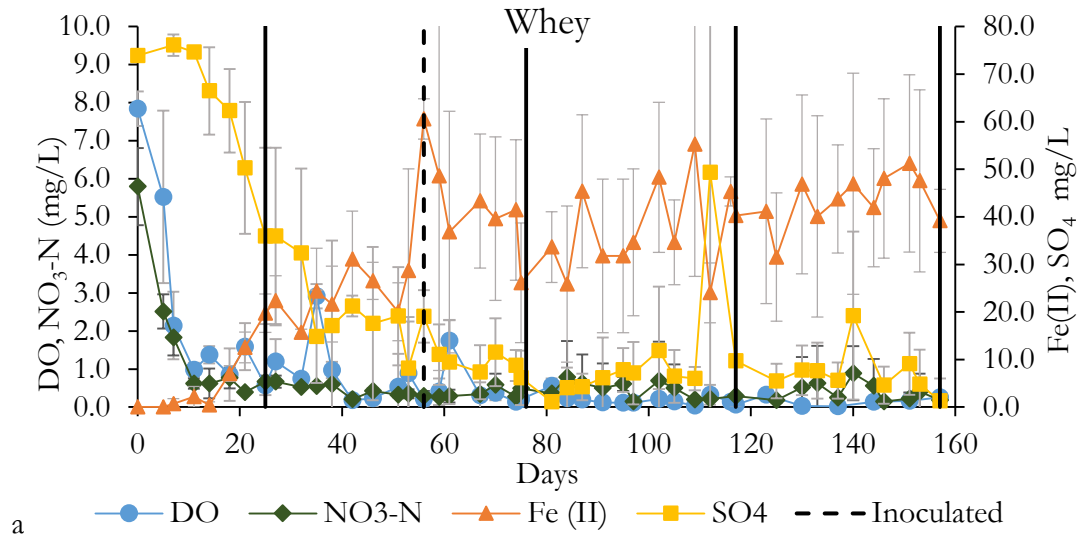
Figure 9. Oxidation/reduction potential (ORP) measured as Eh (mV). Error bars represent 95% confidence intervals of replicate measurements.

Upon carbon addition there was a depletion of terminal electron acceptors. In the whey treatment dissolved oxygen (DO) and nitrate (NO_3-N) were depleted by Day 11, and at approximately Day 14 sulfate was being reduced and Fe(II) was being released due to Fe(III) reduction (Figure 10). Sulfate reducing conditions were met in both lactate and whey treatments at Day 56, and the columns were inoculated with the derived DBR culture on

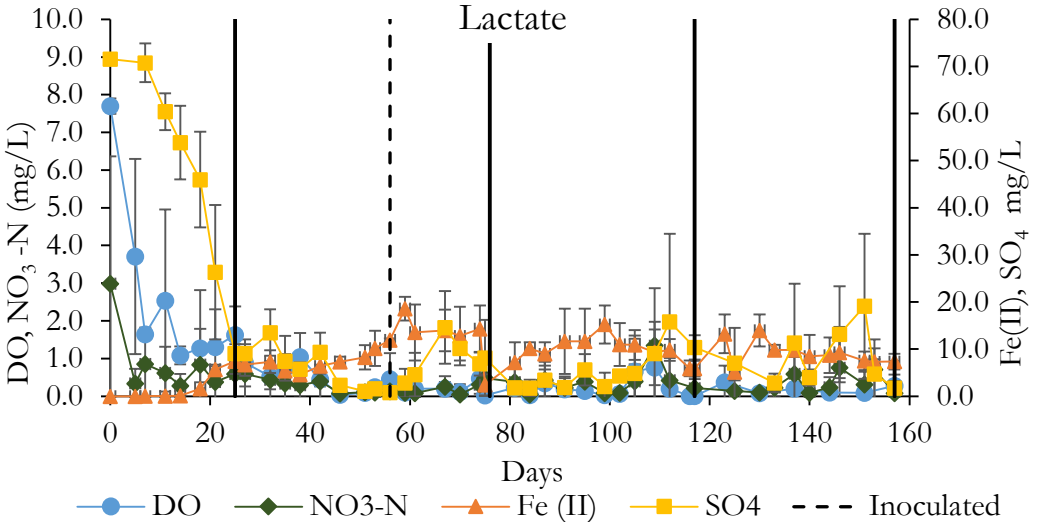
Day 56, as described in Section Bioaugmentation. After inoculation, redox conditions, and the analytes DO, NO_3-N, Fe (II), and SO_4, stabilized in the whey treatment (Figure 10) and Fe(III) and SO_4 were being reduced simultaneously. An average of 40.0 ± 3.4 mg/L of Fe(II) was being released, and SO_4 concentrations dropped from an initial average of 74.0 ± 0.23 mg/L during the first week to 9.2 ± 2.8 mg/L by Day 76.

By Day 56 sulfate reducing conditions were met in the lactate treatment (Figure 10). After inoculation, reducing conditions also stabilized in the lactate treatment, but Fe(II) never reached the level that was observed in the whey treatment. After inoculation, average effluent concentrations of SO_4 and Fe(II) from the lactate treatment were 7.2 ± 2.0 mg/L and 10.2 ± 0.92 mg/L, respectively. After inoculation on Day 56, the effluent Fe(II) concentrations remained significantly higher in the whey ($F_{(2,258)}=404.6$, MSE=93.25, Tukey HSD $p<0.05$) treatment than the concentrations measured in the lactate and control treatments. The control columns remained aerobic throughout the study, except during inoculation, when the approximately 10 mL of the DBR culture was injected in all columns (Figure 11).

At Sacrifice 1 the average dissolved inorganic carbon (DIC) was higher in the lactate treatment than the whey and control treatments (Tukey HSD, $p<0.05$, $n=3$) (Figure 13). At Sacrifices 2, 3, and 4, there was not a statistical difference in the concentration of DIC among any of the treatments. Due to the low pH in the lactate and whey treatments the carbonate speciation is partitioned between bicarbonate and carbonic acid or carbon dioxide. The average pH of the lactate treatment ranged from a low of 6.4 at Sacrifice 2 to a high of 7.0 at Sacrifice 4, with the DIC calculated speciation consisting of approximately 50%HCO_3, 50% H_2CO_3, and 80% HCO_3, 20% H_2CO_3, respectively



a



b.

Figure 10 . Reducing conditions in the (a) whey and the (b) lactate treatment prior to inoculation. DO and NO_3-N on the primary axis, Fe(II) and SO_4 on the secondary axis. Times of sacrifices designated by solid line at Days 25, 76, 117, and 157. Error bars display 95% confidence intervals of replicate measurements.

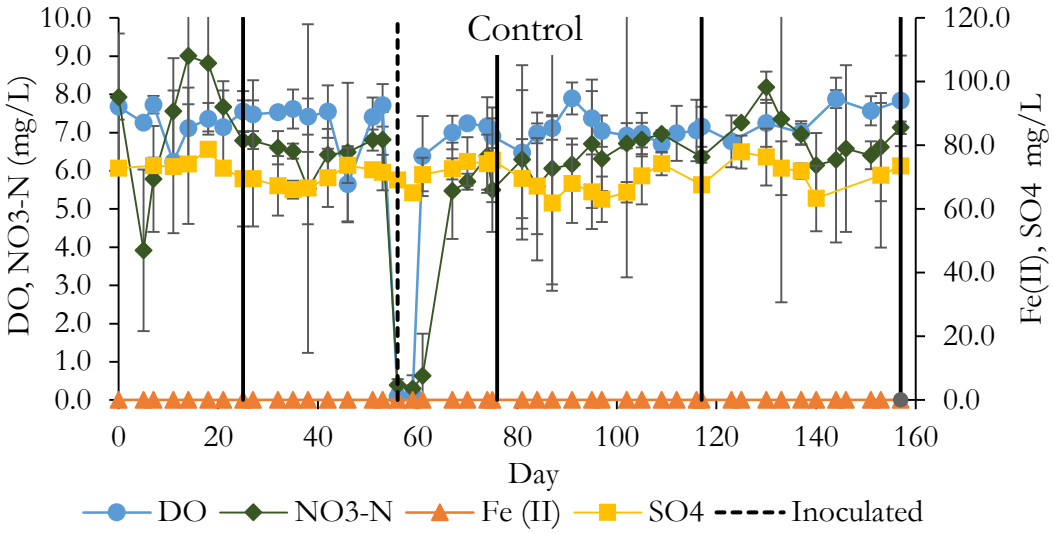


Figure 11. Terminal electron acceptor concentrations in the control treatment over the entire study. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

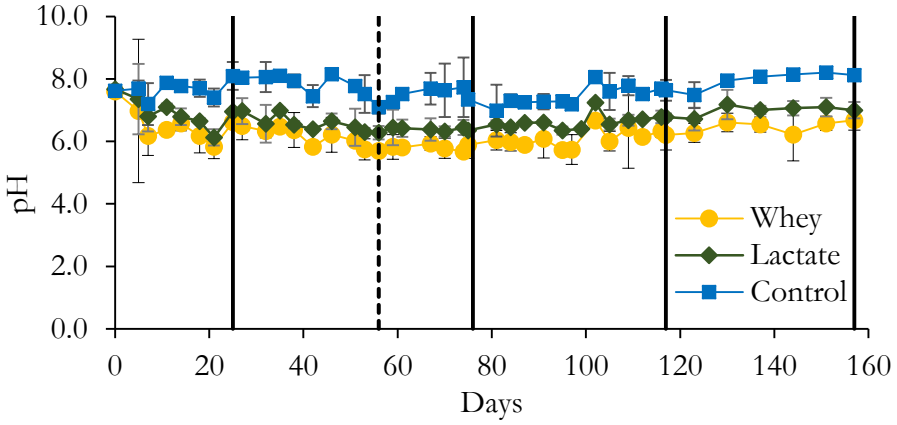


Figure 12. pH in the whey, lactate, and control treatments throughout study. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

The average pH measured in the whey treatment ranged from 5.9 at Sacrifice 2 to a high of 6.68 at Sacrifice 4, and the DIC consisted of approximately 25% HCO_3, 75% H_2CO_3

at Sacrifice 2 and 65% HCO_3, 35% H_2CO_3 at Sacrifice 4. In the control treatment the pH ranged from minimum of 7.3 at Sacrifice 2 and a high of 8.3 at Sacrifice 4, with approximately 90-98% of the DIC in the form of HCO_3. An increase in dissolved inorganic carbon would be expected due to the production of CO_2 during fermentation.

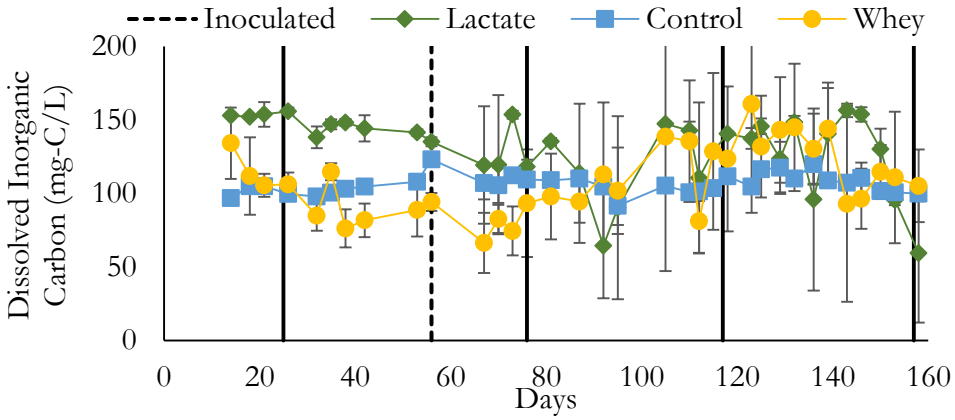


Figure 13. Dissolved inorganic carbon concentrations, measured as HCO_3 (mg/L). Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements

When comparing the concentration of total LMWOAs in the effluent of the reactors, concentrations were higher in the lactate treatment (Figure 14) than in the whey treatment. The difference between the two treatments may be due to an increase in metabolic activity in the whey treatment, resulting with a higher consumption of carbon, and a different composition of LMWOAs in the reactor's effluent.

The LMWOAs in the whey treatment mostly consisted of acetate and butyrate (Figure 15a). Acetate and butyrate are most likely a product of lactose fermentation, as whey consists of 60 to 70% lactose. Throughout the study the carbon metabolites measured in the lactate treatment consisted of acetate and propionate (Figure 15b).

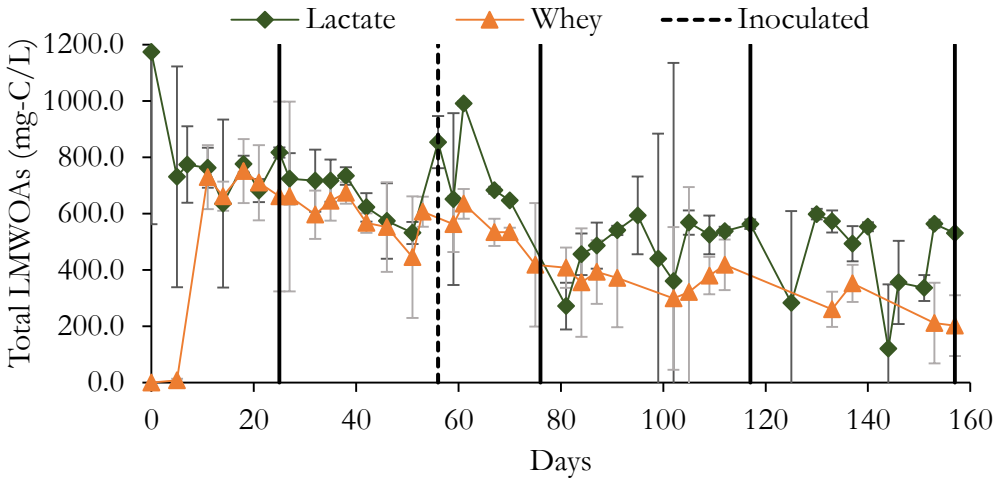


Figure 14. Comparison of total LMWOAs measured in the lactate and whey treatments. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

When comparing the amount of carbon measured as dissolved organic carbon and the sum of acetate, propionate, and butyrate, the LMWOAs accounted for (average ± 95% CI) 101.65 ± 12.84 % of the dissolved organic carbon in the lactate treatment, and 81.33 ± 10.04% in the whey treatment, which is shown in Appendix B. We were unable to capture CO_2 using the Tedlar gas bags, so a full carbon balance was not possible.

The two different carbon sources supported the production of different carbon metabolites. The concentration of propionate measured in the effluent of the lactate treatment reactors was significantly higher than the whey treatment at all 4 sacrifices, while the concentration of butyrate was significantly higher in the effluent of the whey treatment at all 4 sacrifices than the lactate treatment throughout the study (Figure 16). There was no statistical difference between the concentrations of acetate measured in the effluent of the lactate and whey treatments at each sacrifice, but there was a decrease in the concentration of acetate measured at Sacrifice 1 versus Sacrifice 4 in the whey treatment.

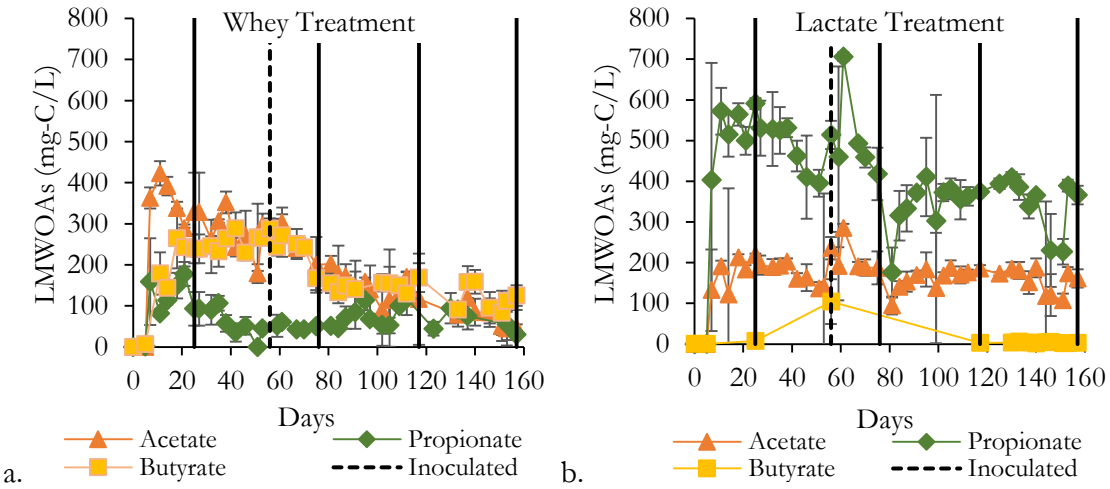


Figure 15. LMWOAs present in the effluent of the (a) whey and (b) lactate treatment over the entire study. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

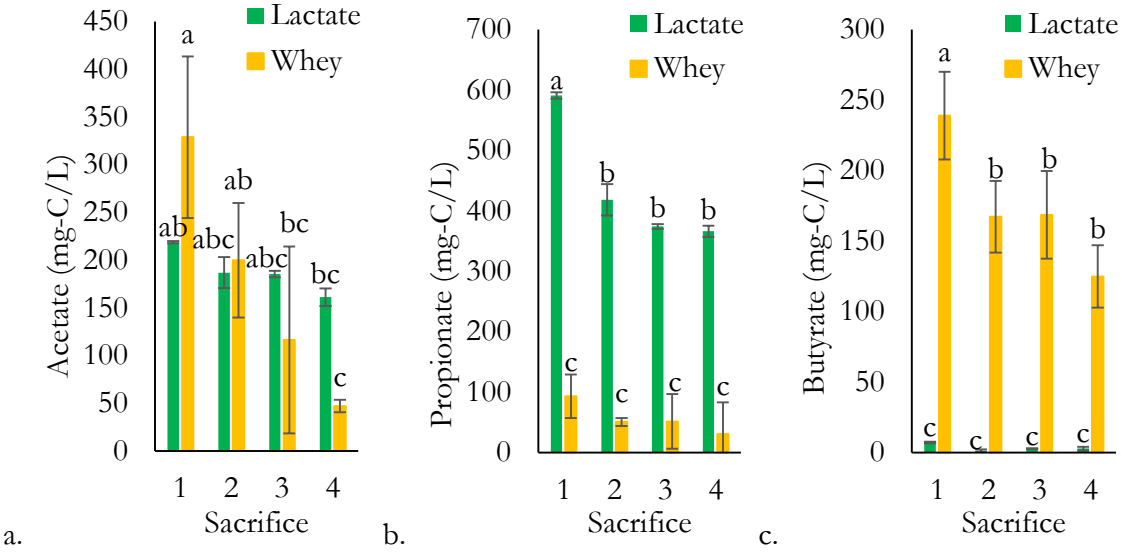


Figure 16. (a) Acetate, (b) propionate, and (c) butyrate concentrations in the effluent of the whey and lactate treatments. Error bars equal standard deviation. Letters distinguish statistical differences between concentrations measured in each treatment at each sacrifice (Tukey HSD).

The effluent TCE concentrations (mean ± 95% CI, N=120) in the control treatment had an average of 0.072 ± 0.004 mmol/L. There was not a statistical different between the

average effluent and influent TCE concentration of 0.079 ± 0.0064 mmol/L (t(224.49) =-1.379, p>0.16, Welch's two sample t-test) in the control treatment (Figure 17). In addition, no TCE or carbon degradation byproducts were detected in the control treatment.

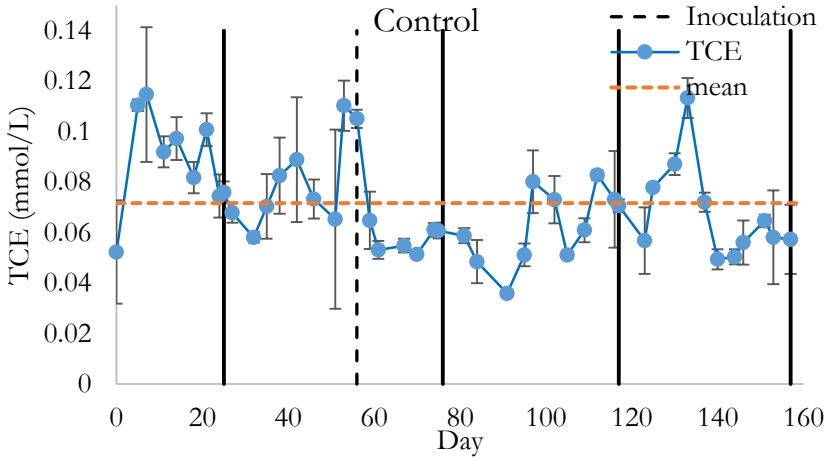


Figure 17. TCE effluent concentrations in the control treatment over the entire study. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

In the whey treatment, TCE reduction began early in the study, resulting in detectable concentrations of DCE on five occasions prior to its inoculation on Day 56 (Figure 18). Once the whey reactors were amended with the DBR culture TCE was reduced to DCE by Day 76, when the second Sacrifice occurred (Figure 18). The concentration of DCE remained persistent through Sacrifice 3 (Day 117), and vinyl chloride was measured only at low concentrations. At the final sacrifice at the end of the study, DCE and VC (at very low concentrations) were still detected, and ethene concentrations approached levels representing nearly complete TCE dechlorination.

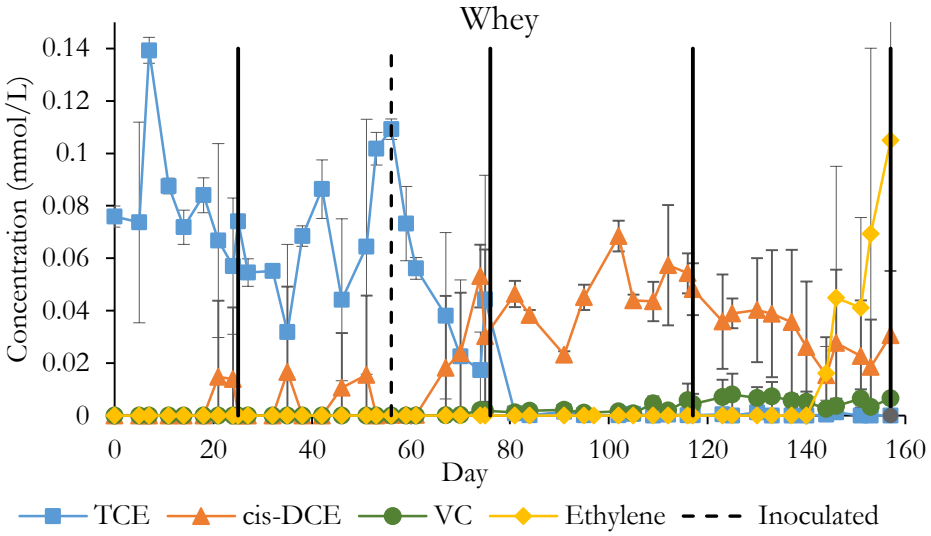


Figure 18. TCE and degradation byproducts in the whey treatment over the entire study. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

In the Lactate treatment DCE was first detected at Day 59, close to the time of inoculation. After inoculation, TCE was entirely converted to DCE by the second sacrifice on Day 76 (Figure 19). Between the second and third sacrifice DCE was reduced to VC, with concentrations as high as 0.08 ± 0.027 mmol/L. Accumulation of VC was significantly greater in the lactate treatment than in the whey treatment. After the third sacrifice VC concentrations dropped, as it was reduced to ethene. Ethene was the predominant degradation product in lactate columns by the end of the study.

At the Sacrifice 3 and 4, DCE and VC were the predominant TCE degradation products. At Sacrifice 3, the whey treatment had significantly greater concentrations of DCE than the lactate treatment and the lactate treatment had significantly more VC than the whey treatment (Tukey HSD, $p<0.05$, $n=3$), as shown in Figure 20.

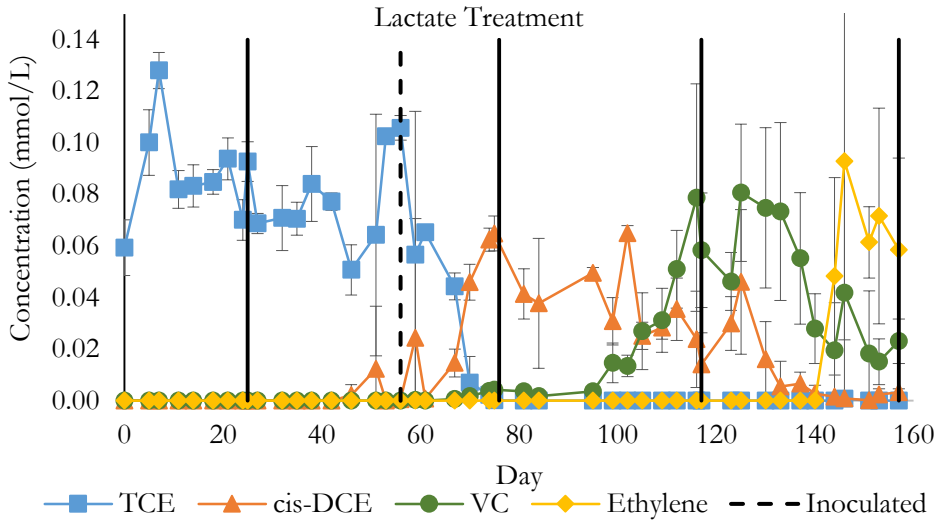


Figure 19. TCE and degradation byproducts in the lactate treatment over time. Times of sacrifice designated by solid line at Days 25, 76, 117, and 157. Error bars represent 95% confidence intervals of replicate measurements.

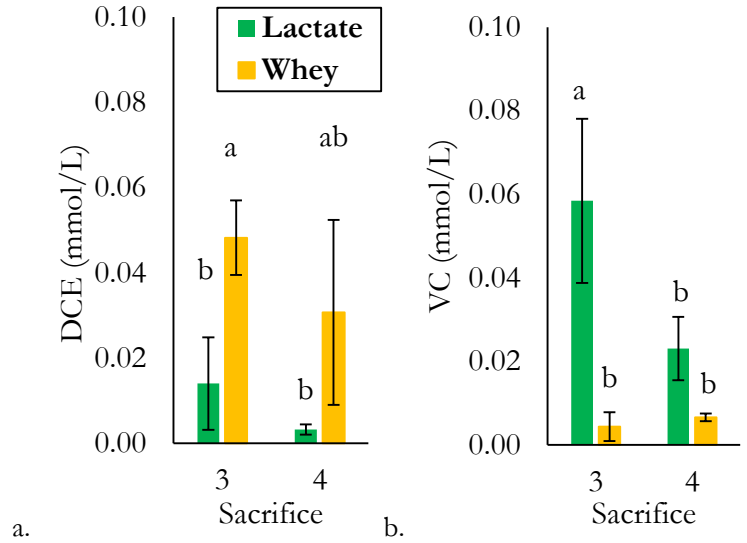


Figure 20. (a) DCE and (b) VC concentrations in all treatments at Sacrifices 3 and 4. Error bars equal standard deviation. Letters represent Tukey HSD, $p<0.05$, $n=3$.

The chlorine number was used to determine when reactor sacrificing occurred. The first sacrifice occurred when reducing conditions were met, and Fe(II) was being reduced at

Day 25 (Figure 21). Inoculation occurred when sulfate was being reduced at Day 56. The second sacrifice occurred on Day 76, when the chlorine number decreased from 3 to 2 or below. The third sacrifice occurred when the chlorine number in one of the three treatments decreased from 2 to 1. At the third sacrifice (Day 117) the lactate treatment had reached a chlorine number of 1.5 or less. At Sacrifice 3, a lower chlorine number was calculated from the lactate treatment than the whey treatment ($p<0.05$, Tukey HSD, $n=3$) due to the vinyl chloride accumulation. The final sacrifice occurred when TCE was being reduced to ethene, resulting in a chlorine number of 0.5 or less. At the final sacrifice there was not a statistical difference between the average chlorine number of the whey treatment (0.57 ± 0.48) and the lactate treatment (0.37 ± 0.07). The variance of the chlorine number calculated for the whey treatment was due to higher levels of DCE and ethene without vinyl chloride accumulation.

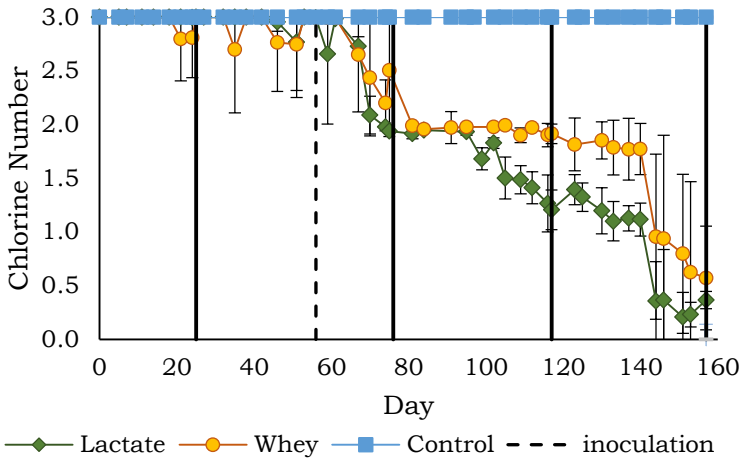


Figure 21. Chlorine Number of each treatment throughout the study. Chlorine Number of 2 at Day 76 (Sacrifice 2), 1 at Day 117 (Sacrifice 3), and 0.5 or below at Day 157 (Sacrifice 4). Error bars represent 95% confidence intervals of replicate measurements

As shown in column studies performed by Ballapragada et al. (1997) and serum bottle studies performed by Men et al. (2014), with the addition of carbon, concentrations of

hydrogen can be produced at levels over two orders of magnitude greater than that required for reductive dechlorination. Average concentrations of hydrogen (nmol/L) measured in this study were significantly higher in the whey treatment than in the control or lactate treatments after inoculation at Sacrifices 3 and 4 (Figure 22). An increase in the concentration of hydrogen is beneficial for dechlorinating bacteria by supplying energy, as the electron donor, along with stimulating a diverse microbial community. However, an increase in available hydrogen may also hinder dechlorination by creating competition with bacteria such as methanogens or iron reducers.

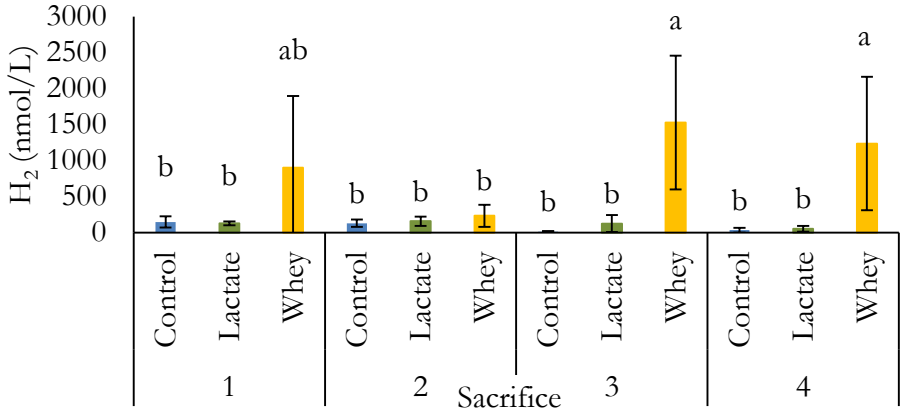


Figure 22. Dissolved hydrogen concentrations (nmol/L) at each Sacrifice in the control, lactate and whey treatments. Error bars show standard deviation and Tukey HSD (whey: Sac. 1-4, lactate: Sac. 4, control: Sac 3-4, n=6; lactate: Sac. 1-3, control: Sac. 1-2, n=5).

Soil Results of Each Sacrifice

At each sacrifice event, soil cores were collected from each column being sacrificed to determine the concentrations of chlorinated solvents included in the pore water and partitioning to the soil. Concentrations of chlorinated solvents in the soil cores did not reflect the dechlorination pathways as well as the effluent data (Figure 23). The average TCE measured in the cores collected from the control columns remained constant over all

sacrifices (Figure 23a). At Sacrifice 1, there was no statistical difference in the TCE concentrations found in the soil of all three treatments (Tukey HSD, $p>0.05$, $n=3$). At Sacrifice 2, there was no TCE reduction in the control treatment and the average concentration (1.6 ± 0.43 mmol/L) was significantly higher than measured in the soil of the lactate and whey treatment (Tukey HSD, $p<0.05$, $n=3$). Prior to sacrifice 2, all reactors from all treatments were inoculated with the culture containing *Dhc*, and TCE reduction was observed in the lactate and whey treatments.

When comparing the concentrations of DCE in the soil at sacrifices 2 through 4 in the lactate and whey treatments, there was no statistical difference between the treatments or between sacrifices (Tukey HSD, $n=3$, $p>0.05$) (Figure 23b). When comparing the concentrations of VC in soil of the lactate and whey treatments at sacrifices 2 through 4, there was not a significant difference between the treatments at any of the sacrifices (Tukey HSD, $n=3$, $p>0.05$) (Figure 23c). The soil conductivity (EC) of all reactors decreased from Sacrifice 1 to Sacrifice 2 and stabilized between Sacrifices 3 and 4 (Figure 24).The soil is made up of 8.7% $CaCO_3$, resulting with a higher pH than the pH measured in the porewater and effluent. There was decrease in the pH of the solids of each treatment between Sacrifice 1 and 4 (Figure 25). The effluent pH of the lactate and whey treatments was often lower than the pH measured in the solids and porewater, which may be a result of TCE reduction, organic carbon metabolism with the production of LMWOAs. Differences in the pH measurements of the effluent and the porewater may be due to the development of preferential flow paths in the soil columns over time, resulting in a lack of mixing in between the pore spaces of the soil and flowing groundwater.

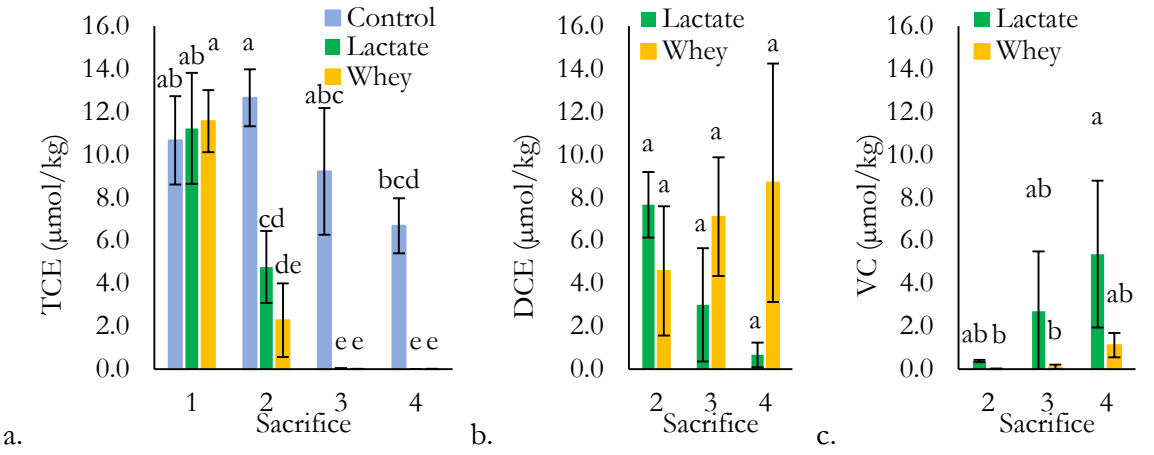


Figure 23. (a) TCE, (b) DCE, and (c) VC concentrations measured in the soil cores collected from the control, lactate and whey samples at each sacrifice Error bars represent one standard deviation of average replicate measurements with Tukey HSD, n=3.

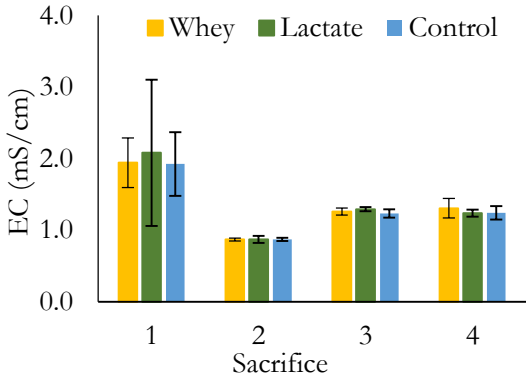


Figure 24.Conductivity the soils collected from the whey, lactate, and control treatments for each sacrifice. Error bars represent 95% confidence intervals of replicate measurements.

Studies show, that depending on the mineralogy, the presence of Fe (III) can inhibit the reduction of TCE and sequential degradation byproducts, as iron reducing bacteria may competitively consume available hydrogen, preventing reductive dechlorination (Paul et al. 2013). Reduced iron can also form compounds such as iron sulfides, or sorbe to other iron minerals, which can then reduce TCE, producing byproducts such as DCE and acetylene (Y

T He et al. 2015; Paul et al. 2013; Wei and Finneran 2011). As iron is reduced, the formation of different iron minerals may occur, such as iron oxides, carbonates and sulfides (Howell et al. 1998).

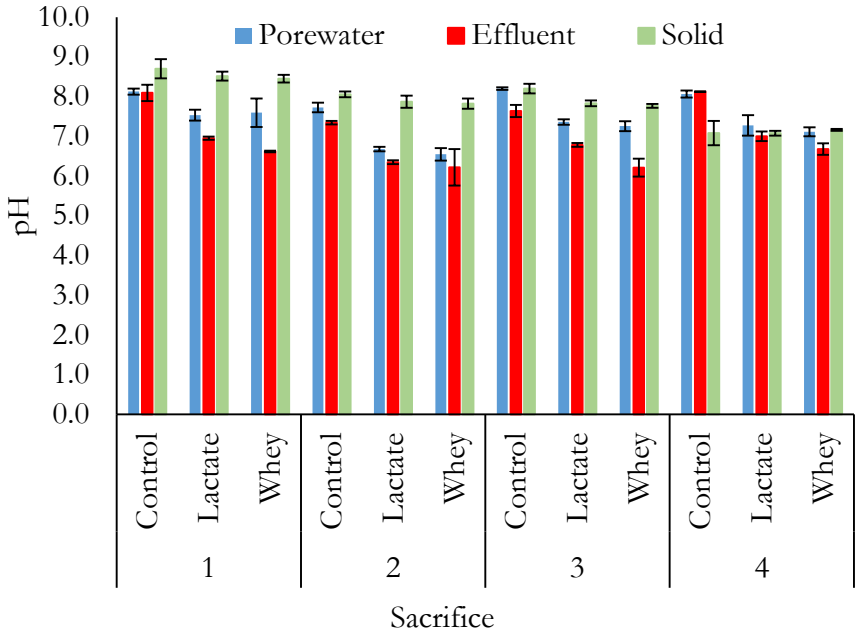


Figure 25. pH measurements of porewater, effluent, and solids collected from each treatment at each sacrifice. Error bars= 95% confidence intervals.

At each sacrifice event, a sequential extraction was performed on each sacrificed column to evaluate the changes in iron mineralogy throughout the study. The amount of iron in each phase included the exchangeable (F1); carbonates (F2); Mn oxide minerals, sulfides, and very amorphous iron hydroxides (F3); amorphous iron oxides (F4); crystalline sulfides (F5); and crystalline iron oxides (F6). There were no significant differences between the iron mineralogy of the lactate and whey treatments (Appendix C). Differences occur when comparing concentrations of average iron measured in each extraction of the carbon treatments and control.

Figure 26 displays the significant differences in the iron mineralogy of the aquifer material between carbon (lactate and whey) and control treatments at each sacrifice, negative values show a loss in the mass of iron when compared to the control and a positive value shows a gain in the mass of iron when compared to the control. The carbon treatments (lactate and whey) show an increase in total and reduced iron in the F2 extraction when compared to control at all four sacrifices. An increase in the total iron measured in the F3 extraction of the carbon treatments when compared to the control occurs at sacrifices 2 and 3, and an increase in the reduced iron measured in the F3 extraction of the carbon treatments when compared to the control occurs at sacrifices 2 through 4. The simultaneous increase in total iron and reduced iron occurring in extraction F2 and F3 may be due to the sorption of aqueous Fe(II). More crystalline forms of iron such as pyrite, magnetite, mackinawite can be reduced directly through microbial processes or indirectly during sulfate reduction and the formation of sulfides, and have been shown to reduce TCE (Badin et al. 2016; Lentini et al. 2012). Acetylene was not detected in any of the treatments, and abiotic transformation of TCE by iron was not observed.

Although concentrations of aqueous Fe(II) were significantly higher in the effluent of the whey treatment than the lactate treatment, the effluent concentrations of Fe(II) only accounted for a small fraction of the total iron in the reactors. Only a small fraction of aqueous Fe(II) is measured in the effluent because most Fe(II) precipitates or sorbes to other minerals (Paul et al. 2013). Changes in the overall concentration of iron minerals susceptible to biotransformation may not be distinct because during iron reduction and the release of Fe(II), aqueous Fe(II) sorption to other iron minerals may form new biogenic iron minerals (He et al. 2015).

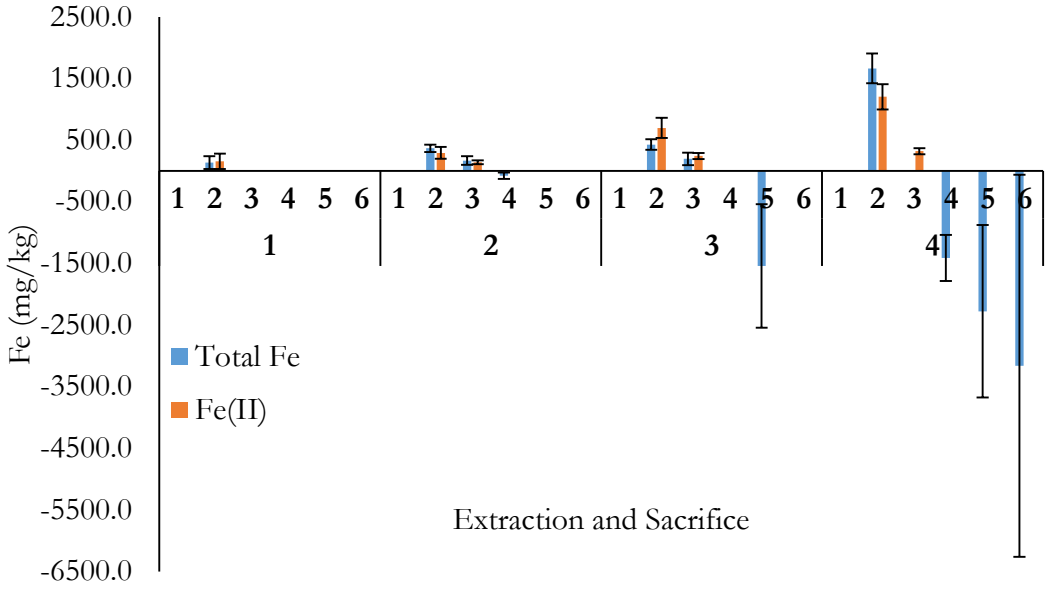


Figure 26. Difference between the concentration of iron measured the control and carbon treatments at each sacrifice. Error bars represent 95% confidence.

The concentration of bioavailable iron and biogenic Fe(II) measured in the carbon and control treatments through HCl extraction at each sacrifice is displayed in Figure 28. At Sacrifices 3 and 4 the total amount of bioavailable iron was greater in the carbon treatments, and 100% was in the form of Fe(II). There was no acetylene detected in any of the treatments, and TCE was degraded following the biological reductive sequence (DCE, VC, and Ethene) in the carbon treatments.

Sulfide concentrations in the soil were not detectable in the control treatment, and were also insignificant in the whey and lactate treatments during the first two sacrifices (Figure 29). The average sulfide concentrations of the soil in the lactate and whey treatments increased at Sacrifice 3, but there was no statistical different between Sacrifices 3 and 4. The formation of FeS occurs as sulfide and ferrous iron are produced. FeS has been shown to abiotically reduce TCE and generate the abiotic degradation byproduct, acetylene. It should

be noted that no acetylene was detected in the effluent of any of the treatments measured in this study suggesting that abiotic TCE degradation via FeS was not a significant pathway in any of these reactors.

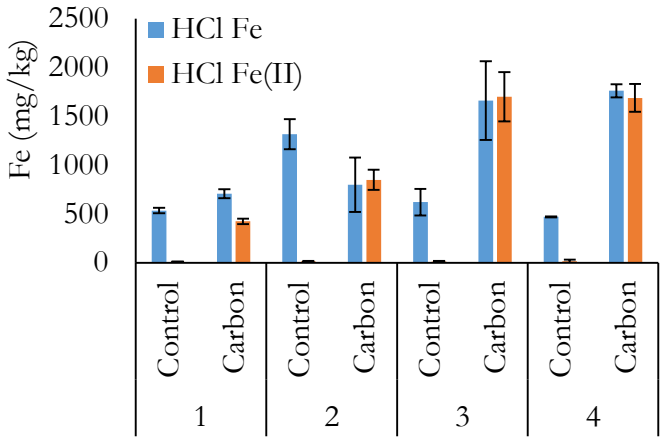


Figure 27. (a) Biogenic iron determined from HCl extraction of solids in control and carbon treatments at each sacrifice. Error bars represent 95% confidence intervals

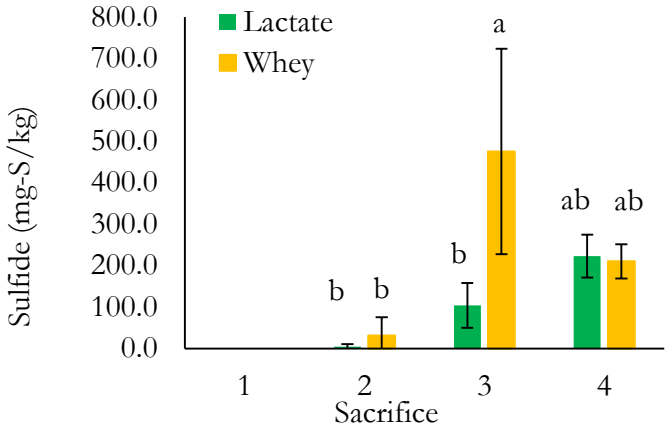


Figure 28. Sulfide concentrations in the whey and lactate treatments for all sacrifices. Sulfide in the control treatments were non-detect. Error bars represent one standard deviation with Tukey HSD (n=3, n=2 whey, Sacrifice 3).

Microbiology

At each sacrifice, core samples were taken from each column and processed through DNA extraction and qPCR. The concentrations of *tceA, vcrA*, and *Dhc* were compared among treatments, but the concentrations of *bvcA* were below the MDL (2.25 log copies/g of dry soil) in all the treatments. Concentrations of *tceA* and *vcrA* were below the MDL (1.56 and 2.41 log copies/g dry soil, respectively) in the lactate and control treatments at Sacrifice 1. When comparing the amount of *Dhc* (log copies/g of dry soil) in each treatment during the period of TCE reduction (Sacrifices 2 through 4), significantly more *Dhc* DNA was measured in the lactate treatment than the control treatment at Sacrifice 3, and the whey treatment contained significantly more *Dhc* than the control treatment at Sacrifice 4 (Figure 29).

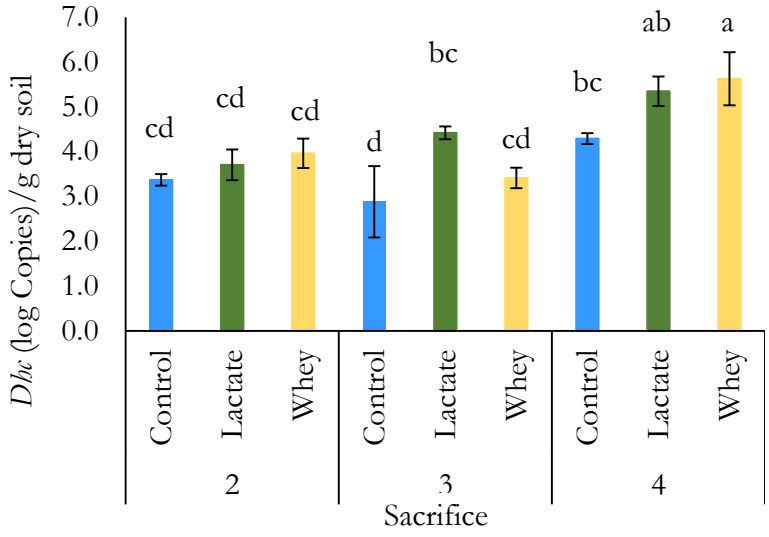


Figure 29. Concentration of *Dhc* (16S) DNA measured in the all treatments at Sacrifices 2 through 4. Error bar represent one standard deviation with Tukey HSD.

The concentration of the functional gene *tceA* (log copies/g dry soil) was statistically greater in the lactate than the control treatment at Sacrifice 3 and 4 (Figure 31), and a statistical increase in the concentration of *tceA* was measured in the lactate treatment between Sacrifice 2 and 4. There was no statistical difference between the concentrations of *tceA* measured in the lactate and whey treatments at Sacrifices 2 through 4. At Sacrifice 4, the carbon treatments contained significantly more *vcrA* than the control treatment, and at Sacrifices 2 through 4 there was not a statisitcal difference in the concentration of *vcrA* measured in the whey and lactate treatments.

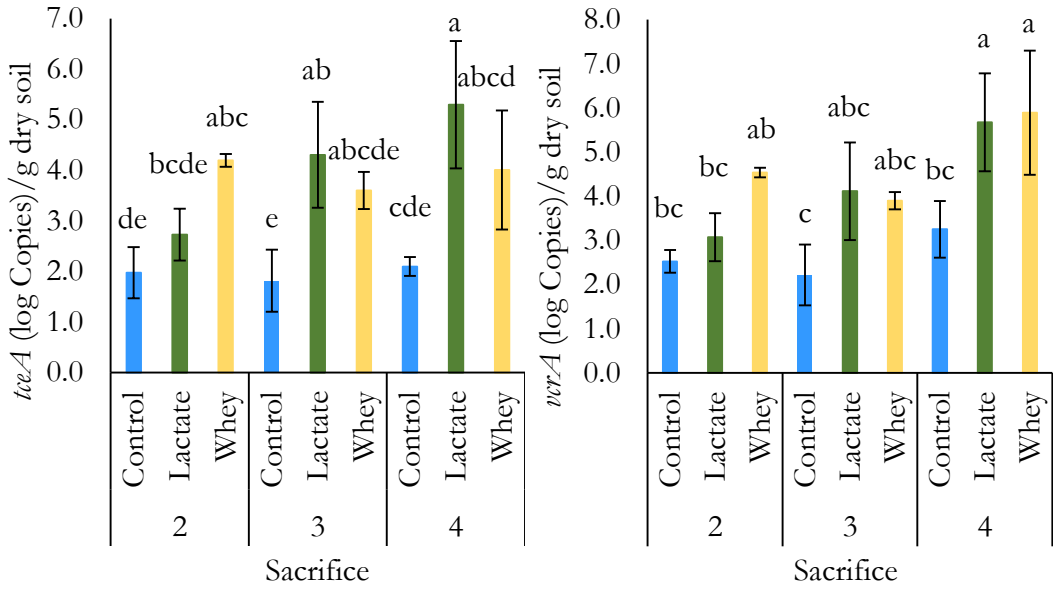


Figure 30. Concentrations of functional genes (a) *tceA* and (b) *vcrA* in each treatment at Sacrifices 2 through 4. Error bar represent one standard deviation with Tukey HSD.

Redundancy Analysis

Through a redundancy analysis (RDA) using the statistical software R, the results collected from the effluent, including LMWOAs (acetate, propionate, and butyrate),

dissolved inorganic carbon (DIC), hydrogen, pH, and Eh, trace elements measured in the porewater, and characteristics associated with the solids, including iron mineralogy and concentrations of *Dhc*, and reductase genes (*tceA* and *vcrA*) were correlated with the TCE degradation byproducts measured in each treatment. Values associated the ordination or TCE and degradation byproducts, along with vectors describing the environmental variables are described in Appendix D. Figure 31 and Figure 32 include constituents that were significantly correlated ($p<0.05$) with the concentration of TCE and degradation byproducts measured in each treatment at all four sacrifices. Correlations between variables that proved to be significant during the RDA are shown in Appendix E.

At all four sacrifices, the control treatment was associated with oxidized conditions (higher Eh) (Figure 31 and Figure 32). At Sacrifices 3 and 4, the control treatment was also associated with higher concentrations of Se, pH, and iron in the F4 and F5 extraction (Figure 33). Higher levels of Se associated with the control may be due to the redox state or the consumption of Se in the lactate and whey treatments. Consumption of selenoproteomes (proteins containing selenium) by *Dhc* has been correlated with an increase in Co consumption, which is found in cobalamins, such as vitamin B, required for *Dhc* metabolism during dehalorespiration (Zhang and Gladyshev 2010). Increased levels of acetate, propionate, Co, and genes *Dhc* (16S), *vcrA*, and *tceA*, along with reduced iron are measured in the lactate and whey treatments at Sacrifice 3 and 4 (Figure 33).

Based on the results of the RDA, cobalt (Co) correlated with carbon addition and TCE reduction ($p<0.05$). As shown in Figure 33, during the stages of TCE reduction (Sacrifices 2 through 4), concentrations of Co measured in the porewater of the carbon treatments were significantly higher than in the control treatment, until Sacrifice 4. At

Sacrifice 4, there was not a statistical difference between Co measured in the porewater of the whey and control treatments. The decrease in Co in the whey treatment may be due to an increase in microbial metabolism during VC reduction. Results from the lactate and whey treatments at all four sacrifices show that carbon fermentation and the production of acetate, measured in the effluent, were correlated with an increase in Co measured in the porewater.

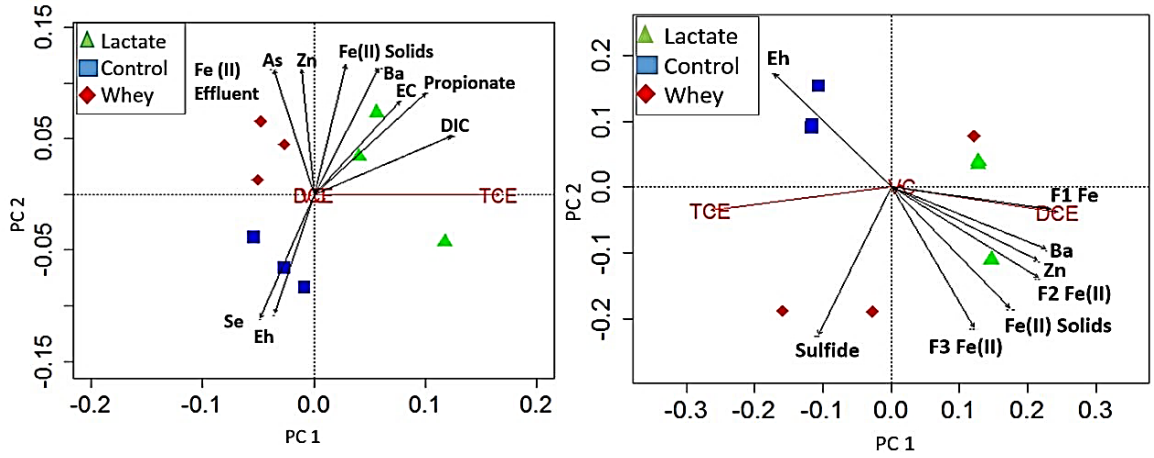


Figure 31. Redundancy analysis of constituents measured at Sacrifice 1 (left) and Sacrifice 2 (right). Plotted, are triplicates measurements of TCE and degradation byproducts of the whey (♦), lactate (▲), and control (■) treatments. Significant (p<0.05) effluent, porewater, and soil characteristics associated with TCE degradation byproducts are overlaid.

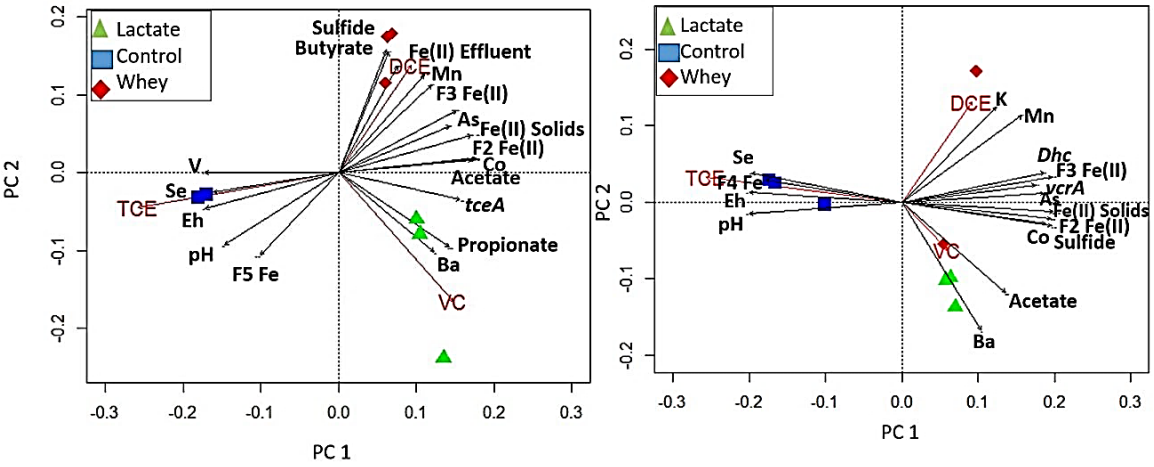


Figure 32. Redundancy analysis of constituents measured at Sacrifice 3 (left) and Sacrifice 4 (right). Plotted, are triplicates measurements of TCE and degradation byproducts of the whey (♦), lactate (▲), and control (■) treatments. Significant (p<0.05) effluent, porewater, and soil characteristics associated with TCE degradation byproducts are overlaid.

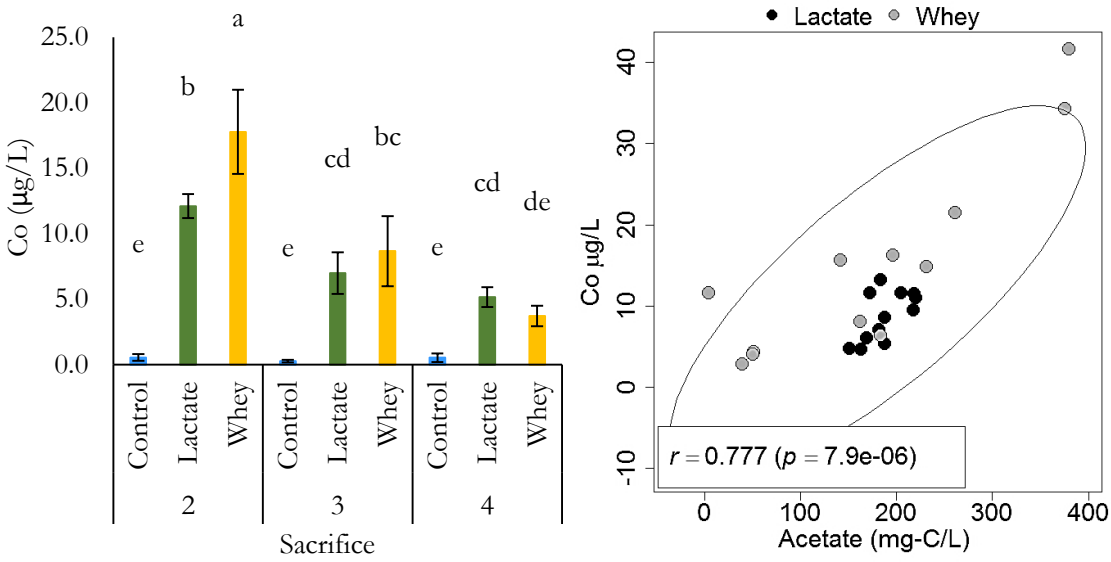


Figure 33. (a) Cobalt measured in the porewater of each treatment at each sacrifice. Tukey HSD with error bars displaying standard deviation (n=3) (b) Correlation between Co and acetate measured in the effluent of the lactate and whey treatments at all four sacrifices. Pearson correlation coefficient (r) and 95% confidence ellipse displayed.

Rates

The whey treatment had significantly less vinyl chloride than the lactate treatment. At Sacrifice 4, both lactate and whey treatments were producing ethene. In the whey treatment the TCE was reduced to DCE, with very little VC accumulation, and ethene was measured as the final product. The biogeochemical conditions in the whey treatment supported high rates of VC reduction, preventing its accumulation.

The original hydraulic residence times were calculated based on the bromide tracer study, which took place prior to the column study (Appendix A). During the study, changes in the flow through the columns occurred as tubing was replaced or biomass built up. The

hydraulic residence times (HRT_{Br}) that were originally calculated for each column were adjusted for the change in flow, as shown in Appendix F.

The operating HRT of each column was then adjusted with the estimated retardation factor of TCE and the sequential degradation byproducts to determine the amount of time each compound would remain in each column. A first order degradation rate was found to best fit the change in TCE concentrations with respect to time (Appendix F). First order rates for determining TCE degradation have been used previously during the column study performed by McLean et al. (2015), with a first order sequential degradation model and during the analysis performed by Murch (2003).

At the time of sacrifice, the effluent TCE and degradation byproduct concentrations represent the extent of TCE dechlorination that occurred over the period of one HRT in the column. The TCE, DCE, VC, and ethene concentrations measured in the effluent of a column is representative of the transformation of influent TCE taking place within each column, and the sum was used as the initial concentration of TCE (TCE_{in}). At the end of the study the total concentration of TCE and degradation byproducts measured in the effluent exceeded the concentration of TCE measured in the influent due to the travel time of ethene, and more accumulation of ethene at the end of the column.

The TCE influent concentration and the HRT were used to determine the initial model input of the first order degradation rate constant (rate=$[\ln(TCE_{HRT})-\ln(TCE_{in})]/HRT$). The model estimated the concentrations of DCE, VC, and ethene based on the rate of TCE degradation and the change in concentrations of the sequential compounds (e.g., $DCE=DCE_0 + [TCE_0 \times K_{TCE} \times (t_1-t_0)] - [DCE_0 \times K_{DCE} \times (t_1-t_0)]$). The first order degradation rates for each compound (K_{TCE}, K_{DCE}, K_{VC}) were solved for using Excel's

Solver function, where estimated rate constants were iterated until the modeled final concentrations matched the measured final concentrations of TCE and its degradation byproducts. The rate of TCE, DCE, and VC degradation determined for each column at the time of sacrifice were averaged for a given treatment, and the average rates were compared among sacrifices and treatments.

The predicted change in concentrations of TCE and degradation byproducts can be plotted with respect to the column length using the degradation rates determined from fitting the sequential first order decay model. An example of the concentrations of TCE and degradation byproducts which were calculated from the estimated rate constants, for a column in the lactate and whey treatment, are shown in Figure 34. At the time of the last sacrifice, TCE was being reduced to ethene in both carbon treatment, but little VC accumulation occurred in the whey treatment.

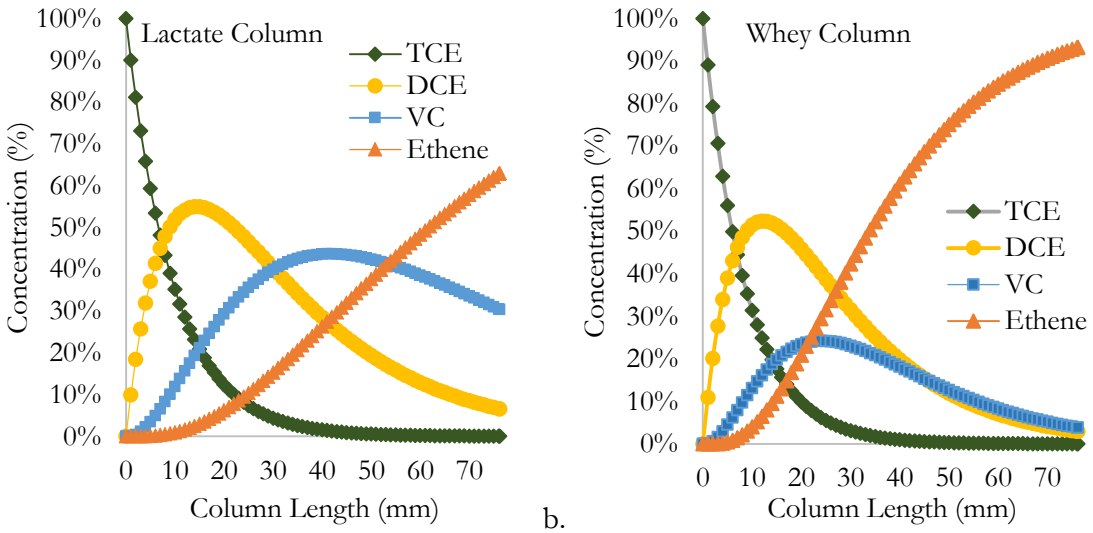


Figure 34. Comparing TCE degradation pathways between a column of the (a) lactate treatment and (b) whey treatment at Sacrifice 4.

The rate of TCE, DCE, and VC reduction was estimated for each column at the time of sacrifice and the average degradation rate of each chlorinated solvent is summarized in Table 11. Only a small amount of TCE degradation was detectable at Sacrifice 1. Degradation of DCE and a small amount of VC degradation began at Sacrifice 2.

Table 11. Degradation rates of TCE, DCE, and VC for lactate and whey with 95% confidence intervals.

Treatment	Sacrifice	K_{TCE} (d^{-1})	K_{DCE} (d^{-1})	K_{VC} (d^{-1})
Lactate	1	2.0E-4±4.0E-5		
	2	3.30±0.86	0.05±0.02	
	3	3.48±1.14	1.14±0.58	
	4	4.24±2.25	2.25±0.33	1.38±0.31
Whey	1	1.0E-4±1.0E-5		
	2	1.17±1.70	0.05±0.03	
	3	4.07±0.80	0.08±0.07	
	4	4.59±0.58	1.23±0.74	5.94±1.78

The average TCE degradation rate (Figure 35) increased from Sacrifice 1 to 4 in the whey and lactate treatments, but there was no statistical difference between the two treatments (Tukey HSD, $p>0.5$, $n=3$). The DCE degradation rate in the lactate treatment increased between Sacrifices 1 and 4 (Tukey HSD, $p<0.05$, $n=3$). The greatest difference between treatments occurred at Sacrifice 4, when VC was reduced at a significantly greater rate in the whey treatment than in the lactate treatment.

The rates of TCE, DCE, and VC degradation in this study were at least an order of magnitude greater than rates generated in studies summarized by Aronson and Howard (1997), Schaerlaekens et al. (1999), and Schneidewind et al. (2014), which consisted of microcosm, column, and field studies, respectively. In this study there was a continuous feed

of highly a concentrated carbon and nutrient solution, as well as very short sampling distances used for transformation measurements which resulted in high degradation rates of TCE, DCE, and VC. The long-term column study performed by McLean et al. (2015) observed complete TCE (10 mg/L) reduction of in the top 3" of the columns receiving whey as the carbon source, which had a residence time of 24 hours, yielding TCE degradation rates comparable to those measured in this study at Sacrifice 4.

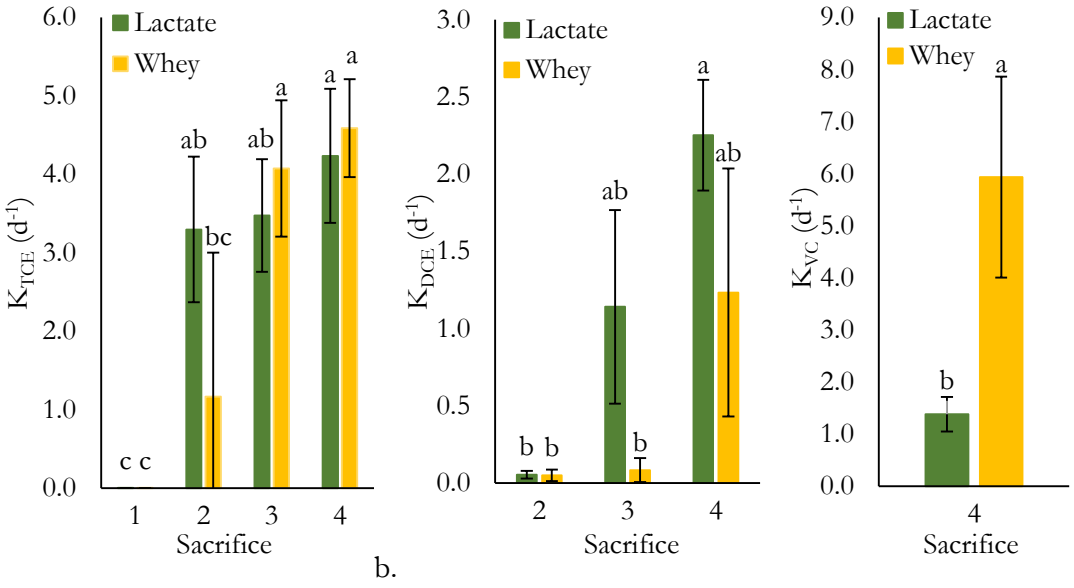


Figure 35. Average rates of (a) TCE degradation, (b) DCE degradation and (c) VC degradation at each Sacrifice in the lactate and whey treatments. Error bars represent one standard deviation with Tukey HSD distinguishing significant differences.

DISCUSSION

The main objectives of this study were to compare the effect of different carbon sources on TCE dechlorination within aquifer material, and to characterize the microbiology and biogeochemistry that supported full dechlorination. Based on the results collected from the effluent throughout the study and soil analyses at each sacrifice, differences among the biogeochemistry of the whey, lactate, and control treatments were identified. While both the lactate and whey treatments were producing ethene at the final sacrifice, the degradation pathway of TCE was significantly different between these two treatments. The lactate treatment had significantly more accumulation of vinyl chloride than did the whey treatment. Results were analyzed to identify the characteristics in biogeochemistry that were associated with the different concentrations of TCE degradation byproducts.

As expected, without carbon addition, the control treatment remained aerobic throughout the study with a higher redox state (Eh) compared to the lactate and whey treatments. Within the first month, both the lactate and whey treatments were operating under reduced conditions, with Fe(III) and SO_4 reduction evident from effluent measurements. The lactate was being degraded to acetate and significantly greater concentrations of propionate than in the whey treatment. whey was being degraded to acetate, along with significantly greater concentrations of butyrate than in the lactate treatment. The butyrate in the whey treatment may be due to the fermentation of lactose, which is found in whey. Lactate (lactic acid) is a simpler form of carbon than lactose, and propionate contains fewer carbons than the metabolite butyrate that was produced from the whey. At the beginning of the study there was a significantly higher concentration of acetate

measured in the whey treatment than the lactate treatment, but the concentration decreased over the course of the study, which was most likely caused by an increase in microbial metabolism. As shown in studies performed by David et al. (2014) and Wei and Finneran (2013), the low concentration of acetate did not have an negative impact on the rate or extent of TCE degradation, but did correspond to a difference in concentration of *Dhc* between the carbon treatments.

As shown by Mirza et al. (2015), when analyzing gene abundance within the columns receiving different carbon sources in the study performed by McLean et al. 2015, carbon type did not lead to a statistical difference in the concentration of *vcrA*, *tceA*, or *Dhc*. Both carbon treatments did have significantly greater concentrations of *vcrA* during DCE and VC reduction (Sacrifices 3 and 4) than the control treatment in this study. The high concentrations of hydrogen produced during the fermentation of whey, may have been the main factor that led to higher rates of VC reduction than measured in the columns receiving lactate.

In this study, reduced iron in the aqueous form was measured at significantly greater concentrations in the effluent of the whey treatment than that in the lactate treatment. This aqueous reduced iron only accounted for a small fraction of the total reduced iron, however, as most Fe(II) precipitates or sorbes to other minerals. While Fe(III) is often considered an inhibitor of TCE reduction, studies have shown that the inhibition of iron on TCE reduction may be dependent on the mineralogy. In this study, there were no significant differences in the iron mineralogy that correlated with the different TCE degradation pathways between treatments. With carbon addition, iron reduction occurred in the solids of

both treatment, and there was no difference in the concentration of biogenic iron between carbon treatments.

The lactate and whey treatments were both continuously fed with a highly concentrated carbon and nutrient supply, which resulted with few differences between the treatments. Both treatments were fully dechlorinating TCE by the end of the study. In the study performed by McLean et al. (2015) there was not a continuous supply of easily fermentable carbon in the emulsified oil treatments, along with no nutrient addition. Similarly, to the results reported by McLean et al. (2015), when comparing the two carbon sources, the whey treatment did prove to be a more robust carbon source, and in this study whey supported full TCE dechlorination without the accumulation of VC.

CONCLUSION

Lactate and whey were substrates added during biostimulation and bioaugmentation of aquifer material in continuous flow-through columns. The impact of these substrates on the rate and extent of TCE dechlorination, along with carbon metabolism, microbial composition and supporting biogeochemical conditions was determined, and based on the results of this study, the following conclusions were made.

1. The type of carbon used to drive reductive dechlorination in this column study had a significant impact on the pathway of TCE transformation to ethene. With the addition of lactate and whey, TCE was reduced to concentrations below the drinking water MCL of 5 μg/L. After inoculating each treatment with the DBR culture, both carbon substrates facilitated TCE reduction to ethene within approximately 100 days. However, the TCE degradation pathways were significantly different between the two carbon treatments. The rate of VC reduction in the lactate treatment ($1.4 \pm 0.3\ d^{-1}$) was significantly less than the whey treatment ($5.9 \pm 1.8\ d^{-1}$), resulting in significantly less VC accumulation in the whey reactors.
2. The type of carbon used during biostimulation in this column study had a significant impact on the microbial community function that developed in the columns over time. Carbon metabolites in the whey treatment were significantly different than the lactate treatment, including greater concentrations of butyrate and hydrogen measured in the whey treatment, while greater concentrations of propionate were measured in the lactate treatment.

3. The type of carbon used to drive reductive dechlorination in this column study did not have a significant impact on the biogeochemistry. With carbon addition, reduced conditions were achieved, and iron and sulfate reduction occurred. There was no statistical difference in the amount of biogenic iron measured in the carbon treatments, and there was no significant differences in the iron mineralogy that related to the difference in TCE degradation pathways between the lactate and whey treatments. Iron reduction did not present competition for TCE reduction, as both treatments were fully dechlorinating by the end of the study.
4. The different carbon treatments did not have an impact on the DNA based molecular biology indicators of *Dhc* and its functional genes. There was no difference in the concentration of reductase genes, *vcrA*, *tceA*, and *Dhc* between the lactate and whey treatment. In the future, analyzing the concentration of RNA to determine the active functional gene pool in each treatment may be more useful in understanding active community differences than simple DNA qPCR methods.

ENGINEERING SIGNIFICANCE

Trichloroethylene is one of the most prevalent environmental contaminants, and is estimated to cost site owners billions of dollars to remediate (USEPA 2000). TCE is a human carcinogen and it is important to prevent human exposure to it by eliminating it from the environment. The remediation method of biostimulation with bioaugmentation is an effective, low cost, low maintenance treatment option. In most contaminated aquifers, dechlorinating microbial communities are stimulated and dechlorinating conditions are met simply with addition of carbon. The cost requirements of biostimulation and bioaugmentation are low compared to those associated with physical-chemical or thermal treatments technologies. Costs associated with biostimulation and bioaugmentation would include initial site characterization, such as analyzing physical characteristics such as groundwater flow path, which influences the transport of contaminants and degradation byproducts, and determines the fate and changes that may occur to surrounding biogeochemical conditions. During biostimulation and bioaugmentation, with the addition of a carbon and the introduction of exogenous bacteria, the biogeochemistry of the site may be altered and the release of other harmful chemicals may be a risk.

During this study, the substrates whey and lactate, were used to determine if one could provide the ideal biogeochemical conditions for complete TCE dechlorination more effectively than the other. whey, as a complex form of carbon, may supply the optimal amount of energy, nutrients, and hydrogen to allow all reductive processes to occur simultaneously, including complete dechlorination. Complete dechlorination will prevent the remediation process from stalling at the carcinogenic degradation byproduct, VC. Whey is not only a waste product from the cheese industry; it also is a complex carbon and energy

source, containing carbon and nutrients in the form of proteins. It is more cost effective to use a waste product purchased from farms, rather than the highly refined carbon sources from chemical manufacturers.

In this study, the calcium lactate pentahydrate (Fisher Scientific, Santa Clara, CA) had a cost of 20¢/g, while the whey (Barry Farms, Wapakoneta, OH) had a cost of only 1¢/g. Whey is an economical substrate that can facilitate a rapid change in redox state and allow for TCE to be used as a terminal electron acceptor. With the addition of whey, carbon and hydrogen are provided to support a dechlorinating community that efficiently achieves full dechlorination. For site operators where TCE is present, whey may provide a simple remediation solution that requires low operation and maintenance costs associated with implementation and monitoring.

During this study, the whey and lactate treatments were able to support full reduction of TCE, with 100±0.01% percent removal in each of the carbon treatments within the same time frame. Vinyl chloride accumulated in the lactate treatment at significantly higher concentrations than the whey treatment. This difference in the amount of VC accumulation between the treatments is an important consideration during the remediation process, because exposure to VC can potentially be avoided with the careful selection of the carbon donor type.

FUTURE STUDIES

The whey and lactate treatments were both reducing TCE to the final degradation byproduct, ethene, but the lactate treatment accumulated a significantly greater concentration of VC. With the addition of whey, high concentrations of hydrogen were produced and the electron donor may have been present in excess, supporting high rates of VC reduction. The difference in hydrogen concentrations between the carbon treatments did not result in a difference in the concentration of reductase genes measured using DNA based techniques. Further research evaluating the microbial RNA concentrations, an indication of actual gene expression rather than gene presence, may provide more information in determining the cause of high rate of VC reduction in the whey treatment. Evaluating the difference in the microbial community and the active genes through RNA analyses may assist with determining carbon and biogeochemical conditions requirements that are specific to the dechlorinating community in order to achieve full dechlorination without the accumulation of VC.

REFERENCES

Ahsanul Islam, M., Edwards, E. A., and Mahadevan, R. (2010). "Characterizing the metabolism of *Dehalococcoides* with a constraint-based model." *PLoS computational biology*, 6 (8).

American Conference of Governmental Industrial Hygienists (ACGIH) "*TLVs and BEIs 2007, Trichloroethylene*." Cincinnati

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) (2012). *Standard methods for the examination of water and wastewater*, American Public Health Association Washington DC.

Agency for Toxic Substances and Disease Registry (ASTDR) (2009). "Public Health Assessment: Hill Air Force Base." <http://www.atsdr.cdc.gov/HAC/pha/pha.asp?docid=795&pg=2>.

Agency for Toxic Substances and Disease Registry (ASTDR) (2012). "Environmental Health and Medicine Education Trichloroethylene Toxicity."Atlanta, GA, 8-10.

Aronson, D., and Howard, P. (1997). Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies. North Syracuse, NY.

Badin, A., Broholm, M. M., Jacobsen, C. S., Palau, J., Dennis, P., and Hunkeler, D. (2016). "Identification of abiotic and biotic reductive dechlorination in a chlorinated ethene plume after thermal source remediation by means of isotopic and molecular biology tools." *Journal of Contaminant Hydrology*, Elsevier, 192, 1–19.

Ballapragada, B. S., Stensel, H. D., Puhakka, J. A., and Ferguson, J. F. (1997). "Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes." *Environmental Science & Technology*, American Chemical Society, 31(6), 1728–1734.

Blair, a., Hartge, P., Stewart, P. a., McAdams, M., and Lubin, J. (1998). "Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow up." *Occupational and Environmental Medicine*, 55, 161-171.

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

Brown, R. A., Mueller, J. G., Seech, A. G., Henderson, J. K., and Wilson, J. T. (2009). "Interactions between biological and abiotic pathways in the reduction of chlorinated solvents." *Remediation J.*, 20(1), 9–20.

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

CH2M Hill (2008). "2008 Five-Year Review for Hill Air Force Base."Salt Lake City.

Chiao, F., Currie, R., and McKone, T. (1994). *Intermedia transfer factors for contaminants found at hazardous waste sites: Trichloroethylene (TCE)*. California Environmental Protection Agency.

Civil-Environmental Survey Group (CES) (2012). "Electrical Resistance Heating." <http://www.cesgroup.co/services>. (May 5, 2015).

Darlington, R. and Rectanus, H. (2015). *Biogeochemical Transformation Handbook*. Naval Facitilies Engineering Command, Engineering and Expeditionary Warfare Center.

David, M. M., Cecillon, S., Warne, B. M., Prestat, E., Jansson, J. K., & Vogel, T. M. (2014). Microbial ecology of chlorinated solvent biodegradation. *Environmental Microbiology*, 17, 4835–4850. http://doi.org/10.1111/1462-2920.12413

Deweerd, K. I. M. A., Concannon, F., and Suflita, J. M. (1991). "Relationship between Hydrogen Consumption , Dehalogenation , and the Reduction of Sulfur Oxyanions by Desulfomonile tiedjei." *Applied and Environmental Microbiology*, 57, 1929-1934.

Dionex (2006). "Application Note 123 Determination of Inorganic Anions and Organic Acids in Fermentation Broths." Thermo Scientific.

Doucette, W., Klein, H., Chard, J., Dupont, R., Plaehn, W., and Bugbee, B. (2013). "Volatilization of trichloroethylene from trees and soil: Measurement and scaling approaches." *Environmental Science & Technology*, 47(11), 5813–5820

Dupont, R. R., Sorensen, D. L., Mclean, J. E., Doucette, B. J., and State, U. (2003). "Enhancement of trichloroethylene degradation via carbon donor and microbial amendment addition." *Proceedings of the Seventh International In Situ and On-site Bioremediation Symposium*, (June).

El Fantroussi, S., Naveau, H., and Agathos, S. N. (1998). "Anaerobic dechlorinating bacteria." *Biotechnology Progress*, 14, 167-188.

Ellis, D. E., Lutz, E. J., Odom, J. M., Buchanan, R. J., Bartlett, C. L., Lee, M. D., Harkness, M. R., and Deweerd, K. A. (2000). "Bioaugmentation for accelerated in situ anaerobic bioremediation." *Environmental Science & Technology*, 34(11), 2254–2260.

Ensley, B. D. (1991). Biochemical diversity of trichloroethylene metabolism. *Annual Review of Microbiology*, 45, 283–299. http://doi.org/10.1146/annurev.micro.45.1.283

Environmental Management Directorate (1998). "Hill Air Force Base Five Year Review." Ogden Air Logistics Center, Hill Air Force Base, UT.

Environmental Security Technology Certification Program (ESTCP) (2005). "Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs." U.S. Department of Defense.

Fowler, T., and Reinauer, K. (2013). "Enhancing Reductive Dechlorination With Nutrient Addition." *Remediation Journal*, 21, 23-35.

Freedman, D.L. and Gosett. J. (1989). "Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions." *Applied and Environmental Microbiololgy*, American Society for Microbiology, 55, 2144-2151.

Harkness, M., Fisher, A., Lee, M. D., Mack, E. E., Payne, J. A., Dworatzek, S., Roberts, J., Acheson, C., Herrmann, R., and Possolo, A. (2012). "Use of statistical tools to evaluate the reductive dechlorination of high levels of TCE in microcosm studies." *Journal of Contaminant Hydrology*, 131, 100-118.

GSI Environmental. (2009). "TCE Physical Properties." http://www.gsi-net.com/en/publications/gsi-chemical-database/single/547.html (May 5, 2015).

He, J., Ritalahti, K. M., Aiello, M. R., and Löffler, F. E. (2003). "Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species." *Applied and Environmental Microbiology*, 69(2), 996–1003.

He, Y. T., Wilson, J. T., Su, C., and Wilkin, R. T. (2015). "Review of Abiotic Degradation of Chlorinated Solvents by Reactive Iron Minerals in Aquifers." *Groundwater Monitoring and Remediation*, 35(3), 57–75.

Howell, J. R., Donahoe, R. J., Roden, E. E., and Ferris, F. G. (1998). "Effects of microbial iron oxide reduction on pH and alkalinity in anaerobic bicarbonate-buffered media : implications for metal mobility." *Mineralogy Magazine, 62A(2)*, 657–658.

Huang, J.-H., and Kretzschmar, R. (2010). "Sequential extraction method for speciation of arsenate and arsenite in mineral soils." *Analytical Chemistry*, American Chemical Society, 82, 5534-5540.

Ibrahem, A. K., Abdel Moghny, T., Mustafa, Y. M., Maysour, N. E., Mohamed Saad El Din El Dars, F., and Farouk Hassan, R. (2012). "Degradation of Trichloroethylene Contaminated Soil by Zero-Valent Iron Nanoparticles." *ISRN Soil Science*, 2012, 1–9.

International Agency for Research on Cancer (IARC) (2014). "Agents Classified by the IARC Monographs."Lyon, 1-25.

Interstate Technology and Regulatory Cooperation Work Group (ITRC) In Situ Bioremediation Work Team and Industrial Members of the Remediation Technologies Development Fourm (RTDF) (1999). "Technical/Regulatory Guidelines Natural Attenuation of Chlorinated Solvents in Groundwater : Principles and Practices."Washington D.C.

Interstate Technology Regulatory Council (ITRC). (2005). "Overview of In Situ Bioremediation of Ethene Chlorinated DNAPL Source Zones". Washington D.C.

Kaifas, D., Malleret, L., Kumar, N., Fétimi, W., Claeys-Bruno, M., Sergent, M., and Doumenq, P. (2014). "Assessment of potential positive effects of nZVI surface modification and concentration levels on TCE dechlorination in the presence of competing strong oxidants, using an experimental design." *Science of the Total Environment*, 481, 335-342.

Kennedy, L. G., Everett, J. W., and Gonzales, J. (2006). "Assessment of biogeochemical natural attenuation and treatment of chlorinated solvents, Altus Air Force Base, Altus, Oklahoma." *Journal of Contaminant Hydrology*, 83(3-4), 221–236.

Klassan, C. D. (1996). *Casarett & Doull's Toxicology The Basic Science of Poisons*, McGraw-Hill, New York.

Lacroix, E., Brovelli, A., Maillard, J., Rohrbach-Brandt, E., Barry, D. a., and Holliger, C. (2014). "Use of silicate minerals for long-term pH control during reductive dechlorination of high tetrachloroethene concentrations in continuous flow-through columns." *Science of the Total Environment*, 482-483, 23-35.

Lawrence, S. (2006). "Description , Properties , and Degradation of Selected Volatile Organic Compounds Detected in Ground Water — A Review of Selected Literature." U. S. Geological Survey. Open-File Report 2006-1338, 62 p., a Web-only publication at http://pubs.usgs.gov/ofr/2006/1338/

Lee, P. K. H., Macbeth, T. W., Sorenson, K. S., Deeb, R. a., and Alvarez-Cohen, L. (2008). "Quantifying genes and transcripts to assess the in situ physiology of *Dehalococcoides* spp in a trichloroethene-contaminated groundwater site." *Applied and Environmental Microbiology*, 74, 2728-2739.

Lee, P. K. H., Warnecke, F., Brodie, E. L., MacBeth, T. W., Conrad, M. E., Andersen, G. L., and Alvarez-Cohen, L. (2011). "Phylogenetic microarray analysis of a microbial community performing reductive dechlorination at a TCE-contaminated site." *Environmental Science & Technology*, 46, 1044-1054.

Lee, P. K. H., Cheng, D., West, K. a., Alvarez-Cohen, L., and He, J. (2013). "Isolation of two new *Dehalococcoides mccartyi* strains with dissimilar dechlorination functions and their characterization by comparative genomics via microarray analysis." *Environmental microbiology*, 15, 2293-2305.

Lentini, C. J., Wankel, S. D., and Hansel, C. M. (2012). "Enriched iron(III)-reducing bacterial communities are shaped by carbon substrate and iron oxide mineralogy." *Frontiers in Microbiology*, 3(DEC), 1–19.

Lewis, J., Qvarfort, U., and Sjöström, J. (2015). "Betula pendula : A Promising Candidate for Phytoremediation of TCE in Northern Climates." *International Journal of Phytoremediation*, 17(1), 9–15.

Li, Y., Li, B., Wang, C.-P., Fan, J.-Z., and Sun, H.-W. (2014). "Aerobic Degradation of Trichloroethylene by Co-Metabolism Using Phenol and Gasoline as Growth Substrates." *International Journal of Molecular Sciences*, 15(5), 9134–9148.

Löffler, F. E., Sun, Q., Li, J., and Tiedje, J. M. (2000). "16S rRNA gene-based detection of tetrachloroethene-dechlorinating Desulfuromonas and *Dehalococcoides* species." *Applied and Environmental Microbiology*, 66, 1369-1374.

Löffler, F. E., Yan, J., Ritalahti, K. M., Adrian, L., Edwards, E. a., Konstantinidis, K. T., Müller, J. a., Fullerton, H., Zinder, S. H., and Spormann, A. M. (2013). "*Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Dehalococcoidales* ord. nov. and family *Dehaolococcoidaceae* fam. nov., within the phylum *Chloroflexi*." *International Journal of Systematic and Evolutionary Microbiology*, 63, 625-635.

Lojkasek-Lima, P., Aravena, R., Stash-Shouakar, O., Frape, S. K., Marchesi, M., Fiorenza, S., and Vogan, J. (2011). "Evaluating TCE Abiotic and Biotic Degradation Pathways in a Permeable Reactive Barrier Using Compound Specific Isotope Analysis." *Ground Water Monitoring & Remediation*, 32(1), 59–72.

Lovley, D. R., and Phillips, E. J. (1986). "Organic matter mineralization with reduction of ferric iron in anaerobic sediments." *Applied and Environmental Microbiology*, 51, 683-689.

Lovley, D. R., Phillips, E. J. P., and Lonergan, D. J. (1989). "Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by Alteromonas putrefaciens." *Applied and Environmental Microbiology*, 55(3), 700–706.

Lovley, D. R. (1991). "Dissimilatory Fe (III) and Mn (IV) Reduction." *Microbiological Reviews*, 55(2), 259–287.

Major, D. W., McMaster, M. L., Cox, E. E., Edwards, E. a., Dworatzek, S. M., Hendrickson, E. R., Starr, M. G., Payne, J. A., and Buonamici, L. W. (2002). "Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene." *Environmental Science and Technology*, 36(23), 5106–5116.

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

McCarty, P. L., Chu, M.-Y. Y., and Kitanidis, P. K. (2007). "Electron donor and pH relationships for biologically enhanced dissolution of chlorinated solvent DNAPL in groundwater." *European Journal of Soil Biology*, 43, 276-282.

McLean, J. E., Ervin, J., Zhou, J., Sorensen, D. L., and Dupont, R. R. (2015). "Biostimulation and Bioaugmentation to Enhance Reductive Dechlorination of TCE in a Long-Term Flow Through Column Study." *Groundwater Monitoring & Remediation*, National Ground Water Association, 1–13.

Men, Y., Lee, P. K. H., Harding, K. C., and Alvarez-Cohen, L. (2013). "Characterization of four TCE-dechlorinating microbial enrichments grown with different cobalamin stress and methanogenic conditions." *Applied and Environmental Microbiology*, 97, 6439-6450.

Men, Y., Feil, H., VerBerkmoes, N. C., Shah, M. B., Johnson, D. R., Lee, P. K. H., West, K. a., Zinder, S. H., Andersen, G. L., and Alvarez-Cohen, L. (2012). "Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with Desulfovibrio vulgaris Hildenborough and Methanobacterium congolense: global transcriptomic and proteomic analyses." *The International Society for Microbial Ecology Journal*, 6, 410-421.

Men, Y., Seth, E. C., Yi, S., Allen, R. H., Taga, M. E., and Alvarez-Cohen, L. (2014). "Sustainable growth of *Dehalococcoides* mccartyi 195 by corrinoid salvaging and remodeling in defined lactate-fermenting consortia." *Applied and Environmental Microbiology*, 80(7), 2133–2141.

Mészáros, É., Imfeld, G., Nikolausz, M., and Nijenhuis, I. (2013). "Occurrence of *Dehalococcoides* and reductive dehalogenase genes in microcosms, a constructed wetland and groundwater from a chlorinated ethene contaminated field site as indicators for in situ reductive dehalogenation." *Water, Air, and Soil Pollution*, 224.

Mirza, B. S., Sorensen, D. L., Dupont, R. R., and McLean, J. E. (2015). "*Dehalococcoides* abundance and alternate electron acceptor effects on large, flow-through trichloroethene dechlorinating columns." *Applied Microbiology and Biotechnology*. http://doi.org/10.1007/s00253-015-7112-1

Murch, K. L. (2003). "One-dimensional Modeling of Bromide Tracer and Trichloroethylene Transport Based on Laboratory Experiments in Vertical Soil Columns." Utah State University, Department of Geology.

NAVFAC. (2014). "In Situ Biogeochemical Transformation Processes for Treating Contaminated Groundwater." (September), 1–6.

Pant, P., and Pant, S. (2010). "A review: Advances in microbial remediation of trichloroethylene (TCE)." *Journal of Environmental Sciences*, The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 22(1), 116–126.

Paul, L., Herrmann, S., Koch, C. B., Philips, J., and Smolders, E. (2013). "Inhibition of microbial trichloroethylene dechorination by Fe (III) reduction depends on Fe mineralogy: a batch study using the bioaugmentation culture KB-1." *Water Research*, Elsevier, 47(7), 2543–54.

Parsons Corporation (2004). "Principles and practices of enhanced anaerobic bioremeidation of chlorinated solvents." 1-457.

Pöritz, M., Goris, T., Wubet, T., Tarkka, M. T., Buscot, F., Nijenhuis, I., Lechner, U., and Adrian, L. (2013). "Genome sequences of two dehalogenation specialists - *Dehalococcoides mccartyi* strains BTF08 and DCMB5 enriched from the highly polluted Bitterfeld region." *Federation of European Microbiological Societies*, 343, 101-104.

Russell, H. H., Matthews, J. E., and Sewell, G. W. (1992). "TCE Removal from Contaminated Soil and Ground Water." *EPA Ground Water Issue*, USEPA, 1-10.

Schmidt, M., Lege, S., and Nijenhuis, I. (2014). "Comparison of 1,2-dichloroethane, dichloroethene and vinyl chloride carbon stable isotope fractionation during dechlorination by two *Dehalococcoides* strains." *Water Research*, 52, 146-154.

Schaerlaekens, J., Mallants, D., Šimûnek, J., Van Genuchten, M. T., and Feyen, J. (1999). "Numerical simulation of transport and sequential biodegradation of chlorinated aliphatic hydrocarbons using CHAIN_2D." *Hydrological Processes*, 13(17), 2847–2859.

Schneidewind, U., Haest, P. J., Atashgahi, S., Maphosa, F., Hamonts, K., Maesen, M., Calderer, M., Seuntjens, P., Smidt, H., Springael, D., and Dejonghe, W. (2014). "Kinetics of dechlorination by *Dehalococcoides mccartyi* using different carbon sources." *Journal of Contaminant Hydrology*, Elsevier B.V., 157, 25–36.

Segelken, R. (1997). "Cornell scientists isolate a bioremediation superstar that gobbles up perc and other chlorinated solvents." *Cornell Chronicle*, 1–2.

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

Solutions IES Inc., Terra Systems Inc., and Parsons Infrastructure and Technology Group Inc. (2007). "Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil." U.S. Air Force, Brooks AFB, TX, 6-5.

Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., Johnston, C. T., and Sumner, M. E. (1996). *Methods of Soil Analysis, Part 3-Chemical Methods*, Soil Science Society of America Inc., Madison.

Spirtas, R., Stewart, P. a., Lee, J. S., Marano, D. E., Forbes, C. D., Grauman, D. J., Pettigrew, H. M., Blair, A., Hoover, R. N., and Cohen, J. L. (1991). "Retrospective cohort mortality study of workers at an aircraft maintenance facility. I Epidemiological results." *British Journal of Industrial Medicine*, 48, 515-530.

Steele, B. (2015). "Gene sequencing explains why natural bioremediation ' bug ' has big appetite for chlorinated pollutants , Cornell researchers report." *Cornell Chronicle*, Ithaca, NY, 1-2.

Stroo, H. F., and Ward, C. H. (2010). *In Situ Remediation of Chlorinated Solvent Plumes*. SERDP and ESTCP Remediation Technology Monograph Series, Springer Science Business Media, New York.

Stroo, H. F., Ward, C. H., and Leeson, A. (2013). *Bioaugmentation for Groundwater Remediation*. SERDP and ESTCP Remediation Technology Monograph Series, Springer, New York.

Tang, Y. J., Chakraborty, R., Martín, H. G., Chu, J., Hazen, T. C., and Keasling, J. D. (2007). "Flux analysis of central metabolic pathways in Geobacter metallireducens during reduction of soluble Fe(III)-nitrilotriacetic acid." *Applied and Environmental Microbiology*, 73(12), 3859–3864.

Ulsamer, S. (2011). "A Model to Characterize the Kinetics of Dechlorination of Tetrachloroethylene and Trichloroethylene By a Zero Valent Iron Permeable Reactive Barrier."M.S. Thesis, Worcester Polytechnic Institute Worcester, MA.

U.S. Department of Health and Human Services (2015). "Trichloroethylene TOXMap beta." U.S. National Library of Medicine Bethesda, Maryland.

United States Protection Agency (USEPA) (1991). "Record of Decision System (RODS) Hill Air Force Base." Superfund Information Systems, 2-4.

United States Protection Agency (USEPA). (1994). "Method 6020; Inductively Coupled Plasma- Mass Spectrometry." (September), 1–18.

United States Protection Agency (USEPA) (1996). "Method 5021 Volatile Organic Compounds in Soils and Other Solid Matrices Using Equilibrium Headspace Analysis." 1, 1-13.

United States Protection Agency (USEPA) (1996). "Method 8260b Volatile Organic Compounds by Gas Chomatography/ Mass Spectrometry (GC/MS)."

United State Protection Agency (USEPA). (2000a). "Integrated Risk Information System; Vinyl Chloride." <http://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/1001tr.pdf>

United States Protection Agency (USEPA) (2000b). "Engineered approaches to in situ bioremediation of chlorinated solvents: Fundamentals and field applications." U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Technology Innovation Office, Washington, DC, 1-144.

United States Protection Agency (USEPA) (2004). "In Situ Thermal Treatment of Chlorinated Solvents : Fundamentals and Field Applications." U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Office of Superfund Remediation and Technology Innovation, Washington, DC.

United States Protection Agency (USEPA). (2008). "Five Year Review Report for Hill Air Force Base" United States Protection Agency Region 8. Denver, CO.

United States Environmental Protection Agency. (2009). "Identification and Characterization Methods for Reactive Minerals Responsible for Natural Attenuation of Chlorinated Organic Compounds in Ground Water." (December).

United States Protection Agency (USEPA). (2014). "TSCA Work Plan Chemical Risk Assessment Trichloroethylene : Degreasing , Spot Cleaning and Arts & Crafts Uses." (June).

URS Corporation (2003). "FINAL CERCLA Five-Year Review Report for Hill Air Force Base." U.S. Department of Defense, Hill Air Force Base Environmental Management Directorate, Salt Lake City.

Van Griethuysen, C., Gillissen, F., and Koelmans, A. A. (2002). "Measuring acid volatile sulphide in floodplain lake sediments: effect of reaction time, sample size and aeration." *Chemosphere*, 47, 395-400.

Wei, N., and Finneran, K. T. (2011). "Influence of ferric iron on complete dechlorination of trichloroethylene (TCE) to ethene: Fe(III) reduction does not always inhibit complete dechlorination." *Environmental Science and Technology*, 45(17), 7422–7430.

Wei, N., and Finneran, K. T. (2013). "Low and high acetate amendments are equally as effective at promoting complete dechlorination of trichloroethylene (TCE)." *Biodegradation*, 24(3), 413–25.

Zhang, Y., Rodionov, D. A., Gelfand, M. S., & Gladyshev, V. N. (2009). "Comparative genomic analyses of nickel, cobalt and vitamin B12 utilization." *BMC Genomics*, 10, 78. http://doi.org/10.1186/1471-2164-10-78

Zhang, Y., and Gladyshev, V. N. (2010). "General trends in trace element utilization revealed by comparative genomic analyses of Co, Cu, Mo, Ni, and Se." *Journal of Biological Chemistry*, 285(5), 3393–3405.

Zhou, J. (2008). "The development of molecular tools for the evaluation of the bioremediation of chlorinated solvents." Utah State University, ProQuest.

APPENDICES

Appendix A

Bromide Tracer Study

After each set of 12 columns from lactate, whey and control treatments, groundwater was pumped through the soil to allow the soil to settle and repack if visible channels or cracks formed. Prior to the experiment, a tracer study was performed in order to determine the average residence time of the columns for each treatment. Ideally, the average hydraulic retention time of each treatment would be identical to ensure that each treatment would receive the same carbon loading rate. The tracer solution consisted of bromide (10 mg/L) and Hill AFB (OU-5) groundwater. Based on the total volume of each column (~350 mL), the soil pore volume (~100 mL), and the aquifer flow velocity (0.08 cm/day), the hydraulic retention time of each column should be approximately 24 hours with a flow rate of approximately 125 mL/day. Each column, ideally, will act as a plug flow system.

The column end caps had a pore volume of approximately 50 mL. Once the end caps fill with the feed solution it should act as a plug and move up through the columns. In order to fill the column caps, 600 mL of a 10 mg/L bromide solution was split between 12 columns at a flow of at 125 mL/day for 10 hours, and the flow was then switched to fresh groundwater. The bromide concentrations in the column effluent, along with the effluent mass, were monitored every few hours until the bromide exited the system and was no longer detected. These data assisted with determining if all columns were hydraulically identical, or if are any reaction or accumulation occurred within the column.

Bromide concentrations leaving the lactate (Figure A.1), control (Figure A.2), and whey (Figure A.3), columns were recorded. The concentration versus time tracer response curves were determined by following the procedure described in Chapter 4 of Waste Water Engineering Treatment and Reuse (Metcalf & Eddy, Inc. McGraw Hill 2003). The mean HRTs of the 12 columns were averaged to determine the average HRT of the entire treatment. Based on the flow and concentrations exiting each column, the lactate treatment had an average (mean ± 95% CI) hydraulic residence time of 30.1 ± 3.8 hours, controls had an average residence time of 30.7 ± 1.1 hours, and the whey treatment had an HRT of 25.2 ± 1.1 hours.

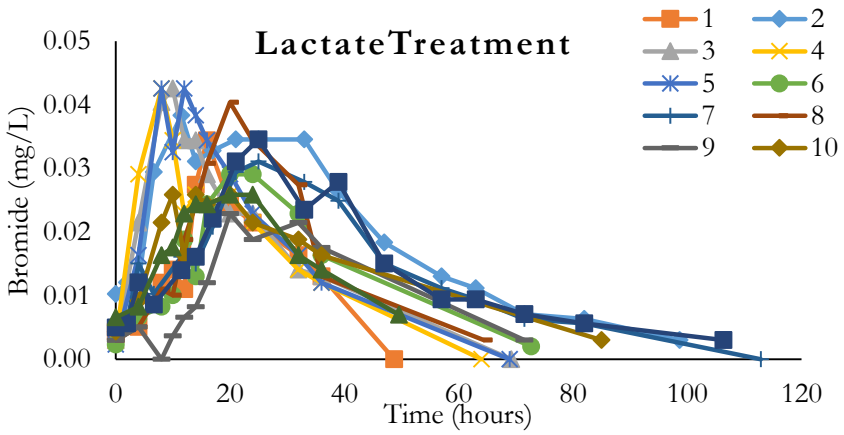


Figure A.1 Effluent bromide tracer concentrations of each column (1-12) of the lactate treatment with time.

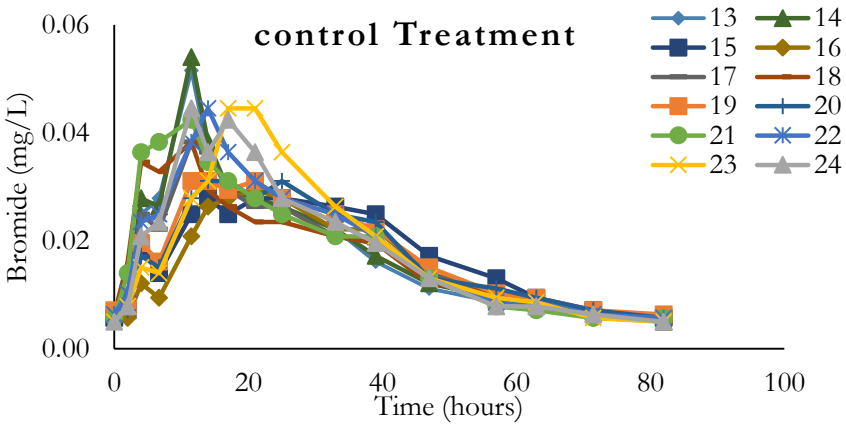


Figure A.2 Effluent bromide tracer concentrations of each column (13-24) over time in control treatment.

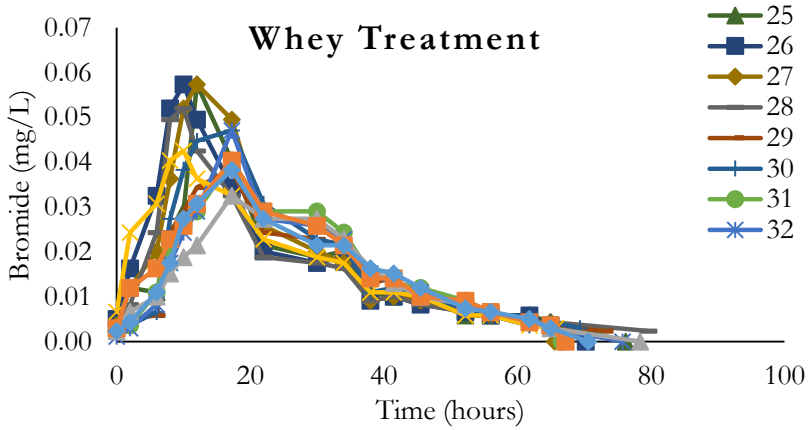


Figure A.3 Effluent bromide tracer concentrations of each column (25-36) over time in whey treatment.

Appendix B

Effluent Dissolved Organic Carbon vs. Total Low Molecular Weight Organic Acids

For most the of study there was not a statistical difference at each sampling event between the effluent total dissolved organic carbon (DOC) and total dissolved carbon in the form of LMWOAs (mg-C/L) in the whey (Figure B.1) and lactate treatments (Figure B.2).

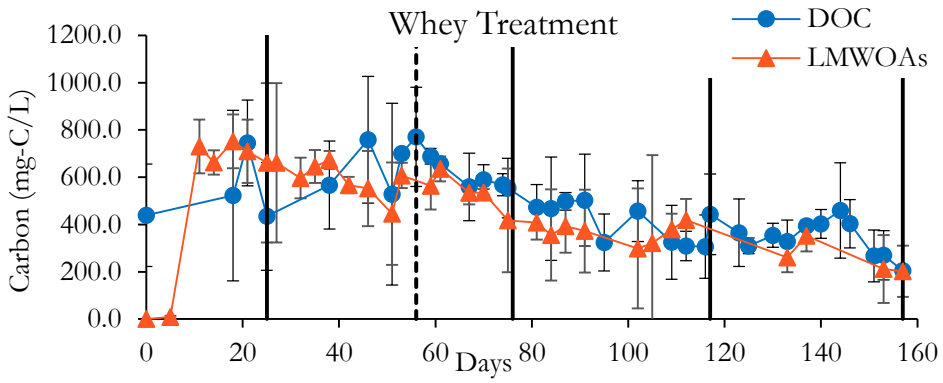


Figure B.1 Comparison between total dissolved carbon measured as DOC or sum of LMWOAs in the whey treatment. Solid lines represent Sacrifice times. Error bars represent 95% confidence intervals of replicate measurements

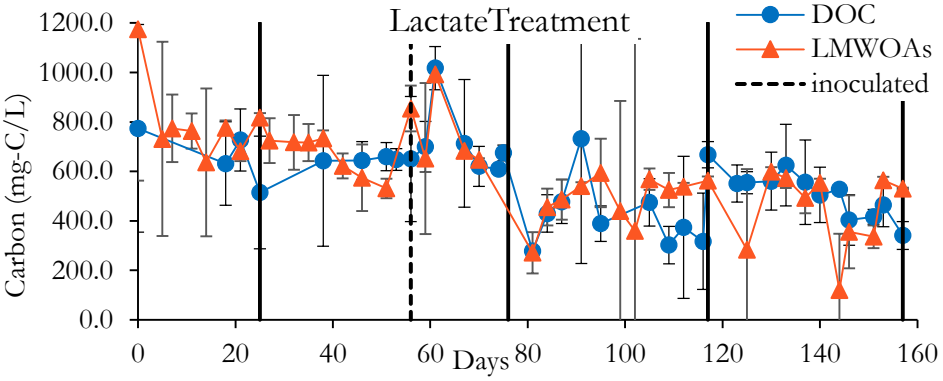
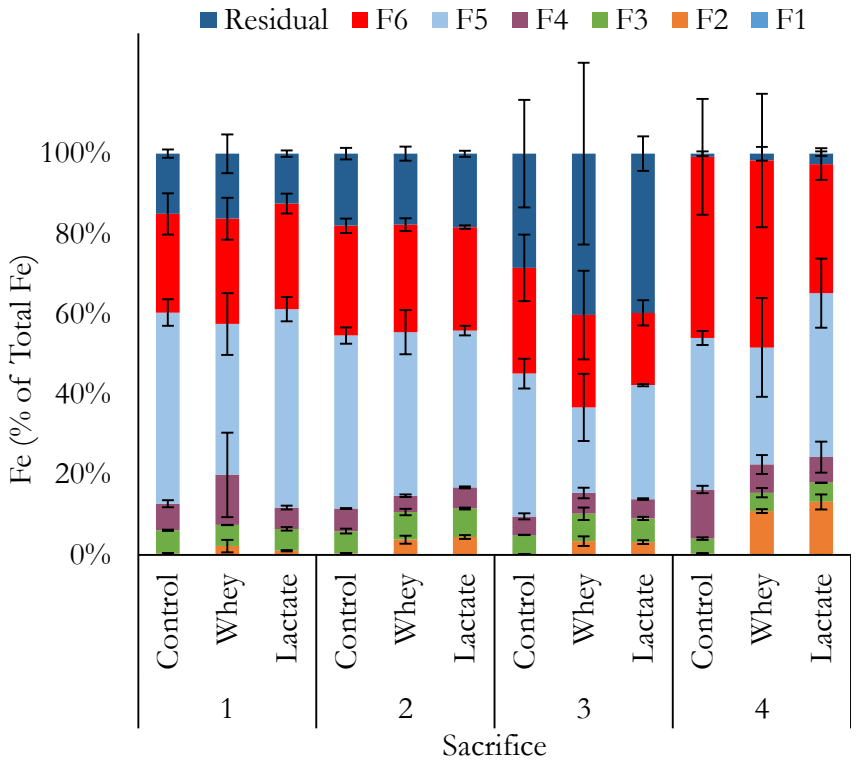


Figure B.2 Comparison between total dissolved carbon measured as DOC or sum of LMWOAs in the lactate treatment. Solid lines represent Sacrifice times. Error bars represent 95% confidence intervals of replicate measurements

Appendix C

Table C.1 Iron mineralogy of each treatment at each sacrifice, measured during the sequential extraction. Iron minerals as percent of total extracted iron. Error bars= 95% confidence intervals.



Appendix D

Redundancy Analysis

Using the statistical software R, and the package "vegan," a redundancy analysis (RDA) using the results that are specific to each triplicate sample collected from each treatment at Sacrifice 1 are shown below. The ordination plot includes the triplicates of each treatment and their association with TCE and degradation byproducts measured in the effluent. The "environmental factors" correlated with the ordination of each treatment are overlaid, only those that have a significance (p-value ≤0.05) are displayed.

Table D1. R output of the resulting RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 1, using "Vegan" package with "rda".

```
Call:
rda(X = sac.lwc, distance = "bray", trace = FALSE, trymax = 100)

Partitioning of variance:
               Inertia Proportion
Total         9.608e-05          1
Unconstrained 9.608e-05          1

Eigenvalues, and their contribution to the variance

Importance of components:
                            PC1      PC2
Eigenvalue            9.608e-05 1.13e-10
Proportion Explained  1.000e+00 0.00e+00
Cumulative Proportion 1.000e+00 1.00e+00

Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  0.1665083
```

Table D2. R output of resulting principal components from the RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 1, using "Vegan" package with "rda".

```
Species scores

          PC1         PC2
TCE 0.1665081 -1.950e-07
DCE 0.0001798  1.805e-04
VC  0.0000000  0.000e+00


Site scores (weighted sums of species scores)

          PC1      PC2
C1A -0.026585 -0.06584
C1B -0.053971 -0.03857
C1C -0.008628 -0.08372
L1A  0.056234  0.07324
L1B  0.040078  0.03394
L1C  0.117312 -0.04297
W1A -0.026525  0.04488
W1B -0.047665  0.06593
W1C -0.050248  0.01311
```

Table D3. R output of the "environmental factors" correlating with TCE degradation byproducts associated with each treatment at Sacrifice 1, using "Vegan" package with "envfit".

```
***VECTORS

                PC1      PC2     r2 Pr(>r)
aceC        0.06841  0.99766 0.5843  0.083 .
propC       0.92175  0.38778 0.9083  0.003 **
butC       -0.67299  0.73965 0.5741  0.083 .
FeII.Eff   -0.30612  0.95199 0.7042  0.039 *
EC          0.74199  0.67041 0.9378  0.001 ***
pH         -0.27747 -0.96073 0.6332  0.052 .
Eh         -0.31641 -0.94862 0.6441  0.046 *
DIC         0.92081  0.39000 0.9328  0.002 **
H2          0.54517 -0.83832 0.0966  0.718
DHC        -0.58979  0.80755 0.1596  0.629
tceA       -0.99688  0.07890 0.3535  0.259
vcrA       -0.93684  0.34977 0.4356  0.182
Sulfide     0.00000  0.00000 0.0000  1.000
FeII.Solid  0.45659  0.88968 0.8268  0.019 *
F1.Fe       0.78488  0.61964 0.4511  0.172
F4.Fe      -0.64156  0.76707 0.2789  0.305
F5.Fe       0.98936 -0.14550 0.3211  0.310
F6.Fe       0.84212  0.53929 0.1535  0.608
F2.Fe.II.  -0.22760  0.97375 0.4499  0.145
F3.Fe.II.  -0.60351  0.79735 0.2561  0.409
K          -0.83337  0.55271 0.1965  0.515
V          -0.89465 -0.44676 0.6176  0.089 .
Mn         -0.08367  0.99649 0.5066  0.152
Co         -0.38200  0.92416 0.4641  0.177
As         -0.09846  0.99514 0.6505  0.049 *
Se         -0.39970 -0.91665 0.7442  0.030 *
Ba          0.67722  0.73578 0.6658  0.048 *
Zn          0.23790  0.97129 0.7333  0.018 *
Mg         -0.08746  0.99617 0.2220  0.456
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999
```

Table D4. R output of the resulting RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 2, using "Vegan" package with "rda".

```
Call:
rda(X = sac.lwc, distance = "bray", trace = FALSE, trymax = 100)

Partitioning of variance:
              Inertia Proportion
Total         0.002195          1
Unconstrained 0.002195          1

Eigenvalues, and their contribution to the variance

Importance of components:
                          PC1       PC2       PC3
Eigenvalue            0.00215 4.435e-05 5.505e-07
Proportion Explained  0.97955 2.020e-02 2.500e-04
Cumulative Proportion 0.97955 9.998e-01 1.000e+00

Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  0.3640358
```

Table D5. R output of resulting principal components from the RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 2, using "Vegan" package with "rda".

```
Species scores

         PC1       PC2        PC3
TCE -0.26420 -0.035013  3.800e-04
DCE  0.24433 -0.038026 -4.851e-06
VC   0.01766  0.002281  5.752e-03


Site scores (weighted sums of species scores)

         PC1      PC2       PC3
C2A -0.10589  0.15377 -0.092098
C2B -0.11525  0.09361 -0.039505
C2C -0.11578  0.09021 -0.036532
L2A  0.14667 -0.11033 -0.145526
L2B  0.12716  0.03440 -0.002224
L2C  0.12732  0.03857  0.098201
W2A  0.12174  0.07710  0.159180
W2B -0.02734 -0.18909 -0.148609
W2C -0.15862 -0.18823  0.207113
```

Table D6. R output of the "environmental factors" correlating with TCE degradation byproducts associated with each treatment at Sacrifice 2, using "Vegan" package with "envfit".

```
***VECTORS

               PC1       PC2     r2 Pr(>r)
aceC        0.73314 -0.68008 0.6943  0.110
propC       0.90650 -0.42220 0.7075  0.062 .
butC       -0.10515 -0.99446 0.1976  0.568
FeII.Eff    0.92354 -0.38350 0.1399  0.795
EC          0.99920  0.04000 0.5928  0.191
pH         -0.43328  0.90126 0.6989  0.109
Eh         -0.70502  0.70918 0.8543  0.032 *
DIC         0.59730  0.80202 0.6696  0.125
H2         -0.72373 -0.69008 0.4342  0.340
DHC        -0.18600 -0.98255 0.6895  0.087 .
tceA        0.28570 -0.95832 0.3482  0.409
vcrA        0.23069 -0.97303 0.2383  0.590
Sulfide    -0.43092 -0.90239 0.8884  0.021 *
FeII.Solid  0.68737 -0.72631 0.9180  0.031 *
F1.Fe       0.98978 -0.14260 0.8077  0.036 *
F4.Fe       0.18888  0.98200 0.3692  0.419
F5.Fe       0.07569  0.99713 0.5514  0.205
F6.Fe      -0.58388  0.81184 0.0269  0.966
F2.Fe.II.   0.84371 -0.53679 0.9411  0.007 **
F3.Fe.II.   0.49504 -0.86887 0.8702  0.028 *
K          -0.35450 -0.93505 0.3517  0.487
V          -0.95398  0.29989 0.7631  0.058 .
Mn          0.28187 -0.95945 0.2255  0.602
Co          0.70753 -0.70668 0.5777  0.189
As          0.30350 -0.95283 0.4928  0.249
Se         -0.71548  0.69863 0.5087  0.263
Ba          0.92361 -0.38334 0.8670  0.022 *
Zn          0.88738 -0.46104 0.8456  0.026 *
Mg          0.87817  0.47835 0.1799  0.726
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999

2 observations deleted due to missingness
```

Table D7. R output of the resulting RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 3, using "Vegan" package with "rda".

```
Call:
rda(X = sac.lwc, distance = "bray", trace = FALSE, trymax = 100)

Partitioning of variance:
              Inertia Proportion
Total         0.002651          1
Unconstrained 0.002651          1

Eigenvalues, and their contribution to the variance

Importance of components:
                            PC1       PC2       PC3
Eigenvalue            0.001763 0.0008805 7.124e-06
Proportion Explained  0.665160 0.3321500 2.690e-03
Cumulative Proportion 0.665160 0.9973100 1.000e+00

Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  0.3816052
```

Table D8. R output of resulting principal components from the RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 3, using "Vegan" package with "rda".

```
Species scores

          PC1       PC2       PC3
TCE -0.25771 -0.04432 -0.010351
DCE  0.09369  0.13800 -0.014207
VC   0.14721 -0.16541 -0.009078


Site scores (weighted sums of species scores)

          PC1       PC2       PC3
C3A -0.17012 -0.02831  0.06360
C3B -0.18029 -0.03181 -0.03742
C3C -0.18082 -0.03199 -0.04271
L3A  0.09982 -0.05994  0.05059
L3B  0.10449 -0.07891  0.01469
L3C  0.13541 -0.23862 -0.07130
W3A  0.06241  0.17491 -0.02939
W3B  0.06041  0.11557  0.27923
W3C  0.06870  0.17910 -0.22729
```

Table D9. R output of the "environmental factors" correlating with TCE degradation byproducts associated with each treatment at Sacrifice 3, using "Vegan" package with "envfit".

```
***VECTORS

               PC1       PC2     r2 Pr(>r)
aceC       0.99451  0.10462 0.9959  0.001 ***
propC      0.83100 -0.55627 0.9228  0.021 *
butC       0.38458  0.92309 0.8863  0.008 **
FeII.Eff   0.66025  0.75105 0.8894  0.008 **
EC        -0.96391 -0.26622 0.3858  0.276
pH        -0.84443 -0.53567 0.9495  0.001 ***
Eh        -0.96651 -0.25663 0.9975  0.001 ***
DIC        0.63421 -0.77316 0.5190  0.183
H2         0.58549  0.81068 0.4851  0.153
DHC        0.88033 -0.47435 0.6685  0.059 .
tceA       0.97618 -0.21694 0.8080  0.020 *
vcrA       0.99624 -0.08658 0.6874  0.073 .
Sulfide    0.48660  0.87363 0.7778  0.036 *
FeII.Solid 0.96238  0.27171 0.9923  0.002 **
F1.Fe     -0.99715 -0.07538 0.3140  0.359
F4.Fe      0.45597 -0.88999 0.1358  0.720
F5.Fe     -0.69096 -0.72289 0.6687  0.040 *
F6.Fe     -0.84322 -0.53757 0.4633  0.187
F2.Fe.II.  0.99527  0.09714 0.9896  0.008 **
F3.Fe.II.  0.88438  0.46677 0.9242  0.002 **
K          0.36456  0.93118 0.9077  0.001 ***
V         -1.00000  0.00112 0.9304  0.007 **
Mn         0.72869  0.68484 0.8618  0.007 **
Co         0.99522  0.09770 0.9330  0.011 *
As         0.92135  0.38873 0.7614  0.039 *
Se        -0.98864 -0.15031 0.8441  0.020 *
Ba         0.77030 -0.63768 0.7995  0.038 *
Zn         0.99995  0.00963 0.5387  0.127
Mg        -0.99652 -0.08338 0.4277  0.261
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999

1 observation deleted due to missingness
```

Table D10. R output of the resulting RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 4, using "Vegan" package with "rda".

```
Call:
rda(X = sac.lwc, distance = "bray", trace = FALSE, trymax = 100)

Partitioning of variance:
              Inertia Proportion
Total         0.001311          1
Unconstrained 0.001311          1

Eigenvalues, and their contribution to the variance

Importance of components:
                             PC1       PC2       PC3
Eigenvalue            0.0009953 0.0002834 3.201e-05
Proportion Explained  0.7593500 0.2162300 2.443e-02
Cumulative Proportion 0.7593500 0.9755700 1.000e+00

Scaling 2 for species and site scores
* Species are scaled proportional to eigenvalues
* Sites are unscaled: weighted dispersion equal on all dimensions
* General scaling constant of scores:  0.3199978
```

Table D11. R output of resulting principal components from the RDA and initial ordination of TCE degradation byproducts associated with each treatment at Sacrifice 4, using "Vegan" package with "rda".

```
Species scores

          PC1       PC2       PC3
TCE -0.25648  0.03227 -0.01636
DCE  0.09164  0.13112 -0.01700
VC   0.05981 -0.06251 -0.04410


Site scores (weighted sums of species scores)

          PC1        PC2       PC3
C4A -0.10111 -0.003587  0.09248
C4B -0.16589  0.025037 -0.03595
C4C -0.17411  0.028666 -0.05224
L4A  0.06333 -0.098839 -0.01934
L4B  0.07012 -0.137492 -0.20206
L4C  0.05759 -0.102571  0.06389
W4A  0.05448 -0.054168  0.20693
W4B  0.09712  0.171877 -0.01508
W4C  0.09848  0.171076 -0.03863
```

Table D12. R output of the "environmental factors" correlating with TCE degradation byproducts associated with each treatment at Sacrifice 4, using "Vegan" package with "envfit".

```
***VECTORS

               PC1       PC2     r2 Pr(>r)
aceC       0.75592 -0.65466 0.7794  0.050 *
propC      0.59223 -0.80577 0.6395  0.108
butC       0.71598  0.69812 0.7286  0.056 .
FeII.Eff   0.83482  0.55053 0.7169  0.051 .
EC        -0.99557  0.09400 0.4289  0.251
pH        -0.99708 -0.07635 0.9643  0.001 ***
Eh        -0.99809  0.06170 0.9644  0.002 **
DIC       -0.21244  0.97717 0.5663  0.057 .
H2         0.99545 -0.09532 0.1152  0.862
DHC        0.97911  0.20335 0.8685  0.006 **
tceA       0.94774 -0.31904 0.6611  0.094 .
vcrA       0.99164  0.12904 0.7466  0.035 *
Sulfide    0.99397 -0.10965 0.9242  0.012 *
FeII.Solid 0.99795 -0.06395 0.9404  0.023 *
F1.Fe     -0.98066  0.19574 0.5232  0.110
F4.Fe     -0.98668  0.16266 0.7838  0.037 *
F5.Fe     -0.98151 -0.19141 0.6573  0.073 .
F6.Fe     -0.98172 -0.19034 0.3171  0.411
F2.Fe.II.  0.98798 -0.15461 0.9470  0.016 *
F3.Fe.II.  0.98637  0.16457 0.9534  0.004 **
K          0.70634  0.70788 0.7412  0.047 *
V         -0.94313  0.33243 0.6591  0.085 .
Mn         0.81098  0.58507 0.8984  0.009 **
Co         0.98919 -0.14666 0.8718  0.019 *
As         0.99814  0.06100 0.8329  0.015 *
Se        -0.98170  0.19045 0.9667  0.005 **
Ba         0.52832 -0.84904 0.9295  0.009 **
Zn         0.69602 -0.71802 0.3015  0.455
Mg        -0.70255  0.71164 0.3541  0.311
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999

1 observation deleted due to missingness
```

Appendix E

Correlations

Tables E.1 through E.5 describe correlations that were found to be significant through redundancy analyses between TCE and degradation byproducts (DCE and VC), carbon metabolites (acetate, propionate, butyrate), pH, EC, Eh, and HCO3, which were measured in the effluent, along with iron measured in the solids, and metals measured in the porewater.

Sacrifice 1 Correlations

Table E.1. Correlation matrix comparing statistically significant variables from the redundancy analysis of Sacrifice 1, along with carbon metabolites. The Pearson R correlation coefficient is displayed below the diagonal and the p-values associated with correlations are displayed above the diagonal.

	TCE	DCE	Acetate	Propionate	Butyrate	pH	EC	Eh	Fe(II) Eff	HCO_3	Fe (II) Solid	Na	V	Zn	Se	Ba
TCE		**0.02**	0.89	**0.00**	0.16	0.65	**0.03**	0.42	0.50	**0.00**	0.27	0.55	**0.03**	0.60	0.37	0.12
DCE	**0.73**		0.10	**0.00**	0.99	**0.04**	**0.00**	**0.02**	0.35	**0.00**	**0.00**	0.24	**0.01**	**0.02**	**0.01**	**0.00**
Acetate	0.05	0.58		0.38	**0.02**	**0.00**	0.05	**0.00**	**0.00**	0.46	**0.00**	**0.02**	0.39	**0.00**	**0.00**	**0.05**
Propionate	**0.88**	**0.91**	0.33		0.37	0.24	**0.00**	0.14	0.98	**0.00**	**0.04**	0.72	0.01	0.17	0.13	**0.04**
Butyrate	-0.51	0.00	**0.76**	-0.34		**0.04**	0.83	0.12	**0.00**	0.34	0.26	0.06	0.59	0.18	0.10	0.59
pH	-0.17	**-0.70**	**-0.94**	-0.43	**-0.68**		**0.02**	**0.00**	**0.00**	0.30	**0.00**	**0.04**	0.15	**0.01**	**0.00**	**0.02**
EC	**0.71**	**0.97**	**0.66**	**0.90**	0.08	**-0.75**		**0.01**	0.26	**0.00**	**0.00**	0.22	**0.03**	**0.03**	**0.01**	**0.01**
Eh	-0.31	**-0.74**	**-0.89**	-0.53	-0.56	**0.93**	**-0.78**		**0.02**	0.21	**0.00**	0.09	0.10	**0.01**	**0.00**	**0.02**
Fe(II) Effluent	-0.26	0.35	**0.92**	0.01	**0.93**	**-0.86**	0.42	**-0.76**		0.98	**0.03**	**0.01**	0.90	**0.02**	**0.01**	0.21
HCO_3	**0.89**	**0.91**	0.28	**0.99**	-0.36	-0.39	**0.90**	-0.47	-0.01		0.06	0.73	**0.02**	0.22	0.16	0.06
Fe (II) Solid	0.42	**0.88**	**0.88**	**0.70**	0.42	**-0.93**	**0.91**	**-0.95**	**0.70**	**0.65**		0.06	0.05	**0.00**	**0.00**	**0.01**
Na	-0.23	0.44	**0.75**	0.14	**0.65**	**-0.69**	0.45	-0.59	**0.81**	0.14	**0.64**		0.72	**0.01**	0.06	0.14
V	**-0.70**	**-0.81**	-0.33	**-0.82**	0.21	0.52	**-0.73**	0.58	-0.05	**-0.75**	**-0.67**	-0.14		0.15	0.10	**0.01**
Zn	0.20	**0.74**	**0.85**	0.50	0.49	**-0.82**	**0.72**	**-0.78**	**0.74**	0.46	**0.85**	**0.82**	-0.52		**0.00**	**0.00**
Se	-0.34	**-0.80**	**-0.91**	-0.54	-0.58	**0.97**	**-0.82**	**0.95**	**-0.80**	-0.51	**-0.95**	**-0.64**	0.58	**-0.85**		**0.01**
Ba	0.55	**0.85**	**0.67**	**0.69**	0.21	**-0.74**	**0.79**	**-0.75**	0.47	**0.64**	**0.81**	0.54	**-0.78**	**0.88**	**-0.83**	

Sacrifice 2 Correlations

Table E.2. Correlation matrix comparing statistically significant variables from the redundancy analysis of Sacrifice 2. The Pearson R correlation coefficient is displayed below the diagonal and the p-values associated with correlations are displayed above the diagonal.

	TCE	DCE	VC	Acetate	Propionate	Butyrate	pH	Eh	Sulfide	Fe(II) Solid	F2 Fe(II)	F3 Fe(II)	Fe(II) PW	Na	V	Zn	Se	Ba
TCE		**0.00**	**0.00**	0.08	**0.01**	0.62	0.06	0.07	0.17	0.06	0.20	0.36	0.15	0.06	**0.01**	**0.01**	0.21	**0.00**
DCE	**-0.96**		**0.00**	**0.02**	**0.01**	0.93	**0.01**	**0.01**	0.42	**0.01**	0.05	0.10	**0.03**	**0.02**	**0.00**	**0.00**	0.08	**0.00**
VC	**-0.95**	**0.91**		**0.02**	**0.02**	0.97	**0.05**	**0.04**	0.39	**0.04**	0.25	0.30	0.09	0.05	**0.02**	**0.00**	0.16	**0.01**
Acetate	**-0.62**	**0.75**	**0.75**		0.14	0.12	**0.00**	**0.00**	0.45	**0.00**	**0.05**	**0.01**	**0.00**	**0.01**	0.11	**0.00**	**0.01**	0.09
Propionate	**-0.78**	**0.81**	**0.74**	0.53		0.30	0.20	0.05	0.68	**0.01**	0.29	0.31	0.16	0.15	**0.00**	0.05	0.18	**0.00**
Butyrate	0.19	-0.04	-0.02	0.56	-0.39		0.16	0.26	0.09	0.50	0.26	0.08	0.11	0.42	0.43	0.52	0.21	0.42
pH	**0.65**	**-0.80**	**-0.67**	**-0.89**	-0.47	-0.52		**0.00**	0.87	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	0.08	**0.01**	**0.00**	0.11
Eh	**0.63**	**-0.79**	**-0.70**	**-0.95**	**-0.66**	-0.42	**0.92**		0.47	**0.00**	**0.01**	**0.00**	**0.00**	**0.02**	**0.03**	**0.01**	**0.00**	**0.04**
Sulfide	0.50	-0.31	-0.33	0.29	-0.16	0.60	-0.06	-0.27		0.53	0.84	0.26	0.37	0.79	0.55	0.99	0.44	0.60
Fe(II) Solid	**-0.64**	**0.81**	**0.68**	**0.88**	**0.77**	0.26	**-0.86**	**-0.98**	0.24		**0.01**	**0.00**	**0.00**	**0.02**	**0.01**	**0.01**	**0.00**	**0.01**
F2 Fe(II)	-0.47	**0.66**	0.43	**0.67**	0.39	0.42	**-0.90**	**-0.80**	0.08	**0.78**		**0.00**	**0.01**	0.10	0.10	0.13	**0.02**	0.22
F3 Fe (II)	-0.34	0.58	0.39	**0.83**	0.38	**0.61**	**-0.90**	**-0.90**	0.42	**0.87**	**0.87**		**0.00**	0.06	0.18	**0.05**	**0.01**	0.21
Fe(II) PW	-0.52	**0.71**	0.59	**0.95**	0.51	0.56	**-0.94**	**-0.97**	0.34	**0.93**	**0.81**	**0.96**		**0.02**	0.09	**0.01**	**0.00**	0.09
Na	**0.65**	**-0.77**	**-0.66**	**-0.78**	-0.52	-0.31	**0.76**	**0.74**	-0.11	**-0.74**	-0.59	**-0.65**	**-0.77**		0.08	**0.00**	0.07	0.06
V	**0.82**	**-0.88**	**-0.74**	-0.56	**-0.96**	0.30	**0.62**	**0.72**	0.23	**-0.82**	-0.58	-0.49	**-0.60**	**0.60**		**0.04**	0.07	**0.00**
Zn	**-0.82**	**0.89**	**0.85**	**0.88**	**0.66**	0.25	**-0.81**	**-0.83**	-0.01	**0.81**	0.55	**0.67**	**0.82**	**-0.86**	**-0.69**		**0.05**	**0.01**
Se	0.46	**-0.62**	-0.52	**-0.82**	-0.49	-0.46	**0.84**	**0.88**	-0.30	**-0.84**	**-0.74**	**-0.79**	**-0.87**	**0.63**	**0.62**	**-0.67**		0.13
Ba	**-0.85**	**0.89**	**0.77**	0.59	**0.96**	-0.31	-0.57	**-0.70**	-0.21	**0.80**	0.45	0.46	0.60	**-0.65**	**-0.96**	**0.79**	-0.54	

Sacrifice 3 Correlations

Table E.3. Correlation matrix comparing statistically significant variables from the redundancy analysis of Sacrifice 3. The Pearson R correlation coefficient is displayed below the diagonal and the p-values associated with correlations are displayed above the diagonal.

	TCE	DCE	VC	Acetate	Propionate	Butyrate	pH	Eh	Sulfide	F2 Total Fe	F5 Total Fe	Fe(II) Solids	F2 Fe(II)	F3 Fe(II)	Fe(II) PW	Co	K	V	Mn	Cu	As	Se	Ba
TCE		**0.04**	0.15	**0.01**	0.09	0.17	**0.00**	**0.00**	0.17	**0.00**	0.08	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.17	**0.00**	**0.01**	**0.00**	**0.01**	**0.00**	0.06
DCE	**-0.69**		0.53	0.24	0.84	**0.00**	**0.00**	**0.02**	**0.00**	0.13	**0.02**	**0.02**	0.07	**0.00**	**0.01**	0.06	**0.00**	0.14	**0.00**	0.13	**0.05**	0.06	0.95
VC	-0.53	-0.24		0.06	**0.00**	0.30	0.66	0.23	0.52	0.15	0.74	0.26	0.32	0.54	0.40	0.27	0.26	0.07	0.95	0.16	0.36	0.18	**0.02**
Acetate	**-0.79**	0.44	**0.64**		**0.01**	0.83	**0.05**	**0.01**	0.23	0.06	0.77	**0.02**	0.23	**0.04**	0.06	0.16	0.83	**0.01**	0.17	0.07	**0.01**	**0.03**	0.22
Propionate	**-0.60**	-0.08	**0.93**	**0.79**		0.31	0.52	0.15	0.67	0.08	0.74	0.16	0.35	0.38	0.28	0.32	0.30	**0.02**	0.94	0.13	0.32	0.17	**0.01**
Butyrate	-0.50	-0.39	**0.89**	0.08	-0.38		**0.01**	0.10	**0.01**	0.35	**0.01**	0.10	0.07	**0.05**	**0.05**	0.07	**0.00**	0.44	**0.00**	0.27	0.17	0.16	0.81
pH	**0.90**	-0.17	**-0.67**	**-0.67**	-0.25	**-0.78**		**0.00**	**0.01**	**0.03**	**0.03**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.01**	**0.02**	**0.00**	**0.01**	**0.00**	**0.00**	0.44
Eh	**0.99**	-0.44	**-0.75**	**-0.77**	-0.53	-0.58	**0.93**		0.11	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.10	**0.00**	**0.01**	**0.00**	**0.01**	**0.00**	0.10
Sulfide	-0.50	-0.25	**0.87**	0.44	-0.16	**0.80**	**-0.80**	-0.57		0.60	0.22	0.11	0.33	0.06	0.10	0.28	**0.01**	0.44	**0.01**	0.44	**0.05**	0.17	0.52
F2 Total Fe	**-0.92**	0.54	0.53	**0.64**	**0.62**	0.36	**-0.73**	**-0.90**	0.20		0.05	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	0.32	**0.00**	0.06	**0.00**	0.08	**0.00**	**0.01**
F5 Total Fe	**0.62**	0.13	**-0.75**	-0.11	0.13	**-0.81**	**0.71**	**0.67**	-0.45	**-0.66**		**0.04**	**0.01**	**0.02**	**0.02**	**0.01**	**0.00**	0.15	**0.01**	0.11	0.15	**0.04**	0.54
Fe(II) Solids	**-0.99**	0.42	**0.76**	**0.76**	0.52	0.59	**-0.93**	**-1.00**	0.57	**0.91**	**-0.68**		**0.00**	**0.00**	**0.00**	**0.00**	0.10	**0.00**	**0.01**	**0.00**	**0.01**	**0.00**	0.09
F2 Fe(II)	**-0.89**	0.38	**0.63**	0.45	0.35	**0.63**	**-0.81**	**-0.90**	0.37	**0.89**	**-0.81**	**0.90**		**0.01**	**0.00**	**0.00**	0.07	**0.01**	**0.00**	**0.00**	**0.05**	**0.00**	**0.05**
F3 Fe(II)	**-0.92**	0.24	**0.84**	**0.69**	0.34	**0.67**	**-0.95**	**-0.95**	**0.64**	**0.83**	**-0.76**	**0.94**	**0.83**		**0.00**	**0.00**	**0.04**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	0.34
Fe(II) PW	**-0.96**	0.32	**0.80**	**0.64**	0.40	**0.68**	**-0.93**	**-0.98**	0.58	**0.90**	**-0.76**	**0.98**	**0.93**	**0.92**		**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.03**	**0.00**	0.09
Co	**-0.91**	0.41	**0.64**	0.51	0.37	**0.63**	**-0.84**	**-0.92**	0.41	**0.88**	**-0.81**	**0.90**	**0.99**	**0.88**	**0.92**		0.08	**0.01**	**0.01**	**0.00**	**0.03**	**0.00**	0.08
K	-0.50	-0.42	**0.90**	0.08	-0.39	**0.99**	**-0.77**	-0.58	**0.79**	0.37	**-0.84**	0.59	**0.62**	**0.69**	**0.67**	**0.62**		0.43	**0.00**	0.28	0.16	0.15	0.78
V	**0.96**	-0.54	**-0.63**	**-0.80**	**-0.74**	-0.30	**0.75**	**0.93**	-0.29	**-0.95**	0.53	**-0.93**	**-0.83**	**-0.82**	**-0.90**	**-0.83**	-0.30		0.08	**0.01**	0.05	**0.00**	**0.01**
Mn	**-0.78**	-0.02	**0.92**	**0.85**	0.03	**0.85**	**-0.94**	**-0.84**	**0.80**	**0.64**	**-0.83**	**0.83**	**0.75**	**0.94**	**0.84**	**0.80**	**0.87**	**-0.61**		**0.04**	**0.00**	**0.01**	0.80
Cu	**0.89**	-0.51	-0.55	**-0.63**	-0.54	-0.41	**0.79**	**0.88**	-0.30	**-0.89**	0.57	**-0.86**	**-0.87**	**-0.86**	**-0.84**	**-0.89**	-0.40	**0.83**	**-0.68**		**0.03**	**0.01**	0.07
As	**-0.80**	0.35	**0.67**	**0.77**	0.38	0.50	**-0.86**	**-0.82**	**0.67**	**0.62**	-0.52	**0.80**	**0.61**	**0.89**	**0.72**	**0.72**	0.51	**-0.67**	**0.86**	**-0.71**		**0.00**	0.68
Se	**0.92**	-0.49	**-0.64**	**-0.72**	-0.50	-0.51	**0.87**	**0.93**	-0.50	**-0.84**	**0.70**	**-0.92**	**-0.85**	**-0.90**	**-0.88**	**-0.91**	-0.52	**0.87**	**-0.82**	**0.80**	**-0.86**		0.15
Ba	**-0.65**	0.02	**0.77**	0.46	**0.78**	-0.09	-0.29	-0.59	-0.25	**0.79**	-0.23	**0.60**	**0.67**	0.36	0.59	**0.61**	-0.11	**-0.79**	0.10	**-0.64**	0.16	-0.52	

Sacrifice 4 Correlations

Table E.4. Correlation matrix comparing statistically significant variables from the redundancy analysis of Sacrifice 4. The Pearson R correlation coefficients are displayed below the diagonal and the p-values associated with correlations are displayed above the diagonal.

	TCE	DCE	VC	Ethene	Sulfide	F2 Fe	F4 Fe	Fe(II) Solids	F2 Fe(II)	Co	Be	Mg	Mn	Cu	Zn	As	Se	Ba
TCE		0.22	0.05	0.11	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.17	**0.04**	**0.00**	0.20	**0.00**	**0.00**	0.11
DCE	-0.46		0.75	0.90	0.16	0.42	0.44	0.17	0.41	0.36	0.37	0.70	**0.00**	0.19	0.69	0.10	0.31	0.35
VC	**-0.66**	-0.13		0.27	**0.04**	**0.02**	0.05	0.07	**0.03**	**0.01**	0.06	0.46	0.62	**0.02**	0.56	0.14	**0.02**	**0.01**
Ethene	-0.57	-0.05	0.41		0.23	0.21	**0.04**	0.08	0.22	0.38	0.13	**0.00**	0.69	0.42	**0.00**	0.50	0.13	0.29
Sulfide	**-0.93**	0.51	**0.68**	0.45		**0.00**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	0.46	**0.02**	**0.00**	0.50	**0.00**	**0.00**	0.19
F2 Fe	**-0.93**	0.31	**0.75**	0.46	**0.95**		**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	0.35	0.09	**0.00**	0.34	**0.00**	**0.00**	**0.03**
F4 Fe	**0.87**	-0.30	**-0.66**	**-0.70**	**-0.73**	**-0.78**		**0.00**	**0.00**	**0.01**	**0.03**	0.06	0.15	**0.02**	0.07	0.06	**0.00**	0.05
Fe(II) Solids	**-0.97**	0.50	**0.63**	**0.61**	**0.94**	**0.93**	**-0.88**		**0.00**	**0.00**	**0.01**	0.16	**0.02**	**0.00**	0.17	**0.00**	**0.00**	0.13
F2 Fe(II)	**-0.92**	0.32	**0.72**	0.46	**0.85**	**0.94**	**-0.90**	**0.92**		**0.00**	**0.02**	0.28	0.09	**0.00**	0.27	**0.01**	**0.00**	**0.02**
Co	**-0.89**	0.35	**0.78**	0.33	**0.88**	**0.95**	**-0.82**	**0.89**	**0.97**		**0.02**	0.53	0.07	**0.00**	0.54	**0.00**	**0.00**	**0.02**
Be	**0.93**	-0.34	**-0.65**	-0.54	**-0.87**	**-0.84**	**0.72**	**-0.83**	**-0.76**	**-0.74**		0.20	0.17	**0.02**	0.29	**0.01**	**0.00**	0.18
Mg	0.50	0.15	-0.28	**-0.95**	-0.28	-0.35	**0.65**	-0.51	-0.41	-0.24	0.48		0.96	0.74	**0.00**	0.70	0.22	0.27
Mn	**-0.68**	**0.91**	0.19	0.16	**0.75**	0.60	-0.52	**0.75**	0.59	**0.64**	-0.50	-0.02		**0.03**	0.94	**0.01**	**0.05**	0.96
Cu	**0.86**	-0.48	**-0.73**	-0.31	**-0.96**	**-0.93**	**0.73**	**-0.89**	**-0.87**	**-0.92**	**0.76**	0.13	**-0.71**		0.76	**0.00**	**0.00**	0.13
Zn	-0.47	-0.16	0.22	**0.90**	0.26	0.36	**-0.62**	0.50	0.41	0.24	-0.40	**-0.97**	0.03	-0.12		0.61	0.23	0.25
As	**-0.88**	0.59	0.54	0.26	**0.94**	**0.91**	**-0.64**	**0.89**	**0.83**	**0.86**	**-0.78**	-0.15	**0.80**	**-0.90**	0.20		**0.00**	0.25
Se	**0.98**	-0.38	**-0.74**	-0.55	**-0.95**	**-0.98**	**0.87**	**-0.98**	**-0.96**	**-0.95**	**0.86**	0.46	**-0.67**	**0.90**	-0.45	**-0.90**		**0.05**
Ba	-0.57	-0.35	**0.82**	0.39	0.48	**0.71**	**-0.66**	0.54	**0.77**	**0.74**	-0.49	-0.41	-0.02	-0.54	0.42	0.42	**-0.67**	

Sacrifice 3 and 4 Correlations

Table E.5. Correlations with results from Sacrifices 3 and 4. Pearson correlation coefficient (below) and p-values (above) describe the significance of each correlation.

	TCE	DCE	VC	Ethene	Aceatate	Propionate	Butyrate	Fe(II) Eff	HCO_3	H_2	Sulfide	F4 Fe	F5 Fe	F2 Fe(II)	F3 Fe(II)	Co	Be	V	Zn	As	Se	Ba
TCE		0.02	0.04	0.15	0.00	0.01	0.04	0.03	1.00	0.09	0.03	0.11	0.05	0.00	0.00	0.00	0.17	0.00	0.03	0.00	0.00	0.01
DCE	-0.56		0.60	0.56	0.27	0.59	0.00	0.01	0.20	0.03	0.00	0.24	0.00	0.20	0.00	0.01	0.47	0.09	0.22	0.03	0.04	0.93
VC	-0.49	-0.13		0.97	0.00	0.00	0.23	0.59	0.44	0.73	0.70	0.25	0.69	0.25	0.47	0.03	0.68	0.00	0.36	0.28	0.04	0.00
Ethene	-0.35	-0.15	-0.01		0.85	0.74	0.57	0.26	0.02	0.13	0.96	0.42	0.77	0.05	0.11	0.86	0.41	0.98	0.37	0.24	0.17	0.93
Aceatate	**-0.73**	0.28	**0.69**	0.05		0.00	1.00	0.59	0.71	0.55	0.04	0.09	0.48	0.03	0.03	0.01	0.48	0.00	0.53	0.01	0.00	0.01
Propionate	-0.57	-0.14	**0.81**	0.08	**0.85**		0.11	0.30	0.62	0.39	0.97	0.28	0.93	0.03	0.20	0.05	0.38	0.00	0.71	0.09	0.01	0.00
Butyrate	-0.49	**0.85**	-0.30	0.14	0.00	-0.39		0.00	0.44	0.00	0.00	0.27	0.01	0.14	0.01	0.03	0.54	0.49	0.03	0.06	0.07	0.66
Fe(II) Eff	-0.51	**0.61**	-0.14	0.28	-0.14	-0.26	**0.83**		0.31	0.02	0.21	0.27	0.00	0.03	0.01	0.01	0.22	0.38	0.00	0.18	0.05	0.53
HCO_3	0.00	0.31	0.19	-0.54	-0.09	-0.13	0.20	0.25		0.89	0.74	0.87	0.03	0.28	0.85	0.22	0.55	0.83	0.24	0.73	0.96	0.38
H_2	-0.41	0.52	-0.09	0.37	0.15	-0.21	**0.73**	0.55	-0.04		0.00	0.23	0.03	0.39	0.13	0.15	0.87	0.79	0.03	0.39	0.16	0.97
Sulfide	-0.50	**0.75**	-0.10	-0.01	0.48	0.01	**0.70**	0.31	-0.08	**0.71**		0.25	0.07	0.26	0.03	0.04	0.93	0.12	0.44	0.03	0.04	0.76
F4 Fe	0.39	-0.29	-0.29	-0.20	-0.41	-0.27	-0.27	-0.28	-0.04	-0.30	-0.29		0.02	0.10	0.21	0.05	0.00	0.04	0.09	0.25	0.01	0.14
F5 Fe	0.46	**-0.65**	-0.10	-0.07	-0.18	0.02	**-0.63**	**-0.67**	-0.52	-0.50	-0.44	0.56		0.54	0.01	0.01	0.01	0.13	0.02	0.22	0.02	0.44
F2 Fe(II)	**-0.84**	0.32	0.28	0.48	0.51	0.51	0.37	0.52	-0.27	0.22	0.28	-0.40	-0.24		0.00	0.00	0.13	0.00	0.09	0.00	0.00	0.04
F3 Fe(II)	**-0.89**	**0.68**	0.18	0.39	0.51	0.32	**0.63**	0.59	0.05	0.37	0.51	-0.31	-0.58	**0.91**		0.00	0.14	0.00	0.22	0.00	0.00	0.38
Co	**-0.81**	0.58	0.51	-0.04	**0.62**	0.46	0.51	0.58	0.31	0.35	0.50	-0.47	-0.59	**0.64**	**0.64**		0.22	0.00	0.00	0.01	0.00	0.00
Be	0.34	-0.18	-0.11	-0.21	-0.18	-0.22	-0.16	-0.30	-0.15	0.04	-0.02	**0.71**	0.58	-0.37	-0.36	-0.31		0.12	0.19	0.24	0.03	0.29
V	**0.83**	-0.41	**-0.64**	-0.01	**-0.86**	**-0.80**	-0.17	-0.22	-0.06	-0.07	-0.38	0.48	0.37	**-0.71**	**-0.65**	**-0.82**	0.38		0.10	0.00	0.00	0.00
Zn	-0.50	0.31	0.23	0.23	0.16	0.09	0.50	**0.69**	0.29	0.51	0.19	-0.41	-0.53	0.41	0.30	**0.68**	-0.32	-0.40		0.75	0.06	0.01
As	**-0.81**	0.52	0.27	0.29	**0.61**	0.42	0.45	0.33	-0.09	0.21	0.51	-0.29	-0.30	**0.77**	**0.92**	0.57	-0.29	**-0.65**	0.08		0.00	0.49
Se	**0.91**	-0.49	-0.48	-0.34	**-0.73**	-0.58	-0.44	-0.46	0.01	-0.34	-0.49	**0.61**	0.54	**-0.85**	**-0.86**	**-0.80**	0.51	**0.85**	-0.46	0.02		0.02
Ba	-0.57	-0.02	**0.78**	0.02	**0.60**	**0.76**	-0.11	0.16	0.22	-0.01	-0.08	-0.36	-0.19	0.49	0.22	**0.67**	-0.26	**-0.75**	**0.62**	0.17	-0.53	

Appendix F

Hydraulic Retention Time Adjustment

Table F.1 displays the original HRT, flow, and calculated pore space determined from the bromide tracer study results, along with the calculated average flows and HRT calculated for the time each column was Sacrificed ($HRT=V_p/Q_{avg}$). Columns 1-12 are part of the lactate treatment and columns 25-36 are part of the whey treatment.

Table F.1. Flow characteristics of each column at the time of Sacrifice. Column 1-12 are under the lactate treatment and 25-36 are under the whey treatment.

		Br Tracer Study			At Sacrifice	
Sacrifice	Column	HRT (hr)	Q (mL/d)	V_p (mL)	Q_{avg}	HRT (hr)
1	1	22.74	111.68	137.42	111.49	29.58
1	7	38.83	94.83	153.42	110.56	33.30
1	12	23.55	103.52	101.58	120.06	20.30
1	28	24.55	116.31	119.00	112.52	25.38
1	32	27.18	99.26	112.43	115.61	23.34
1	33	22.16	114.15	105.41	116.90	21.64
2	3	23.67	142.64	140.68	111.60	30.25
2	5	23.33	89.84	87.34	124.47	16.84
2	9	36.78	98.24	150.56	122.90	29.40
2	27	22.39	101.78	94.94	102.29	22.28
2	34	28.20	119.56	140.47	99.77	33.79
2	36	26.98	106.04	119.19	120.31	23.78
3	2	34.54	95.07	136.81	110.07	29.83
3	8	27.84	112.40	130.41	113.33	27.62
3	11	39.58	107.74	177.70	103.22	41.32
3	29	26.67	112.61	125.12	126.57	23.72
3	31	26.73	97.45	108.56	123.92	21.02
3	35	25.65	119.19	127.40	144.11	21.22
4	4	21.80	111.92	101.67	114.93	21.23
4	6	32.62	125.00	169.88	145.84	27.96
4	10	35.96	110.00	164.81	116.28	34.02
4	25	25.00	145.97	152.08	119.48	30.55
4	26	21.92	141.10	128.87	126.16	24.52

4	30	25.23	112.61	118.38	129.61	21.92

Retardation Factor

Table F.2 includes the soil characteristics used to determine the retardation factor, such as the bulk density (ϱ_b), the porosity ($n=1-\varrho_b/\varrho_p$), and the fraction of organic carbon in the soil (f_{oc}).

Table F.2 Soil characteristics of the packed columns used for determining the retardation factor.

Soil (g)	V_{col} (cm^3)	bulk density (ϱ_b)	Particle Density (ϱ_p)	Porosity (n)	Organic Carbon (f_{oc})
596	347.5	1.72	2.66	0.355	0.002

The retardation factor (R), calculated as $R=1+(K_{oc}f_{oc}\varrho_b)/n$, or $R=1+K_d(\varrho_b)/n$, is applied to the HRT to account the sorption of the chlorinated hydrocarbons onto the soil (Table E.3).

Table F.3. The organic carbon-soil partitioning coefficient (Koc) and organic carbon fraction (f_{oc}) of the soil used to determine the distribution coefficient (K_d) of each compound in the soil and the resulting retardation factor (R).

Compound	K_{oc}	f_{oc}	K_d	R
TCE	76.068	0.002	0.177	1.855
DCE	43.000	0.002	0.100	1.483
VC	29.500	0.002	0.069	1.331
Ethene	26.900	0.002	0.063	1.302

Degradation Rate

The retardation factor is applied to the bromide HRT to determine the compound specific HRT, which is then divided by the length of the column (7.62 cm) to determine the

velocity of each compound (Table F.4. The adjusted HRT and velocity of each compound moving through Column 4 of the lactate treatment.). The change in concentration and first order degradation rates were calculated for each time period required to travel through 1 mm intervals of the column (TableF.6). Horizontal dispersion was insignificant due to the small volume of the column. Shown below is an example of the information used to determine the degradation rates for each column.

Table F.4. The adjusted HRT and velocity of each compound moving through Column 4 of the lactate treatment.

	HRT (hr)	V (mm/hr)
Br	21.23	3.59
TCE	39.38	1.94
DCE	31.49	2.42
VC	28.27	2.70
Ethene	27.65	2.76

The initial K_{TCE} was determined based on the HRT of TCE and the final concentration of TCE measured at the time of Sacrifice. The TCE degradation rate, along with the sequential rates were adjusted sequentially until the predicted final concentration matched the concentration measured at the time of Sacrifice (Table F.5).

Table F.5. Calculated TCE degradation rate (hr^{-1}) and predicted degradation rates (hr^{-1}) based on the effluent concentrations measured at the time of sacrifice.

Column	K_{TCE}	K_{DCE}	K_{VC}
4.0	0.1920	0.1015	0.0704
calculated K_{TCE}:	0.2021		

Table F.6 displays the change in concentration of each compound for every 1 mm interval, and the time required for each compound to travel to each interval. The production

of each sequential degradation byproduct is dependent on the travel time required and the reduction of the preceding compound, as shown by the following equations.

1. $TCE=TCE_0 - [TCE_0 \times K_{TCE} \times (t_1^{TCE}-t_0^{TCE})]$
2. $DCE=DCE_0 + [TCE_0 \times K_{TCE} \times (t_1^{TCE}-t_0^{TCE})]- [DCE_0 \times K_{DCE} \times (t_1^{DCE}-t_0^{DCE})])$.
3. $VC=VC_0 + [DCE_0 \times K_{DCE} \times (t_1^{DCE}-t_0^{DCE})]- [VC_0 \times K_{vc} \times (t_1^{VC}-t_0^{VC})])$.
4. $Ethene=Ethene_0 + [VC_0 \times K_{VC} \times (t_1^{VC}-t_0^{VC})]$

Table F.6 Time intervals required for each compound to move through the column, the resulting concentrations of TCE, DCE, VC and ethene based on the calculated rates and the comparison between the final predicted concentrations and measured concentrations.

L (mm)	t_{TCE} (hr)	TCE	t_{DCE} (hr)	DCE	t_{VC} (hr)	VC	Ethene	Total (μmol/L)
0	0.00	68.17	0.00	0.00	0.00	0.00	0.00	68.17
1	0.52	61.41	0.41	6.76	0.37	0.00	0.00	68.17
2	1.03	55.32	0.83	12.57	0.74	0.28	0.00	68.17
3	1.55	49.83	1.24	17.53	1.11	0.80	0.01	68.17
4	2.07	44.88	1.65	21.74	1.48	1.52	0.03	68.17
5	2.58	40.43	2.07	25.28	1.85	2.39	0.07	68.17
6	3.10	36.42	2.48	28.23	2.23	3.39	0.13	68.17
7	3.62	32.81	2.89	30.66	2.60	4.48	0.22	68.17
8	4.13	29.55	3.31	32.63	2.97	5.65	0.34	68.17
9	4.65	26.62	3.72	34.20	3.34	6.87	0.48	68.17
10	5.17	23.98	4.13	35.40	3.71	8.13	0.66	68.17
11	5.68	21.60	4.55	36.30	4.08	9.40	0.88	68.17
12	6.20	19.46	4.96	36.92	4.45	10.68	1.12	68.17
13	6.72	17.53	5.37	37.30	4.82	11.95	1.40	68.17
14	7.23	15.79	5.79	37.47	5.19	13.20	1.71	68.17
				…..				
73	37.72	0.03	30.17	5.12	27.08	21.85	41.17	68.17
74	38.24	0.03	30.58	4.90	27.45	21.49	41.75	68.17
75	38.76	0.03	30.99	4.70	27.82	21.14	42.31	68.17
76	39.27	0.02	31.41	4.51	28.19	20.78	42.86	68.17
76.2	39.38	0.02	31.49	4.47	28.27	20.71	42.97	68.17
Measured (μmol/L):		0.02		4.48		20.72	42.95	68.17
% difference		0.31		0.22		0.03	-0.04	0.00

Figure E.1 displays the change in TCE and degradation byproducts across the Column 4 of the lactate treatment as Sacrifice 4. In the lactate treatment the VC degradation rates were much slower than the whey treatment, resulting in VC accumulation. The degradation rates determined for each column were used to calculate the averages for each treatment at the time of Sacrifice.

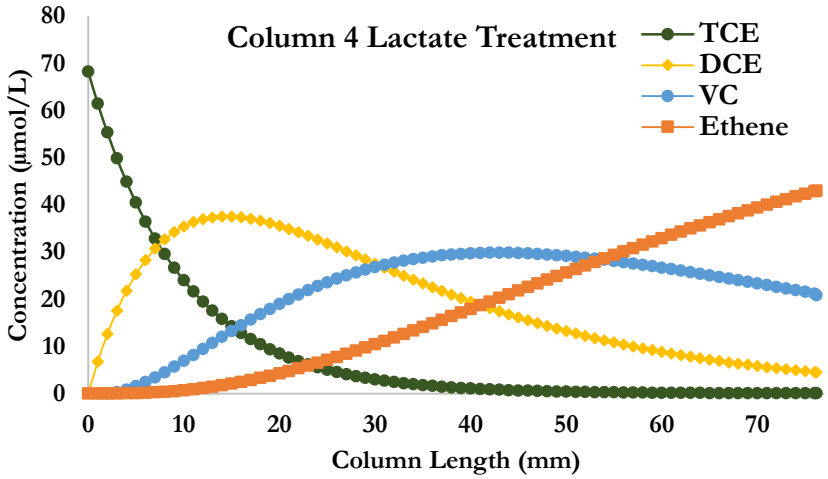


Figure F.1 The predicted reduction of TCE and degradation byproducts along the length of Column 4 of the lactate treatment at Sacrifice 4.