Assessing Microbial Viability and Biodegradation Capabilities in Sandstone

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Earth Sciences (Water)

Waterloo, Ontario, Canada, 2017

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Environmental practitioners have demonstrated enhanced in situ bioremediation (EISB) in homogeneous unconsolidated soils to remediate chlorinated solvents. However, EISB has not been fully investigated in bedrock environments. In addition, there is limited research in the literature that has evaluated bacteria viability in the primary porosity of bedrock for the purpose of reductive dechlorination in the low permeability units of bedrock.

Studies that involve bacterial transport in low permeability geological material are typically limited by slow diffusion rates. In this thesis, electrokinetics (EK) was used to overcome slow diffusion rates, and limited bacteria-contaminant-electron donor interactions, by increasing the hydraulic conductivity within the sandstone, in a paired EK-bioaugmentation (EK-Bio) experiment. Idaho Gray andstone cores were artificially contaminated with the aqueous solvent, trichloroethene (TCE), and KB-1 bacteria, a commercially available reductive dechlorinating bacterial consortium, were transported into the cores to assess the ability of bacteria to reductively dechlorinate the solvent. Three goals were outlined to address the main objectives of bacteria viability assessment and dechlorination capabilities:

1) Develop an apparatus at the bench-scale to test EK in bedrock;
2) Determine if amendments could be transported through the primary porosity of bedrock using EK; and
3) Evaluate whether dechlorination of TCE could be promoted in bedrock following the addition of amendments using EK.

Four columns were treated with EK to deliver and continuously saturate the cores with TCE contaminant, KB-1 bacteria, and lactate electron donor for about ten days. One core was immediately sampled (baseline), one core incubated for five weeks, and two replicate cores incubated for nine weeks in an anaerobic environment. Results showed that as incubation time increased, *vcrA* and *bvcA* reductase gene concentrations increased and fermentation products were metabolized. Although chlorinated ethene concentrations were below detection in the long term incubated cores, dechlorination of TCE was not explicitly observed, as complete mass balance could not be achieved. EK transport was an effective tool to migrate amendments into Idaho Gray sandstone and KB-1 bacteria could thrive within the primary porosity of the sandstone.

Acknowledgements

The successful completion of this thesis would not have been possible without the involvement of countless individuals. Firstly, thank you to my supervisors who made this incredible opportunity possible: Dr. Philippe Van Cappellen and Industrial Supervisors Evan Cox (Geosyntec Consultants, Inc.), and Dr. David Reynolds (Geosyntec). Your support and encouragement as I developed my research, project management, and writing skills throughout this venture was wholeheartedly appreciated. Funding was made possible through the Natural Sciences and Engineering Research Council of Canada Industrial Postgraduate Scholarship (NSERC-IPS) program, Geosyntec Consultants, Inc., and the Canada Excellence Research Chair (CERC) program. I'd also like to thank Dr. David Blowes and Dr. James Longstaffe (University of Guelph) for their valuable input as members of my advisory committee.

I'm extremely grateful for my Industrial Sponsor, without whom my research accomplishments could not have been achieved. From Geosyntec: Dr. David Reynolds, Evan Cox, and Matt Vanderkooy. This research was an internally funded R&D project from Geosyntec in 2015, led by David and Matt. Early work was overseen by Matt, who provided significant feedback and support. Thanks to Carol Cheyne for helping to organize some of the preliminary tests. Especially thank you, David, for always being available to make sure I was on the right track, for teaching me to keep my focus when it seemed as though the data were not cooperating, for believing in me, and for providing last minute feedback in the final hours of compiling this thesis! The whole team at SiREM, but especially: Sandra Dworatzek, Jeff Roberts, Phil Dennis, Jennifer Webb, Michael Healy, Alicia Quintanilla, Ximena Druar, Rita Schofield, Jennifer Wilkinson, and Kela Ashworth, as well as Jason White. Your support, assistance, and constant encouragement was invaluable, and made all the frustrating moments in the lab bearable. Thanks for all the laughs and moments of distraction, for checking on my setups on the weekends when I wasn't able, for fielding my questions, and for being one of the best groups of people I've ever had the pleasure to work alongside. You rock my socks!

Thanks to Caitlin Marshall and Nael Yasri of Environmental Engineering Laboratories of The University of Western Ontario for conducting geophysical analyses, as well as Michael Rossi, Morgan Greenwald, and team at Cascade Drilling (Montpelier, VT) for sampling my rock cores.

To my parents, thank you for your sacrifices over the years, which has made this opportunity possible. No matter where life takes me, may you trust that your little girl will always land on her feet. To my in-laws, thank you for loving me as your own and for your constant encouragement through this entire process.

To my husband, my Bee, Nathan. Thank you for always having my back and loving me for me. Thank you for your encouragement each and every day. You teach me to see beauty in the world, encourage me to be my best version, and to laugh daily. Your princess loves you.

Table of Contents

List of Figures

List of Tables

Chapter 1 Introduction

Chlorinated solvents are one of the most common groundwater contaminants in the world, as a result of their long history of use and improper disposal practices. Remediation of chlorinated ethenes by abiotic (e.g. chemical oxidants) and biotic (e.g. bacteria) processes are possible in overburden soil, and in groundwater. One method of biotic treatment that has proven to be successful for complete dechlorination in groundwater is enhanced in situ bioremediation (EISB), whereby parent solvents (e.g. tetrachloroethene [PCE] or trichloroethene [TCE]), are dechlorinated to non-toxic endpoints (e.g. ethene [ETH]), with the addition of bacteria that are capable of dehalogenation, and any necessary electron donors to aid bacterial fermentation processes.

Although EISB can be effective in relatively homogeneous unconsolidated soils, such as sand aquifers (National Academies of Sciences, Engineering, and Medicine, 2015), the technology is unsuitable for contaminated fractured rock environments. Groundwater flowpaths and contaminant transport in fractured rock tends to be discrete, moving through the higher hydraulic conductivity fractures (i.e. secondary porosity; Reynolds and Kueper, 2002). Depending on how long the contaminant has impacted the bedrock, the contaminant may also intrude the pore spaces of the bedrock matrix (i.e. primary porosity); the longer the exposure time, the more contaminant mass can diffuse into the bedrock. Distribution of amendments (e.g. bacteria and electron donor) would thus be ineffective in fractured rock environments. It is also currently unknown whether bacteria can effectively populate the pore space of the matrix to carry out dechlorination in the low permeability units of bedrock. Even if EISB amendments could be introduced into bedrock environments, complete dechlorination would not occur immediately, due to extremely low diffusion rates and limited interaction between bacteria and the contaminant.

To overcome low diffusion rates, limited bacteria-contaminant-electron donor interactions, and discrete flowpaths due to fracture formations, electrokinetics can be applied. Electrokinetics (EK) is the application of low direct current to mobilize charged and uncharged species in porous medium, but has never been successfully demonstrated in a rock environment to aid in bioremediation of chlorinated solvents.

This research used EK to deliver and transport amendments into TCE contaminated sandstone cores at the bench scale in a paired EK-bioaugmentation (EK-Bio) study. Bioaugmented bacteria were evaluated for their viability in the primary porosity of sandstone and their long-term potential to dechlorinate solvent. The bench scale setup was validated using ionic and non-ionic tracers. After amendments were introduced and distributed in the cores, the cores were incubated for periods that ranged from zero days (baseline), to five weeks (medium-term) and nine weeks (long-term), before analysis.

This document is structured as follows: Chapter 2 includes a literature review of chlorinated solvents in bedrock and specific methods for remediation, as well as a general overview of EK, followed by the research objectives; Chapter 3 provides a comprehensive methodology that includes an overview of the experimental design issues that were addressed in this thesis, the base methodology and analytical methods used, modifications required for the system design, and validation tests and results; Chapter 4 describes the methodology for the final EK-Bio tests; Chapter 5 outlines the results and discussion of the EK-Bio tests; and conclusions follow in Chapter 6.

Chapter 2 Literature Review and Research Objectives

2.1 Presence and Fate of Chlorinated Solvents in Fractured Sedimentary Bedrock

Chlorinated solvents are one of the most prevalent groundwater contaminants in the world. A long history of use and improper disposal practices has resulted in the detection of chlorinated solvents in various media, including groundwater, soils, and air. Chlorinated solvents are used for a wide variety of different purposes, from degreasing agents and dry cleaning solvents, to feedstocks for production of other chemicals or products (Doherty, 2000). Examples of chlorinated solvents include carbon tetrachloride (CTC), tetrachloroethene (PCE), trichloroethene (TCE), and 1,1,1-trichloroethane (TCA) (Doherty, 2000). Chlorinated solvents were initially popularized in the industrial sector because of their high vapour pressure, low flammability and reactivity, and excellent ability to dissolve a wide range of organic substances (Doherty, 2000).

At many manufacturing sites, these chemicals have been released to the environment through spills, leaks, as well as improper storage and disposal. Due to the widespread prevalent use and poor historical disposal practices, PCE and TCE are among the most common groundwater contaminants in the world (Doherty, 2000; Moran et al., 2007; Yu et al., 2015). First synthesized in 1864 (Waters et al., 1977), TCE was discovered to be carcinogenic (Chiu et al., 2013; Vogel and McCarty, 1985), with the first recorded detection in drinking water wells in the 1970s (Lagakos et al., 1986). Since then, there has been widespread awareness of TCE as a groundwater contaminant, and significant efforts implemented to address cleanup. As of 2010, TCE had been identified in soil or groundwater at more than 750 of approximately 1,300 Superfund sites in the United States (Chiu et al., 2013; US EPA, 2011).

Chlorinated solvents possess unique physical and chemical attributes that render them difficult and expensive to remediate in soil and groundwater, especially in some types of subsurface materials, such as bedrock. For example, PCE and TCE are dense non-aqueous phase liquids (DNAPLs), which sink to confining layers in aquifers when released into the environment, where they may accumulate in the primary or secondary porosity of rock (Kao et al., 2016; Rajic et al., 2016; Wadley et al., 2005). Fractures (i.e. secondary porosity) are important entry points for DNAPLs, where the hydraulic conductivity is typically higher than the surrounding material. Compared to the primary porosity (or pore spaces of rock matrix), fracture void space connectivity can be significant, leading to contaminant plumes that cover a substantial volume or depth in the subsurface (Frind et al., 1999). Over time, the DNAPL can spread over large regions (on the order of miles), both vertically and laterally, transported by gravity and groundwater flow (Kueper and McWhorter, 1991; Parker et al., 1994; Reynolds and Kueper, 2002).

Although solvent accumulation in the fractures may be remediated by natural attenuation or engineered processes (e.g. pump-and-treat), a significant portion of the contaminant mass may remain embedded in the primary porosity (Chen et al., 2015). Research into DNAPL diffusion from secondary to primary porosity shows that this process contributes to the decrease of pure-phase DNAPL in a relatively short period of time (Reynolds and Kueper, 2002). However, this partitioning does not necessarily signify that the DNAPL has dissipated, as solvents can remain in the matrix (Parker et al., 1994). Once the contaminant mass in the fractures has been removed, contaminant in the primary porosity can diffuse back into groundwater and even impact the indoor air of buildings through vapour intrusion (Algreen et al., 2015).

The challenge of chlorinated solvent remediation is magnified by the inherent heterogeneity of geological materials, which may have highly conductive fractures, and variable permeability primary porosity that are difficult to delineate, as well as difficulties defining the contaminant mass (Broholm et al., 2016), dissolution mechanisms of pure phase NAPLs, and migration of the aqueous phase NAPLs (McLaren et al., 2012). Although there have been significant attempts in recent decades to address chlorinated solvent remediation in bedrock, elucidating the contaminant mass transfer between primary and secondary porosity continues to hinder remedial efforts.

2.2 Remediation of Chlorinated Solvents in Bedrock

In one study from 2006, chlorinated solvent impacted sites in the United States were evaluated for post-treatment mass reduction efficacy following remediation by chemical oxidation, enhanced bioremediation, thermal treatment, and surfactant/co-solvent flushing (McGuire et al., 2006). Of the 59 sites assessed, only three sites were located in fractured rock hydrogeology; two of these sites used enhanced bioremediation to degrade chlorinated solvents, and the other used chemical oxidation (McGuire et al., 2006). The information gathered for this review were either derived from published literature, from site reports submitted to state regulatory agencies, or from a survey of remediation professionals who worked on DNAPL source-zone remediation projects. The remaining sites were listed as either fine- or coarse-grained hydrogeology, which could be interpreted as soils, since no further information was provided. The limited number of sites evaluated in fractured bedrock environments suggests that either long term post-treatment monitoring data are unavailable at fractured bedrock sites (studies with short term or no post-treatment monitoring data were excluded from the study), or remediation in fractured bedrock environments is limited.

As noted in the 2006 study, in situ treatment options for chlorinated solvents in bedrock is limited. Some of these options include: chemical oxidation, electrochemical (i.e. hydrochlorination) treatment, and

bioremediation. The latter, including a special case of bioremediation, will be addressed in Sections 2.4 and 2.6, respectively.

Chemical oxidation and electrochemical treatment of DNAPL were conducted at the bench scale. In Schaefer et al. (2012), chelated ferrous iron and alkaline activated persulfate oxidation were compared to permanganate oxidation for the treatment of PCE in artificially fractured sandstone blocks (Schaefer et al., 2012). The fractures in the blocks were saturated with PCE solution, followed by a water flush until PCE was no longer displaced. Residual PCE was determined by the difference in PCE injected into the fracture minus what was recovered by flushing (Schaefer et al., 2012). Permanganate treatment removed the most mass, followed by iron activated persulfate; alkaline activated persulfate was least effective. However, permanganate had the drawback of MnO_2 precipitation, which plugged the fracture. At low pH, the authors suggested that natural oxidant demand within the sandstones also competed with PCE for permanganate. The redox reaction with permanganate could produce CO_2 gas, which would also inhibit flow within the fracture. With persulfate, 1000 mg/L Fe-EDTA activated oxidant had comparable mass removal to permanganate, without the side effect of precipitate formation. The authors suggested that PCE mass removal was limited by dissolution into the aqueous phase, rather than oxidant availability, regardless of oxidant used. One important finding was that although early treatment with the oxidants had high rates of DNAPL mass removal, as soon as oxidant delivery into the fracture ceased, the DNAPL mass concentrations increased (Schaefer et al., 2012). Additionally, the DNAPL-water interfacial areas in the fractured rock decreased over time, for example, due to precipitation plugging, which hindered mass removal efficiency.

In the electrochemical, or hydrochlorination, study, removal of TCE in the presence of co-contaminants were evaluated (Fallahpour et al., 2017). Humic acid (which represented natural organic matter), chromate, selenate, and nitrate were paired with TCE in separate tests in a flow-through vertical column reactor. A mixture of the co-contaminants, excluding humic acid, was also mixed with TCE to compare TCE removal when only one other contaminant was present vs. four other contaminants. Electrodes, made of iron or copper, were embedded in a limestone core and current applied. In the absence of any of the co-contaminants, TCE removal of approximately 90% was achieved within four hours of electrochemical treatment. Humic acid interfered with TCE dechlorination either by outcompeting TCE for electrons or for H_2 produced at the cathode, or could have formed Fe-humate precipitates that affected the anode surface (Fallahpour et al., 2017). Co-contaminant introduction into the cores reduced TCE removal efficiency by the reaction with ferrous iron ions at the anode to produce precipitates on the electrode surface and throughout the column. The authors believed that nitrate was mainly removed through electrocoagulation,

rather than competition for H_2, which explained why TCE removal efficiency was approximately 95% (Fallahpour et al., 2017).

Both studies present useful information for dechlorination of solvents in bedrock. In the Schaefer et al. (2012) study, oxidant concentration was not the limiting factor for contaminant mass removal; instead, the size of the DNAPL-water interface, and the dissolution of contaminant into the aqueous phase were controlling factors for contaminant removal. Although oxidant treatment was effective for PCE removal, as soon as the oxidant delivery stopped, the PCE concentration rebounded. The authors did not mention whether they monitored for the formation of any transformation products as PCE was treated, thus complete dechlorination was not determined. Additionally, only the fractures were evaluated; the extent of interconnected pore matrices on treatment efficacy was not assessed.

In Fallahpour et al. (2017), co-contaminant impact on TCE removal efficiency was quantified. As with Schaefer et al. (2012), transformation products were not evaluated. Electric potential was only applied for four days; the question remains whether removal efficiency would improve over longer treatment times, or whether treatment profiles of co-contaminants would change over time.

2.3 Enhanced In Situ Bioremediation (EISB) of Chlorinated Solvents

In addition to abiotic treatment, chlorinated solvents are susceptible to anaerobic biotic reductive dechlorination (Kao et al., 2016; Major et al., 2002; Mao et al., 2012). Although numerous bacterial species have been identified in chlorinated solvent contaminated sites, most dechlorinating bacteria are unable to reduce PCE and TCE completely to non-toxic ethene (ETH), as *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) are rate-limiting intermediates that are toxic and carcinogenic (**Figure 1**; Kotik et al., 2013; Lee et al., 2008). Only a handful of genera, *Dehalococcoides sp.* (*Dhc*) and *Dehalobacter sp.* (*Dhb*), are known to effectively use molecular hydrogen (H_2) to dechlorinate cDCE and VC to ETH (Azizian et al., 2010; Maymó-Gatell et al., 1997). However, *Dhc* and *Dhb* function most efficiently in a consortium with other bacteria, including acetogens (e.g. *Acetobacterium*), fermenters, methanogens (e.g. *Methanomethylovorans*), sulfate-reducers, and iron-reducers (e.g. *Geobacter*; Pérez-de-Mora et al., 2014). The non-dechlorinating species help to maintain reducing environments, as well as synthesize vitamins and other metabolites required by *Dhc* and *Dhb* (Hug et al., 2012). Even within the *Dhc* and *Dhb* genus, only strains containing the reductive genes, *vcrA*, can effectively dechlorinate VC to ETH (Scheutz et al., 2010; Van Der Zaan et al., 2010). The commercially available KB-1 bacterial consortium (SiREM), details described elsewhere (Hug et al., 2012), includes *Dhc* with the *vcrA* and *bvcA* genes, and has been optimized for successful bioaugmentation at field sites.

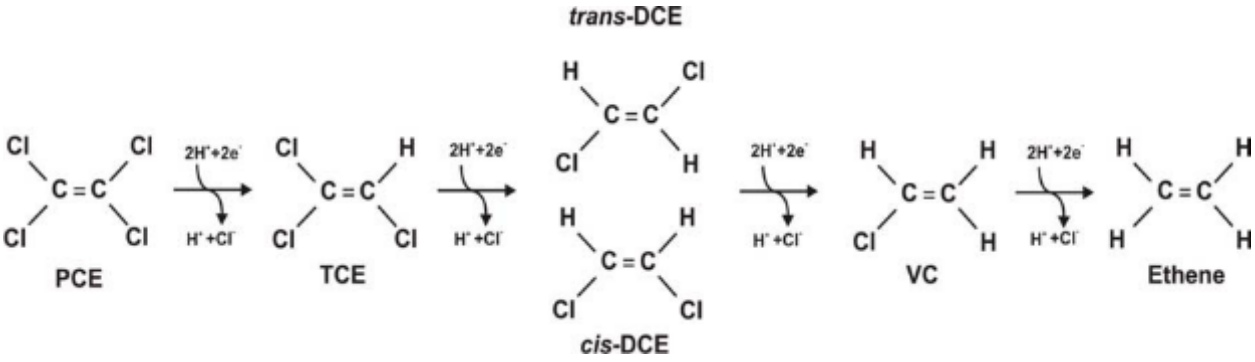


Figure 1 Biotic reductive dechlorination pathway of tetrachloroethene (PCE) and trichloroethene (TCE) typically follows cis-1,2-dichloroethene (cDCE) to vinyl chloride (VC) to ethene (ETH) (Parsons, 2004).

Besides appropriate compositions of bacterial communities that include *Dhc* and/or *Dhb*, electron donors are required to sustain dechlorination (Lu et al., 2002). Examples of carbon sources or volatile fatty acids (VFAs) that have demonstrated ability to promote reductive dechlorination include glucose, sucrose, methanol, ethanol, formate, acetate, proprionate, butyrate, lactate, crotonate, fumarate, and hydrogen (Lu et al., 2002). Other than hydrogen (H_2), all other electron donors serve as precursors for H_2 formation via fermentative metabolism (Hug et al., 2012).

Enhanced in situ bioremediation (EISB) is the addition of any combination of indigenous or non-native bacteria, carbon source, electron donors, nutrients, or other amendments to increase the degradation efficiency of indigenous bacterial populations (Gödeke et al., 2006; Huang et al., 2008). The importance of EISB as an option for complete dechlorination at contaminated sites cannot be overstated, given the abundance of conducted research and regulatory protocols created in recent years. Bioremediation is an attractive option for treatment of chlorinated solvents, as it can reduce the size of persistent contaminant mass long after initial amendment addition has been discontinued, thereby minimizing back diffusion, and potentially reducing overall treatment costs and effort.

Several field studies conducted in the past decade demonstrate the breadth of investigations using EISB. In fact, investigations have evolved to: address the exact growth rate or dechlorination rate of *Dhc* in groundwater (Schaefer et al., 2009); determine the optimal concentrations of *Dhc* necessary to inject in bioaugmentation field tests to observe complete dechlorination (Schaefer et al., 2010a); examine the variability in microbial communities of three different locations within a single site, due to geochemical differences and contaminant concentrations (Kotik et al., 2013); and the impacts of sulfate concentration (a competing electron acceptor), chloroform concentration (toxic to bacteria at high concentrations), VFA availability, and groundwater salinity and pH on organochloride-respiring bacterial densities (Baldwin et

al., 2017). These particular studies evaluated conditions in the groundwater. At the same time, there were several other studies that assessed bioremediation in bedrock environments, as described below.

2.4 Natural Attenuation and EISB of Chlorinated Solvents in Bedrock

A search of the literature reveals only eight studies that evaluated biotic reductive dechlorination in bedrock environments. Two were an analysis of natural attenuation processes, either from sampling the associated contaminated groundwater (Lenczewski et al., 2003), or from collecting both groundwater and sandstone cores from the contaminated plume to prepare microcosms in the laboratory (Darlington et al., 2008); one was a bioaugmentation evaluation of dechlorinating culture to enhance PCE DNAPL dissolution rates in artificially fractured bedrock samples (Schaefer et al., 2010b). Another three bioaugmentation studies used several lines of evidence to determine the processes that controlled biotic reductive dechlorination of TCE, but sampled from groundwater only for all analyses (Pérez-de-Mora et al., 2014; Révész et al., 2014; Verce et al., 2015). Only one paper assessed bacterial viability in a highly fractured DNAPL contaminated site (Lima et al., 2012). A more in-depth evaluation of these studies follows.

The study by Lenczewski et al. (2003) was one of the first well-documented field assessments of natural attenuation of TCE in groundwater at a highly fractured shale bedrock site. Multiple lines of evidence were used to prove dechlorination occurred, including analysis of volatile organic compounds (VOCs), dissolved gases, inorganics concentration and distribution, redox conditions, molecular enumeration, and molecular sequencing. Within the anaerobic zone of the plume, TCE was actively dechlorinated through reductive processes. However, there was also an aerobic zone near a seep where groundwater discharged to the adjacent stream. It was believed that aerobic co-metabolism of chlorinated ethenes may occur at that location (Lenczewski et al., 2003). A comparison of bacterial community within and outside the plume determined that species commonly found in reducing conditions, methanotrophs, methanogens, iron-reducers, and sulfate-reducers, were all found within the plume, but that none of these species were found in the uncontaminated region of similar geologic setting (Lenczewski et al., 2003).

Sandstone cores were extruded from a TCE contaminated industrial site and used to prepare microcosms to evaluate biotic and abiotic transformation processes in Darlington et al. (2008). Electron donor was only supplied from the sandstone material; no further amendments were introduced, to mimic in situ conditions. The results only weakly suggested that biotic reductive dechlorination and abiotic transformation occurred, since only a subset of microcosm bottles had reduced forms of the parent compound. In bottles amended with TCE, only cDCE was observed; VC was not produced. In the abiotic bottles, ^{14}C-labelled solvents (i.e. [^{14}C]TCE , [^{14}C]cDCE, and [^{14}C]VC) were dosed to track degradation through the formation of soluble products, ^{14}C-non-strippable residue (NSR), and $^{14}CO_2$ (Darlington et al., 2008). Acetylene was observed

in the field; glycolate, formate, and acetate were identified as components of NSR in the abiotic microcosm bottles, which were good indicators of abiotic transformation. Iron-bearing minerals had been observed to catalyze abiotic reduction of chlorinated ethenes; the sandstone evaluated in this study was composed of as much as 1.9% iron, which provided further evidence of abiotic transformation (Darlington et al., 2008). The authors could have used molecular sequencing techniques to identify key bacterial species at the site to provide further evidence that biotic reductive dechlorination could occur, or explain why reductive dechlorination stalled at cDCE.

In Schaefer et al. (2010b), batch and fractured rock bench-scale experiments were set up to evaluate biotic reductive dechlorination efficacy under three conditions: in the presence of a PCE DNAPL mass, with bacteria injected at a constant rate; in the presence of dissolved PCE near saturation (no contaminant mass), with bacteria injected at a constant rate; and with dissolved PCE near the saturation limit at a high bacterial injection flow rate. Where DNAPL was present in batch experiments, no dechlorination, no lactate fermentation, and no sulfate reduction occurred. The addition of H_2 gas also did not seem to have any impact on dechlorination. When only dissolved PCE was in the batch microcosms, complete reductive dechlorination occurred within days (Schaefer et al., 2010b). In the artificially fractured sandstone experiments, PCE DNAPL had no impact on dechlorination; *Dhc* could rapidly produce measurable quantities of ETH in both PCE-only and PCE + DNAPL tests. At high bacterial injection flow rates, no ETH was observed, and less dechlorination activity occurred overall, likely due to increased shear stress, which led to *Dhc* detachment and subsequent migration out of the fracture (Schaefer et al., 2010b).

In situ reductive dechlorination (i.e. natural attenuation) was measured using flowpath independent lines of evidence (Bradley et al., 2009). This study was similar to Lenczewski et al. (2003), in that multiple lines of evidence were evaluated, including detection of electron donor, chlororespiring bacteria, and accumulation of chloroethene daughter products. Data was collected via depth-specific discrete borehole packers of crushed geologic material, which were placed in boreholes as in situ microcosms for one year (Bradley et al., 2009). Bench scale microcosms were also assembled using depth-specific geologic material and amended with ^{14}C-labelled chlorinated ethenes. This particular investigation set a precedent for follow-on bioaugmentation evaluations at the same site, such as conducted by Révész et al. (2014).

In the three EISB field studies (Pérez-de-Mora et al., 2014; Révész et al., 2014; Verce et al., 2015), where both bacteria and electron donor were introduced, hydraulic connectivity via fracture systems had a vital role in the efficacy of bioaugmentation. Where connectivity was high, reductive dechlorination activity was also high; where connectivity was poor, minimal reductive dechlorination was observed. This observation was true in mudstone shale (Révész et al., 2014), carbonate limestone and dolostone (Pérez-de-Mora et al., 2014), and cemented conglomerate bedrock (Verce et al., 2015). The addition of electron donor also

promoted dechlorination, especially as competing electron acceptors, other than the chlorinated ethenes, may interfere with biodegradation of chlorinated ethenes (Révész et al., 2014). Initial contaminant concentration did not seem to have an effect on bioremediation efficacy; rather, availability of electron donor, dissolved phase of contaminant, and presence of dechlorinating bacteria, especially *Dhc* with the *vcrA* gene, were more important factors that controlled reductive dechlorination activity.

To date, only one paper could be easily identified in the literature that examined whether dechlorinating bacteria in a contaminated site could be detected within the bedrock primary porosity, not merely in the fractures or suspended in the groundwater (Lima et al., 2012). Delineating whether dechlorinating bacteria are viable within the primary porosity has important implications for remediation, especially bioaugmentation, since DNAPL contaminant mass typically resides in the primary porosity (Parker et al., 1994). Lima et al. (2012) only assessed the sandstone-dolostone site at one point in time, and natural attenuation was evaluated, rather than EISB (no amendments were added). Continuous rock core, 85.3 m total length, was sampled at approximately 5 cm intervals, which allowed for the incorporation of various distances from fractures, to represent a range of geophysical and chemical conditions. Each 5 cm sample was split for microbial sequencing and VOC analyses.

Degradation occurred along the groundwater flowpath, and various electron donors were available (due to co-contaminants released previously on the site) to sustain biodegradation. Although pore size distribution, interconnectivity and pore throats can act as bottlenecks in geological media for microbial growth and migration, the pore radii in the Lone Rock Formation (0.8 – 18.9 μm, as determined by mercury intrusion porosimetry [MIP]; up to 50 μm, as determined by backscattered scanning electron microscopy), where 98% of the contaminant mass was located, were deemed sufficiently large for migration of target species, *Dhc*, which have cell diameters of about 0.5 μm (Maymó-Gatell et al., 1997). The molecular results using end point nested polymerase chain reaction (EP-PCR) and denaturing gradient gel electrophoresis (DGGE) provided convincing evidence for dechlorination in the pore matrix of the Lone Rock Formation, with *Dhc* and other known iron- and sulfate-reducers detected in some samples, even at a distance of 64 cm below the next closest fracture (Lima et al., 2012). Additional DGGE fingerprint analyses indicated highly heterogeneous communities, which could reflect the heterogeneity of the primary porosity (Lima et al., 2012). Thus, the results obtained from Lima et al. (2012) provide considerable evidence that dechlorinating bacteria are viable and actively dechlorinating in the pore matrices of contaminated, highly fractured, bedrock, even as conditions may not be entirely ideal (minor aerobic conditions were also observed; Lima et al., 2012).

Lima et al. (2012) set important groundwork for further evaluation of bacterial viability and dechlorination capabilities in the primary porosity of bedrock, especially for EISB purposes. Of particular

research interest would be bedrock environments where fracture systems are minimal, to understand the role of diffusion as it relates to the viability of bacteria in these systems. However, as eluded to previously, bacterial migration depends on characteristics of the geological material (discussed further in Section 2.7).

Accessibility of electron acceptor (i.e. bioavailability), lack of electron donor or other nutrients within the geological units, bacterial competition (Kotik et al., 2013) or electron acceptor competition, and non-ideal geochemical conditions (e.g. presence of oxygen or extreme pH) could also have negative impacts on EISB in bedrock. To overcome some of the limitations inherent in natural bedrock primary porosity when applying EISB (e.g. discontinuous flow paths, bacterial distribution, electron donor distribution), electrokinetics may be used.

2.5 Principles of Electrokinetics

In a basic EK setup, a pair of electrodes are placed in geological material and low intensity direct current is applied (Gill et al., 2014; Ng et al., 2014; Virkutyte et al., 2002). At the anode, H^+ ions are produced through oxidation, producing an acid front (**Equation 1**), while OH^- ions are produced at the cathode through reduction, resulting in an alkaline front (**Equation 2**), respectively (Acar et al., 1995; Acar and Alshawabkeh, 1993).

$$\textbf{(1)} \quad 2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

$$\textbf{(2)} \quad 2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

There are three types of movement that occur under the influence of an electrical potential (**Figure 2**): (1) electromigration (EM; movement of charged, dissolved, or suspended ions; Gill et al., 2014); (2) electrophoresis (EP; mass flux of charged particles; Acar et al., 1995); and (3) electroosmosis (EO; the mass flux of pore fluid; Acar et al., 1995). Both EO and EM are independent of the hydraulic conductivity of the soil, but highly dependent on voltage gradient, and thus amendments can be effectively distributed with EK, even in geologically heterogeneous or low permeability sites (Alshawabkeh, 2009; Gill et al., 2014; Jones et al., 2011; Saichek and Reddy, 2005). The direction of EO transport depends on the zeta potential of the geological material and the pH of the media.

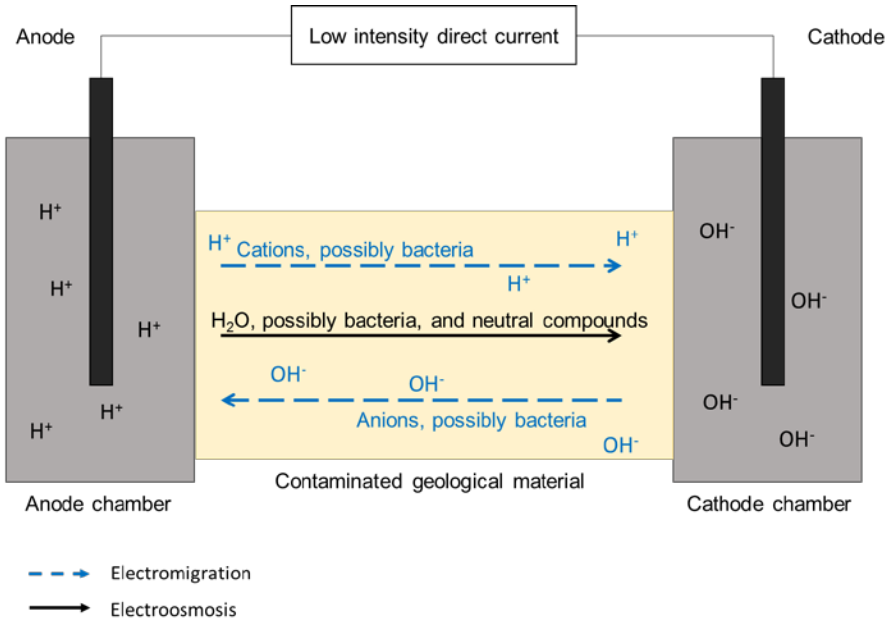


Figure 2 Schematic of bioaugmentation using EK, demonstrating electroosmosis and electromigration movements (modified from Ng et al., 2014).

EK-enhanced remediation is advantageous over non-EK remediation techniques because: (1) the technology can be implemented in the subsurface with minimal disturbance to the overburden material and to buildings at the surface; (2) it is suitable for variable geological materials; and (3) it reduces post-treatment volume of waste material (Alshawabkeh, 2009). In addition, EK can be paired with amendments (e.g. EISB) to enhance remediation success.

With bioaugmentation (the addition of non-native bacteria) or biostimulation (stimulating indigenous bacteria with nutrients), bacteria or amendments can be moved primarily by EM or EO. Bacteria and/or electron donor is consequently well-distributed within the geological media in a manner that cannot be achieved as quickly or effectively in the absence of EK, regardless of natural hydraulic conductivity. Thus, EK-enhanced bioaugmentation (EK-Bio) may be more effective than traditional remediation techniques, given the same site conditions. To date, only one paper has used EK to aid in bioaugmentation of intact sedimentary rock (Hansen et al., 2015). However, the lines of evidence used to confirm successful dechlorination were inconclusive.

2.6 EK-Bio in Bedrock

The experiment conducted by Hansen et al. (2015) suggests EK may be a viable technology to aid in EISB in intact bedrock (also known as EK-Bio), given slow natural diffusion of amendments into the

primary porosity. Besides enhancing amendment transport through bedrock matrix, EK can potentially create undesired electrolysis reactions (Hansen et al., 2015). At the anode, aerobic and acidic conditions are created, which could inhibit or kill the bioaugmented species; at the cathode, anaerobic and alkaline conditions are created, so that, although reducing conditions are optimal for microbes, the pH may be too extreme. In their study, a 20.3 cm long bryozoan limestone core was spiked with 150 mg/L cDCE using negative vacuum pressure and bioaugmented once with KB-1 using EK during the 31-day long experiment. A control experiment, in which no EK was applied, was also performed. The electrode reservoir solutions were periodically recirculated to counter the effects of pH fluctuations at the electrodes. At the end of the experiment, both treatment and control cores were sacrificed and analyzed in the lateral direction for lactate distribution, pH, as well as *Dhc* and *vcrA* concentrations via quantitative PCR (qPCR; Hansen et al., 2015). As the objective of this study was to design an experimental setup to assess the potential of EK aided EISB in contaminated limestone (Hansen et al., 2015), dechlorination products/VOCs were not analyzed.

Lactate concentration of 10 g/L was dosed into the core; 5 g/L lactate was measured in the aqueous mixing reservoir for the duration of the experiment, and only a slight decrease in concentration was observed at the anode, in conjunction with an increase in acetate, a fermentation product of lactate (Hansen et al., 2015). Lactate appeared to be transported by EM as expected because of the negative charge on the ionic donor, and by the absence of lactate at the cathode. The recirculation system appeared to effectively control against large pH fluctuations, as the mixed reservoir solution had near neutral pH (between 6.9 to 8.1). Analysis of pH in the lateral direction of the core within the pore volume indicated slightly more alkaline conditions, especially near the cathode end of the core (pH 7.9 to 8.7). Additionally, the core material (limestone, source of alkalinity) could have provided buffering capacity for extreme pH fluctuations, especially at the anode (Hansen et al., 2015).

There was no *Dhc* above detection limit along the length of the core, which is not surprising, given the small volume (0.5 mL of 10^{11} *Dhc* cells/L) of KB-1 bioaugmented into the core, and minimal acclimatization/incubation time (Hansen et al., 2015). Total DNA analysis, using spectrophotometric Nanodrop, which provides a sum of active and inactive intact cells, indicated that DNA content was highest along the axis boundaries of the core, which is also where lactate concentrations were lowest. The authors hypothesized that EP was the primary transport mechanism for bacterial transport (Hansen et al., 2015), but it is unknown whether the dosage of KB-1 was sufficient to observe dechlorination, especially as lactate concentration only decreased slightly overall, acetate concentration was moderate, which indicates only minimal fermentation activity, and VOCs were not measured. Additionally, the authors did not describe where KB-1 was dosed, for instance, into the mixing reservoir, or directly into the core at one or both electrode interfaces, which would also impact the transport efficacy of the bacteria.

Besides lack of convincing data to suggest EISB was effective in this study, many other issues were encountered with their apparatus. The pumping and recirculation system inadvertently caused the introduction of atmospheric air into the electrode reservoir, which could have detrimental impacts on the anaerobic KB-1 community (Hansen et al., 2015). Geochemical properties, such as pH and redox, were monitored only in the mixed reservoir, rather than at the electrode reservoirs, or were approximated from the porewater when the core was sacrificed. The redox electrode malfunctioned during experimentation, which resulted in no redox measurements. Although this would provide key information on the changes occurring in the pore matrix, pore volume was not sampled, as the authors believed there would be insufficient volume to extract and that sampling could disrupt the electrokinetically enhanced flowpaths (Hansen et al., 2015).

The setup designed by Hansen et al. (2015) demonstrated the first attempt at EK-Bio in bedrock. Although numerous issues were encountered and the resulting data was inconclusive, the authors established a valuable precedent for further investigations of EK-Bio in bedrock primary porosity. Most importantly, the design of their EK apparatus can be adapted, modified, or even drastically changed to address the issues encountered by Hansen et al. (2015). Paired EK-EISB in primary porosity can also be used to further investigate bacteria viability and the possibility of dechlorination once communities have been established in the pore matrix, as experimental times can be shortened significantly.

2.7 Amendment Transport Through Bedrock Primary Porosity

The relationship between sedimentary pore connectivity and solute transport is not a new concept (Löfgren and Neretnieks, 2006; Lu et al., 2015; Robinson et al., 2016). Of note is the lack of agreement between different authors, whereby some argue that matrix diffusion occurs only at the fracture-water interface, and others argue that diffusion occurs indefinitely due to pore connectivity (Löfgren and Neretnieks, 2006). Factors such as geological formation, rock type, stress of pore spaces due to depth, pore throat radii, and dead-end pores all contribute to assessments of pore connectivity.

Löfgren and Neretnieks (2006) used EM to evaluate long range pore connectivity in intrusive igneous rock. In their study, seven cores of variable lengths between 1.5 and 12.1 cm by 4.7 cm diameter were evaluated, including two cores that were split into short and long samples to compare connectivity of different core lengths of the same material. Iodide tracer was applied on one end of the core and direct current was used to mobilize the tracer through the cores. Formation factors, which are geometrical factors dependent only on the geometry of the micropore network, and independent of the diffusing species, were determined from through-diffusion on shorter cores (1.5 and 1.6 cm long), through-electromigration on both long and short cores, and by rock resistivity using both alternating and direct current (AC and DC,

respectively). Results of through-diffusion with through-electromigration formation factors were comparable, after accounting for natural variability of the formation factor of granitic rocks. Evaluation of formation factors derived from rock resistivity tests compared to the through-diffusion and through-electromigration formation factors was approximately two times larger, but could also be due to natural variation in the formation factor. The authors concluded that using these three different methods resulted in comparable formation factors. By extension, it may be reasonable to believe insofar if solutes can be transported into the interconnected pore matrix, EM will help transport the solutes orders of magnitude faster. If there are dead-end pores, no amount of time will distribute solutes beyond that space, whether transported by diffusion or EM.

It is recognized that the sizes and distribution of the primary porosity pores have important implications for biogeophysical and chemical processes (Phadnis and Santamarina, 2011; Stack et al., 2014). Pore size distribution, rather than porosity, was shown to affect changes in microbial activity with grain size and depth (Phadnis and Santamarina, 2011). Larger pores were more conducive for chemical processes to occur (Stack et al., 2014), rather than smaller pores, since solutes could precipitate and interfere with macropores; this could be due merely to size exclusion of solutes from micropores. Bacterial or amendment transport through bedrock may be inhibited by a lack of overall connectivity or increased interaction with the geological material (e.g. attachment). Bacteria may be controlled by the same transport mechanisms as other solutes in the bedrock environment. For instance, pore throat sizes could limit bacterial transport, if the bacteria are larger than the bulk of pore throat sizes (Hansen et al., 2015). In Lu et al. (2015), although larger pore sizes were observed in the Anahuac marine shale, they were mostly isolated by the abundant, nanopore-sized clay matrix. Changes in effective porosity could also occur due to microscale chemical reactions. In one study, exposure of mudstone-sandstone to TCE appeared to reduce effective porosity, possibly due to oxidation of ferrous minerals via an abiotic transformation pathway (Schaefer et al., 2013). Therefore, numerous processes control pore connectivity, and consequently, amendment transport, through primary porosity. These processes must be considered when designing and analyzing results of EK-Bio tests, where pore connectivity has a considerable role in the efficacy of amendment transport through bedrock matrix.

2.8 Research Objectives

There were two main research objectives for this thesis. First, to determine whether bacteria could be viable in the primary porosity of a high porosity sandstone bedrock. Second, if bacteria were observed to be viable, determine whether they could carry out reductive dechlorination within the primary porosity of contaminated sandstone.

Three main goals were identified to address the research objectives:

1) Apparatus development: Develop a column apparatus capable of reliably testing EK in bedrock to study the following two objectives of amendment transport and distribution (goal 2) and dechlorination within the bedrock (goal 3);
2) Amendment distribution: Determine if amendments could be distributed through primary porosity of sandstone using EK; and
3) Dechlorination in bedrock: Determine if biotic dechlorination could be promoted in bedrock following addition of amendments including bacteria.

Each goal was broken down into specific, achievable tasks that could be addressed sequentially:

1) Apparatus development
    a) Develop a method to seal sandstone cores to, and extract cores from, polyvinyl chloride (PVC) column sleeves;
    b) Design an EK column configuration that minimizes advective flow;
    c) Establish a technique to introduce, and retain, TCE in cores;
    d) Construct a method to sample primary porosity porewater effectively; and
    e) Assemble a system to deliver amendments to the cores.
2) Amendment transport and distribution
    a) Identify tracers to distinguish EM and EO transport;
    b) Demonstrate that charged tracers (e.g. chloride) can be forced to migrate through cores to validate EM;
    c) Demonstrate that non-charged tracers (e.g. TCE) can be forced to migrate through cores to validate EO;
    d) Demonstrate lactate migration via EM;
    e) Demonstrate TCE migration via EO; and
    f) Demonstrate KB-1 migration via EO and/or EM.
3) Dechlorination in bedrock
    a) Confirm whether suitable geochemical conditions can be achieved to sustain bacterial growth (e.g. negative ORP, neutral pH);
    b) Determine if TCE can be biotically dechlorinated via cDCE and VC to ETH;
    c) Determine if end products are formed (e.g. DHGs, chloride); and
    d) Assess if there is an increase in growth and activity of vital dehalorespiring bacteria.

There are significant knowledge gaps in the literature regarding amendment and bacterial distribution via EK with respect to remediation of chlorinated solvents in bedrock materials, which this thesis will attempt to address. This research also provides useful information related to bacterial viability in sedimentary bedrock primary porosity.

Chapter 3 Methodology

3.1 Overview of Experimental Design Process

In typical 1-D bench scale experiments, EK tests consist of a column of geological material (e.g. clay) packed into a non-conductive sleeve, with each end of the column inserted into electrolyte "tanks" filled with electrolyte solution (**Figure 3**). An electrode is placed into each of the electrolyte solutions, then connected to a power supply, which delivers low direct current (e.g. 0.05 – 0.5 mA/cm^2) through the column to migrate charged and uncharged species. Pumps are used to redistribute solution between the two EK tanks to collect overflow that accumulates in the cathode due to EO migration, to introduce amendments to the column, or to balance pH changes within the electrolyte tanks. EK is applied to encourage homogeneous distribution of the amendments and/or bacteria throughout the geological matrix, thus, current may be applied for a timespan of a few days or up to several days. After amendment and bacterial distribution, power is turned off, and the column can be left to incubate to encourage bacterial growth and dehalogenation of chlorinated solvents. Depending on the experimental design, amendments and bacteria can be dosed directly into the electrolyte solutions, or in wells cored directly into the geological material.

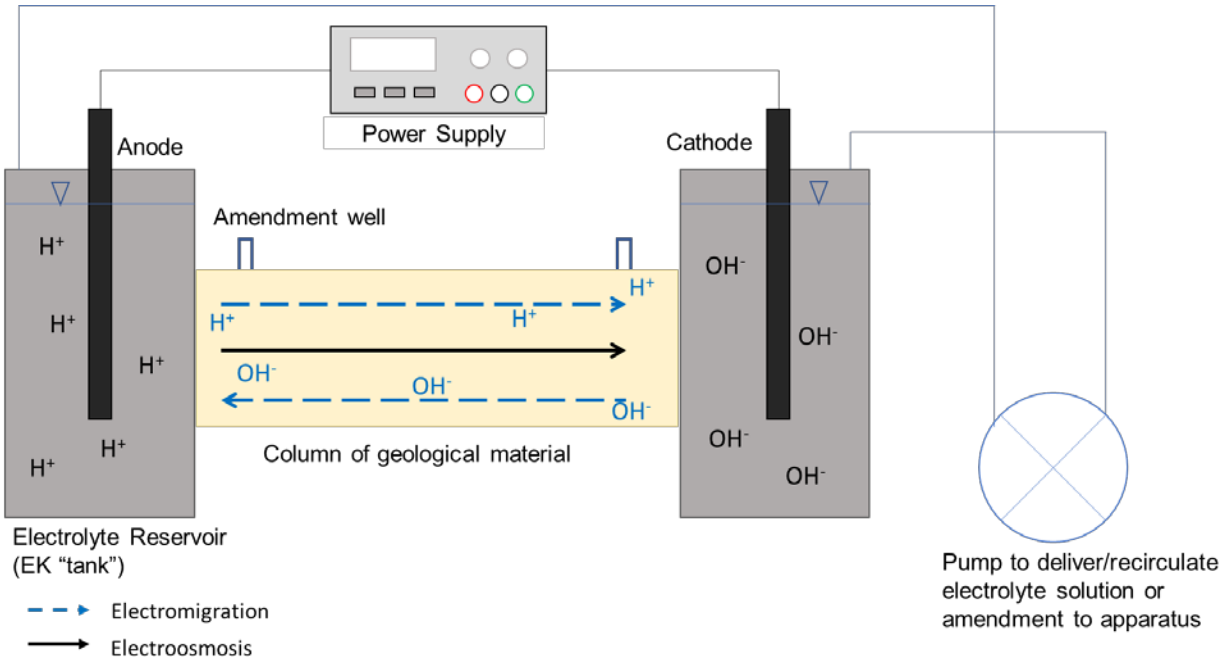


Figure 3 A basic EK setup consists of a column of geological material (e.g. clay), electrolyte tanks, electrodes that are connected to a power supply, which delivers low current density to the column, and a pump to distribute electrolyte buffers or amendments.

The initial EK tests in bedrock in this study were conducted using similar procedures to the abovementioned methods for typical clay EK tests. Sandstone cores were set into PVC sleeves, then inserted into electrolyte tanks containing electrolytic buffer, and EK applied. However, EK tests that incorporated only the sandstone core in the column revealed that modifications were necessary to address several design limitations, including:

- Short-circuiting of electricity and amendments along the outer axis of the core;
- Rapid EO transport, which created significant hydraulic head differences between the electrolyte reservoirs;
- Advective flow, as a result of EO transport, which confounded the interpretation of the mechanism(s) responsible for amendment transport;
- Poor TCE retention in the cores, as spiked solvent was flushed out of the cores when EK was applied; and
- Amendment delivery challenges, related to transfer of consistent amendment concentrations.

In total, as will be described in Sections 3.4, three separate column configurations were tested, validated, and modified as necessary prior to conducting the final EK-Bio experiments. Validation was required to ensure compliance with the overall goal of designing a functional apparatus. When all design limitations were addressed, the final column design (Section 3.4.3) was subjected to validation testing to demonstrate EK migration and to satisfy research goal number two (i.e. amendment transport and distribution). First, chloride tracer tests were conducted to assess EM. The apparatus was also tested to demonstrate migration of TCE via EO. Once the methodological approaches were validated, EK-Bio tests were conducted using the final, validated column configuration (complete details provided in Section 3.4.3). Different sampling timepoints (i.e. baseline, five weeks of incubation, and nine weeks of incubation) were chosen to assess variances in bacterial activity to satisfy the third research objective (i.e. dechlorination in bedrock).

3.2 Initial Materials, Methods, and Analytical Procedures

3.2.1 EK Apparatus

The main components of the EK apparatus used in this thesis consisted of two 10 L prefabricated PVC electrolyte tanks. The tanks housed electrolytic buffer and mixed metal oxide (MMO) electrodes, and were connected by a cylindrical column containing the geological material to be tested (**Figure 3**). A peristaltic pump delivered the amendment solutions to the column.

Each PVC tank had a single hole cut out of one side wall, designed to house a nominal pipe size (NPS) 3” (76.2 mm) ID PVC pipe. The tanks were filled with an electrolytic buffer composed of 15.3 mmol/L monobasic potassium phosphate and 24.7 mmol/L dibasic potassium phosphate buffer solutions (BioShop) targeting a pH of 7 in each tank. Stocks of each buffer solution were made by dissolving 430 g of monobasic potassium phosphate or 208 g of dibasic potassium phosphate in 1 L of reverse osmosis (RO) purified water. The final working buffer solution was made in 10 L batches by mixing 100 mL of both stock solutions into 9.8 L of RO water for each tank. Excess solution not used in the tanks was kept on reserve for top up of the tanks or to neutralize pH as the buffer capacity decreased over time.

A MMO electrode, approximately 30 cm long × 3 mm OD, was suspended in each tank, and connected to a power supply (Agilent E3612A) that was set to deliver a current density of 0.5 mA/cm^2 to the column. The pump (Gilson MINIPULS®3) delivered solutions at a rate of approximately 500 µL/min. Viton tubing and 2-stop peristaltic pump tubing (**Figure 4**; Cole-Parmer) were new or washed and re-used as necessary.

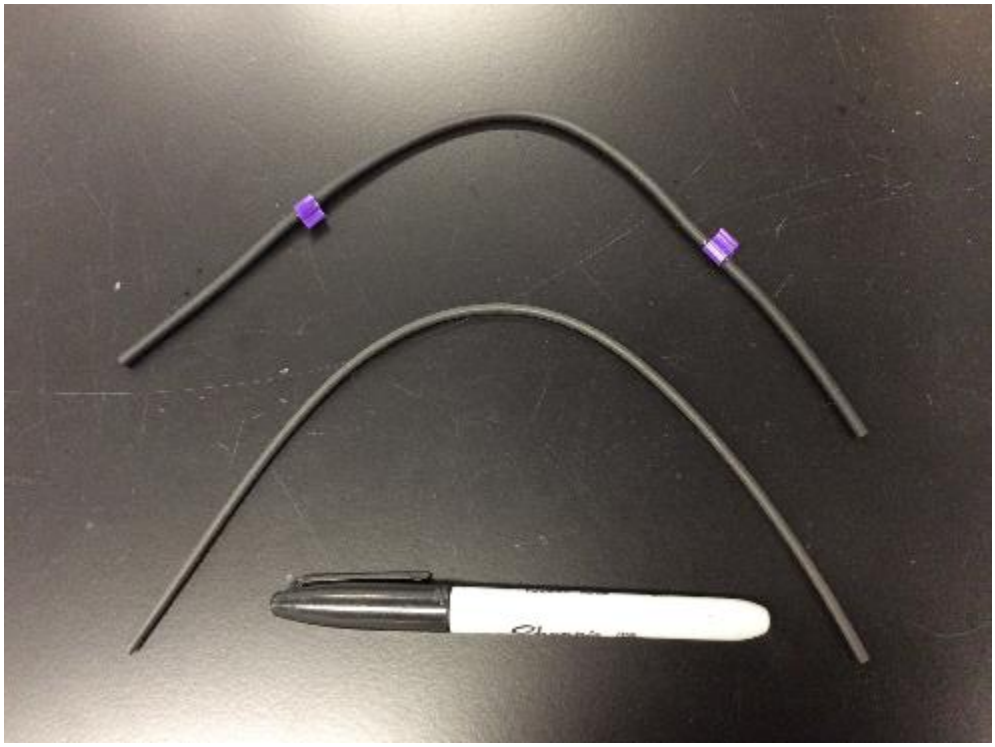


Figure 4 *Top:* Two-stop Viton peristaltic pump tubing for the Gilson MINIPULS®3 peristaltic pump. *Bottom:* Viton tubing used to deliver amendment solution.

3.2.2 Sandstone Core

Preliminary testing had been conducted with Idaho Gray sandstone, Indiana carbonate limestone, and Mancos shale cores (Kocurek Industries, Inc.), which had all been cored perpendicular to their bedding planes, to evaluate ease of amendment migration into the primary porosity of each respective bedrock type.

The cores were vacuum saturated under negative pressure (-23 psi) with an amendment solution containing sodium lactate, KB-1, and aqueous TCE contaminant (**Appendix A**). TCE was not effectively transported into shale, thus dechlorination of TCE was not detected; *Dhc* were not detected in the shale likely because they were not effectively transported into the tight primary porosity. Limited concentrations of chlorinated ethenes and *Dhc* were measured in the limestone. In the sandstone, dechlorination of TCE was evident and *Dhc* were recovered from the cores, which indicated that sandstone would be conducive for further testing in this thesis.

Idaho Gray sandstone was thus chosen for use in this study, as it had the highest porosity and permeability of various sandstone materials available from the commercial supplier. The largest available diameter from the supplier, 63.5 mm, was used; lengths of 127 mm were selected to minimize material costs and avoid inclusion of disturbed matrix that could lead to amendment transport biased along higher hydraulic conductivity pathways (Löfgren and Neretnieks, 2006) during sampling.

The following physical characteristics of the sandstone were provided by Kocurek:

- Porosity – 0.29;
- Brine permeability – 2,150-2,400 mD; and
- Gas permeability – 7,187-7,956 mD.

Additional physical, geochemical, and biological properties were analyzed, including porosity and pore size distribution by MIP on an intact 1 cm^3 sample (Micromeritics AutoPore IV 9500); surface charge at neutral pH to determine EO flow direction by zeta potential on crushed samples (ZetaPlus Analyzer); baseline microbial dehalorespiring capabilities by qPCR for VC reductive genes on a crushed sample.

Porosity determined by MIP was 0.17, lower than that provided by the supplier (method of analysis not provided), and the average pore diameter was about 13 μm (**Appendix B**). The zeta potential, averaged from twenty-five measurements, was -12.56 mV ± 4.93 mV, which indicated that at near neutral pH, EO flow direction would be from anode to cathode (**Appendix C**). The qPCR analysis of the sandstone did not identify any *vcrA* or *bvcA* above the quantification limit (**Appendix D**); therefore, native *Dhc* species were presumed absent from the virgin sandstone material.

Sandstone cores were received from the supplier, rinsed to remove fines accumulated on the surface from the cutting process, and dried in an oven at 60°C overnight to remove residual moisture in the cores. The cores were stored at room temperature until assembly in the EK apparatus.

3.2.3 Initial Column Design

To apply EK through sandstone cores, a suitable column design needed to be developed to encapsulate the sandstone cores, while preventing electricity and amendments from short-circuiting along the outer axis of the cores. The technique also needed to be conducive to sampling the cores (i.e. to extract VOCs). Marine paint and self-sealing plumbing tape were tested for their ability to waterproof the outer axis of the cores, without a sleeve casing. Although the materials did not impede sampling, they were pervious and therefore abandoned.

Non-conductive PVC pipe was a suitable alternative for paint or plumbing tape because it was designed to be utilized with the existing electrolytic tanks and because of its simplicity. The challenge was to devise a method to adhere the cores to the sleeve to prevent short-circuiting, to not impede EK transport through the cores, and to effectively extract the cores after EK treatment. Two methods were compared: concrete masonry silicone and a combination of concrete silicone near the faces of the core, with bentonite clay in the void space between the sleeve and the core. The combined silicone with bentonite clay method was abandoned due to the messiness of the technique and uncertainty that the outer core surface was made impermeable by the clay. Instead, concrete/masonry silicone (GE) was simple and effective. Both the silicone and PVC pipe could be removed by freezing the column, then sawing the pipe and peeling the silicone off the frozen core after EK testing to extract the cores for sampling.

A piece of hardened silicone was extracted in acidified HPLC grade MeOH to evaluate whether VOCs would emanate from the material into the core. There was no detection of any VOCs, including target chlorinated solvents (e.g. TCE, cDCE, or VC), above detection limits. Thus, the silicone was deemed suitable for use in the study, as it would not contribute additional constituents to the sandstone core.

The silicone was applied to the axial surface of sandstone cores in layers, with each layer allowed to dry before each subsequent application. When a silicone thickness of approximately 10 mm had set, the core was sealed with silicone to the edges of the PVC pipe, which extended approximately 10 mm longer than the core at either end, leaving the core faces open for transport, as shown in **Figure 5**. This column setup was the starting point for subsequent modifications (Section 3.4).

Figure 5 A sandstone core sealed along the axial surface with concrete/masonry silicone, then set into PVC sleeve with silicone near the faces of the cores. The PVC pipe extends slightly beyond the face of the core on both ends.

3.2.4 Core Preparation (Saturation) for EK Testing

Once the cores were encased in the PVC pipes, the cores needed to be saturated prior to use in the EK apparatus, otherwise the electrical circuit would not be complete. Cores were submerged in RO water or other solution and placed in a pressure-sealed environment (e.g. door chamber of anaerobic glove box or pressure vessel). Gases in the water and pore spaces of the cores were removed by subjecting the cores to negative vacuum pressure (-23 psi) for 15 min, replacing the negative pressure with N_2 gas (20 psi), then applying vacuum pressure again. The cycle was repeated at least three times or until the water stopped bubbling, which suggested that air pockets within the pore spaces of the cores had been replaced with the aqueous solution.

In initial trials to test the wetting method, the sandstone cores were saturated in deoxygenated RO water only. Cores used in EK-Bio tests (Chapter 8) were saturated in deoxygenated sodium lactate solution (EK-Bio). In addition, CO_2 gas was used to purge the solution instead of N_2, as it has higher solubility in water. The saturation solution needed to be deoxygenated because KB-1 require anaerobic conditions to survive.

3.3 Analytical Procedures

During EK operation, the electrolyte buffers were monitored daily for pH, ORP, and EC. The buffer solutions were adjusted as needed to return pH to near-neutral, or to maintain equivalent hydraulic heads between tanks throughout each experiment.

Aqueous VOCs were analyzed by gas chromatography-flame ionization detector (GC-FID; SiREM); anions and VFAs were analyzed by ion chromatography (IC); and reductive enzyme functional genes, *vcrA* and *bvcA*, were analyzed by qPCR (SiREM). Sandstone cores were sampled and analyzed for VOCs (Cascade Drilling, Montpelier, VT) using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-electron capture detector (GC-ECD). Clay pucks (Sections 6.3 and 6.4) were analyzed for VOCs using GC-MS (ALS Waterloo). A summary of the analytical details is provided in **Table 1**.

Table 1 Analytical details.

Analysis	Instrument	Minimum		External Laboratory
		Volume	Mass	
Aqueous VOCs	GC-FID	1 mL	-	SiREM
Anions (aqueous and extracted from solid)	IC	100 µL	15 g (extraction)	-
VFAs (aqueous and extracted from solid)	IC	100 µL	15 g (extraction)	-
Functional genes, *vcrA* and *bvcA*	qPCR	-	0.25 g	SiREM
Sandstone VOCs	GC-MS, GC-ECD	-	15 g	Cascade
Clay VOCs	GC-MS	-	5 g	ALS

To assess homogeneity in amendment distribution across the sandstone cores, the cores were sectioned into five sample "pucks" after each trial run (**Figure 6**). Each puck was approximately 25 mm thick. The outer two pucks (1 and 5) were typically discarded to eliminate bias caused by proximity to the electrolyte reservoirs or solution delivery wells. The inner two pucks (2 and 4) were sampled in the middle of the puck, with the outer edge trimmed, whereas the centre puck (3) was divided into top, middle, and bottom subsections. Each puck or subsection was analyzed for anions and VFAs. A method was developed to extract the analytes into water. If the cores did not require VOC analyses, cores were sectioned into pucks using a chisel, and puck 3 further divided into the three subsections. A portable rock crusher (**Figure 7**; Sore Thumb, BlackCatMining.com) was used to pulverize the puck pieces, then a subsample of each puck was mixed with an equal amount (by mass) of ultrapure water in a 50 mL centrifuge tube. The centrifuge tubes were placed horizontally on a shaker table and shaken at 100 rpm for 24 hrs to extract analytes into the aqueous phase. Extractant was analyzed directly on the IC. The same crushing procedure was used to sample the cores for molecular analysis. When VOC analyses were required for the cores, entire cores were

sent to Cascade, who performed initial sampling and VOC analyses, and returned the unused portions for anion and VFA analyses. Cascade used a specialized crushing apparatus to simultaneously crush the puck samples in a sealed environment and capture the samples in methanol filled vials for extracting VOCs for analysis.

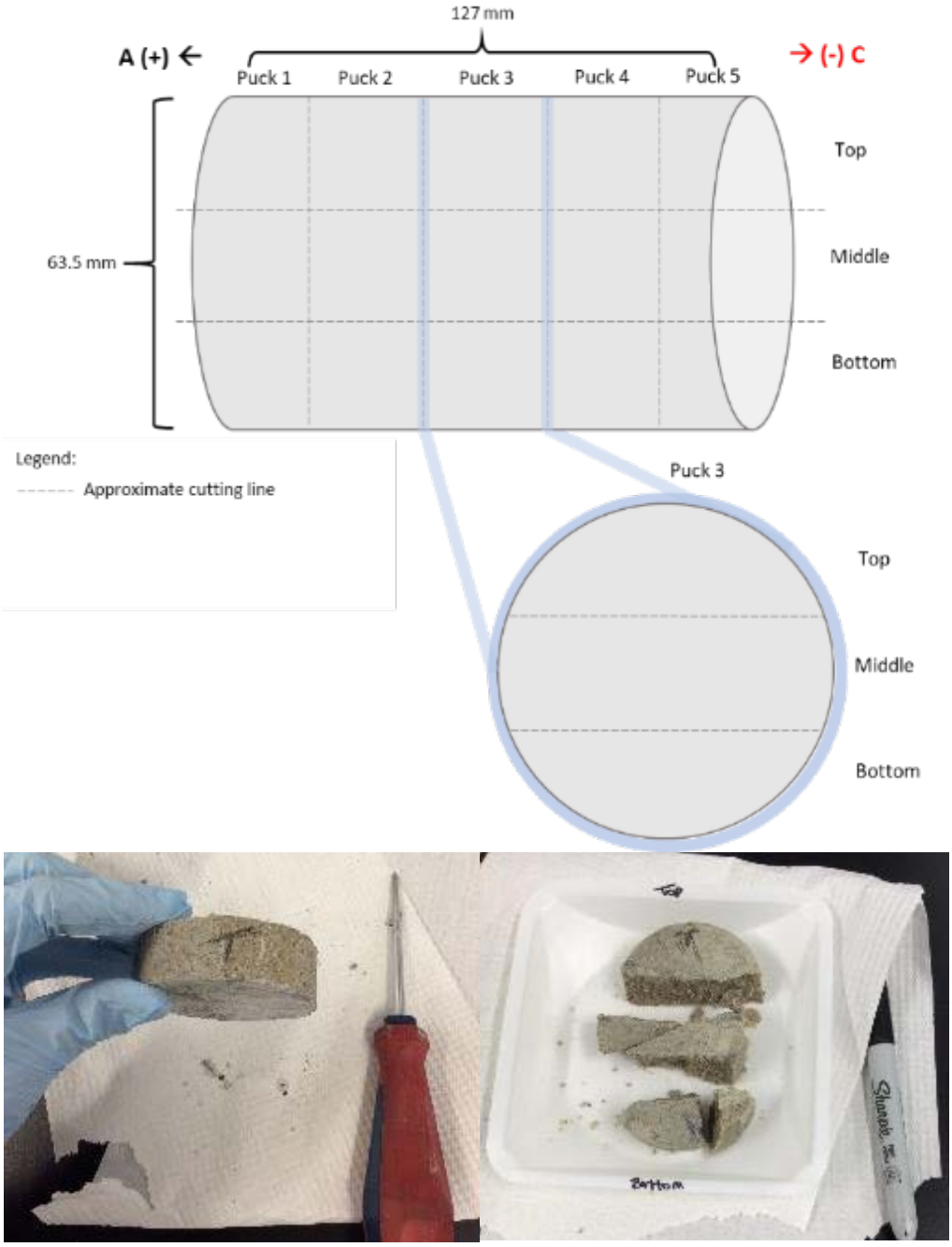


Figure 6 *Top:* Cores sectioned into pucks. *Bottom left:* Example of a puck when sawed off the core. *Bottom right:* Example of a puck sampled into top, middle, and bottom pieces.

Figure 7 Portable or hand-held steel rock crusher used to crush puck samples for extraction.

3.4 Additional Column Modifications

Since very few laboratory studies of EK in bedrock have been attempted, significant column development and testing efforts were required. The initial column design, which consisted of only the sandstone core, was deemed unsuitable and had to be modified, as outlined below. A series of iterations were tested, with each version requiring slight adjustments, until the final version (Design 3) satisfied the requirements of a functional column. This section discusses each column design tested in the development phase. The final validated column setup is discussed at the end of this chapter.

3.4.1 Column Design 1 – Sandstone Core Only

The initial column design consisted of only sandstone core within the PVC column attached to the anode and cathode reservoir tanks (**Figure 8**). Tracer solutions were added to the core directly via vertical wells drilled into the anode and cathode ends of the sandstone core. It was assumed that the sandstone would have sufficiently low permeability, so advective flow would be negligible. However, in the first trials with EK applied, large head differentials between the anode and cathode tanks were observed after only a couple of hours of EK power application (data not shown). The differential was significant enough to cause advective flow of buffer from the cathode to anode tanks, due to the build-up of head in the cathode tank. While tracer results of sodium lactate, sodium bromide, and KB-1 showed effective and generally uniform

distribution of amendments across the sandstone (**Figure 9** and **Figure 10**, respectively), given natural heterogeneity of the sandstone, the contributions of EM and EO could not be isolated from that of advection.

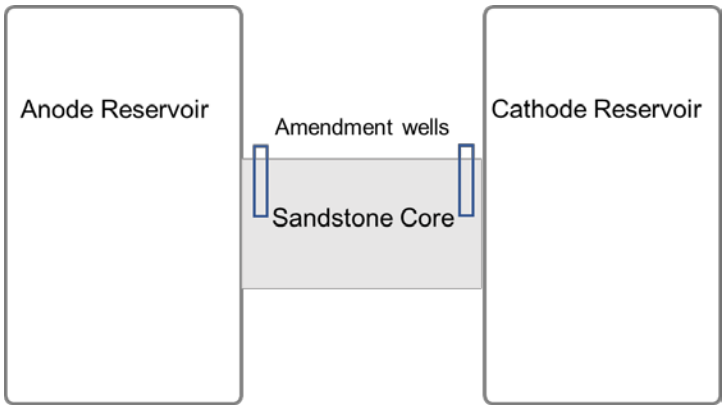


Figure 8 Schematic of initial EK column design.

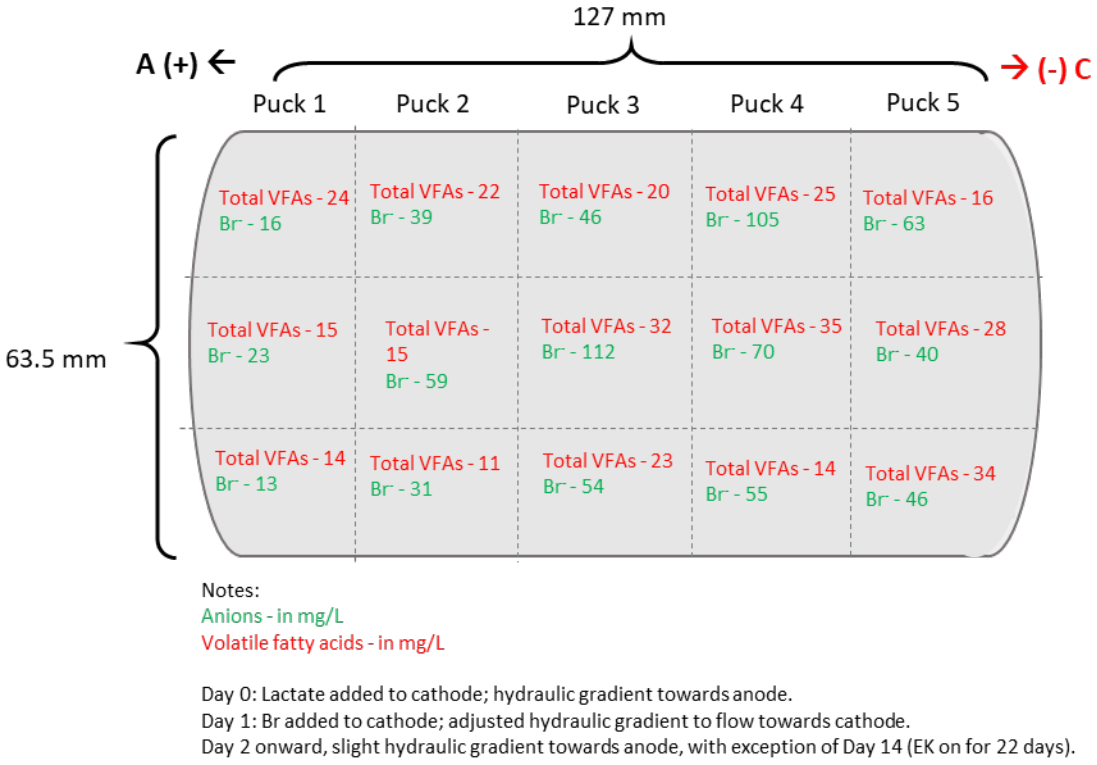
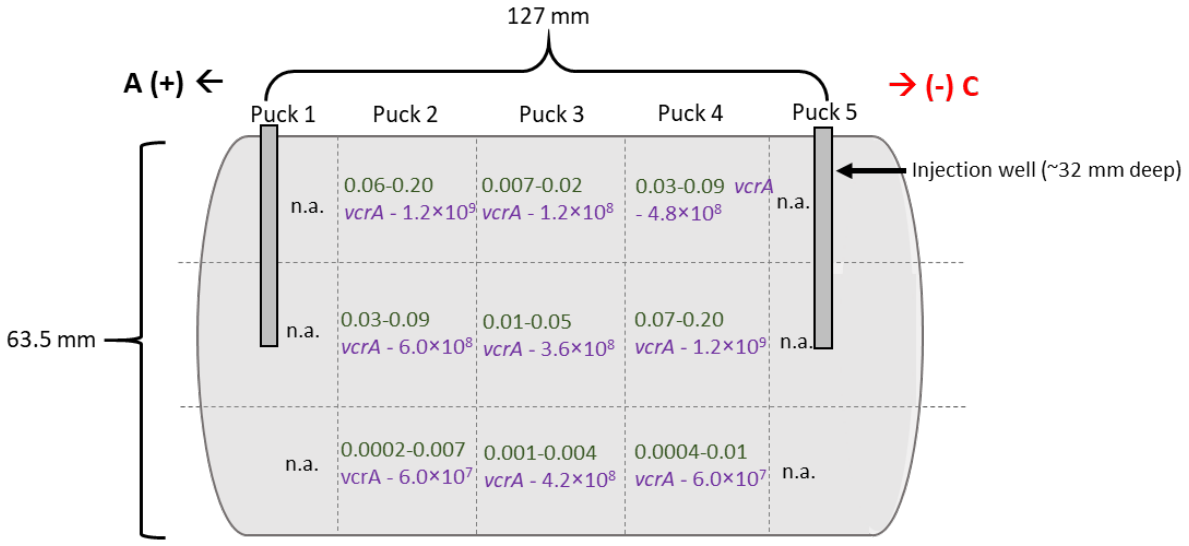


Figure 9 Anion extractions with lactate (analyzed as total VFAs) and bromide injected into the cathode electrolyte reservoir and transported via EM.



Notes:
% *vcrA* in microbial population of extractable DNA
n.a. - not analyzed
Vinyl chloride reductase - in gene copies/L (detection limit ~3.0×10^7 gene copies/L)

1. Below 10^4 gene copies/L, ethene dechlorination will not occur; between 10^4 - 10^6, some dechlorination will occur, but likely stall; between 10^7 - 10^9, complete dechlorination to ethene will occur.
2. Concentration of *Dhc* in KB-1 parent vessel: 3.85×10^{11} *Dhc* gene copies/L; percent *Dhc* compared to all amplifiable rRNA: ~84% of *Dhc* in parent vessel; percent with *vcrA* enzyme: assume 100%.

Figure 10 Reductive gene analysis with 0.5 mL of KB-1 injected directly into wells in the core and migrated via EM and EO.

In addition to the advection issues, several other limitations were encountered with this design. The supply wells in the cores complicated the amendment injection process. In EK-Bio tests, multiple solution amendments and KB-1 would be added to the column, but the limited volume of the wells could hinder solution transfer. Alternatively, injection of multiple well volumes of amendment in a short period of time could force amendment into the primary porosity adjacent to the wells, which would confound the transport mechanism. Porewater sampling could also be challenging if there was insufficient volume available in the wells at each sampling timepoint.

Initial saturation of the cores with aqueous TCE was also complicated with this design. Since clean sandstone cores were used in the study rather than field contaminated cores, a reliable method was needed to saturate the primary porosity of the cores with aqueous TCE. Two techniques were tested using negative vacuum pressure in pressure vessels to force TCE solution into the primary porosity (similar to the saturation method used for core preparation, Section 5.2.1) and using peristaltic pumping to fill pore spaces against gravity (as would be used to saturate unconsolidated soils).

Three issues arose with the pressure saturation method. First, a satisfactory mass balance could not be achieved. In multiple trials, the TCE concentration in saturated cores, even without EK application, was

several orders of magnitude lower than the feed solution. Therefore, a significant loss of contaminant occurred, possibly through volatilization into the headspace of the pressure vessel. The second issue was solution preparation and sampling variability. Although VOC analyses confirmed TCE presence in the feed solution, there was high variability between duplicate samples (**Table 2**).

Table 2 Concentrations of a batch solution spiked at a target of 100 mg/L TCE sampled the specified days after spiking.

Days After Spiking	TCE (mg/L)	
	Rep 1	Rep 2
4	114	68
6	42	42
8	48	-
10	22	-

Further experimentation revealed that initial batches of TCE solution made up in pressure vessels were not given sufficient time for the neat TCE to equilibrate, given the large volumes of solution required (6 L). The method of sampling from the vessel may also have impacted the results. Initial batches of solution were prepared by injecting neat TCE into the feed ports of the vessels without using additional aliquots of water to chase the neat TCE (**Figure 11**); this method allowed the water to remain anaerobic. However, it is possible that when VOC samples were taken from the same port, neat TCE may still have been attached to the walls of the port, leading to biased high detections (data not shown). Later batches of solution preparation addressed these concerns; neat TCE was injected directly into the water from the vessel opening, and the solution was left to equilibrate for at least three weeks prior to use. The solution was also purged with N_2 gas as needed to create anaerobic conditions. The third reason the pressure saturation technique was not deemed acceptable was due to TCE being flushed out of the core as soon as EK was applied, either by EO, advection, or both (post-EK sandstone VOC results, **Table 3**).

Table 3 TCE concentrations extracted from core samples treated with pressure saturation to contaminate the pore spaces.

Solution	TCE Concentration		
	Aqueous (mg/L)	Core Rep 1 (µg/kg)	Core Rep 2 (µg/kg)
Spiked Solution	58	-	-
Pressure Saturated - no EK	-	4,190	2,360
Pressure Saturated - after EK	-	10.3	13.2

The peristaltic pump method could not be configured to function as desired. Teflon coated rubber stoppers were stoppered into both ends of the PVC pipe with the core sealed inside, and pump tubing inserted into the rubber stoppers to allow solution transfer. A peristaltic pump was used to pump solution into the vertically positioned column, by feeding solution from the bottom of the column and out through

the top. However, the force of gravity could not be overcome with the pump. Thus, a column assembly containing only the sandstone core had numerous issues that made the particular design unsuitable for the study. Negative pressure saturation and pumping against gravity also were not viable options to contaminate sandstone cores.

Figure 11 *Left:* Basic components of a pressure vessel include the feed port, which extends to the bottom of the vessel (1), pressure release valve (2), and pressure gauge (3). *Right:* A 6 L pressure vessel in the foreground; additional vessels in the background.

3.4.2 Column Design 2 – Sandstone Core with Clay Caps

To address both the hydraulic head variance and flushing of TCE out of the core, clay caps were placed adjacent to each open end of the sandstone core in the PVC column (**Figure 12**). The clay acted as low permeability barriers to prevent advective flow through the columns due to differing hydraulic head levels in the anode and cathode reservoir chambers. Two different craft modelling clays, chosen initially for economical reasons, were tested to determine compatibility, but were discarded in favour of pure kaolinite clay (Sigma Aldrich), as it provided the greatest reproducibility between column sets. Kaolinite was wetted using RO water and approximately 1.5 g/L KBr (BioShop) solution to increase the ionic strength of the

clay, as well as to maintain the structural integrity of the clay caps. The introduction of sodium ions in the EK column were avoided, where possible, since sodium caused slumping or collapsing of clays when EK power was turned on. The electron donor was the only amendment where sodium was used, since it was provided as a solution from the supplier. For each column that was assembled, new clay was used to avoid cross-contamination of TCE and other experimental constituents, except for the validation columns, as indicated below.

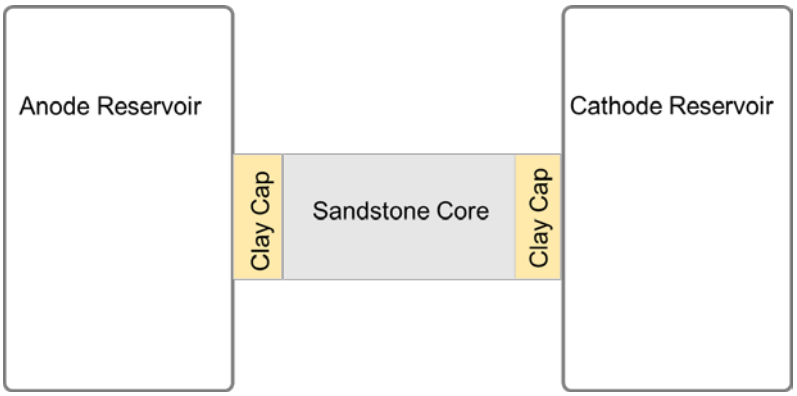


Figure 12 Schematic of EK Column Design 2 with clay caps bounding the core.

A series of permeameter tests were conducted to compare hydraulic conductivity (K) through the column in the absence and presence of clay caps, and in the absence and presence of EK. Examples of K are listed in **Table 4**. Hydraulic head of sandstone only, measured by falling head, was $4.2\times10^{-2} \pm 2.4\times10^{-2}$ cm/s (n=4). Varying clay cap thickness did not appear to alter K significantly when EK was applied and reservoir heights were equal at the beginning of the experiments, indicating consistency in EO permeability. Thus, the addition of clay caps near the cathode, even in the absence of EK, was advantageous for minimizing advective and EO fluxes through the core. Clay was also added near the anode since it was deemed that there were no negative consequences for adding additional clay caps to the design, and the caps could potentially help prevent TCE within the core from flushing out once EK was applied.

Table 4 Changes in the hydraulic head of the column with the introduction of clay caps, with and without EK; n = 4 per each condition.

Clay Location (C - Near Cathode, B - Near Both Electrodes)	Individual Cap Thickness (cm)	Total Cap Thickness (cm)	EK?	K ± SD (cm/s)
-	-	-	no	$4.2\times10^{-2} \pm 2.4\times10^{-2}$
C	1	1	no	$7.8\times10^{-5} \pm 4.6\times10^{-5}$
B	1.75	3.5	no	$3.0\times10^{-6} \pm 3.6\times10^{-6}$
B	1.75	3.5	yes	$1.3\times10^{-5} \pm 3.0\times10^{-6}$
B	2.5	5	yes	$1.7\times10^{-5} \pm 1.8\times10^{-6}$

The negative pressure saturation technique to contaminate the sandstone with aqueous TCE was also tested on a core assembled with two adjacent clay caps. The TCE concentration used to saturate the core was 164 mg/L; immediately after saturating the core, the concentration had dropped to 46 mg/L. The concentration in one core sample immediately after saturation was 7,070 μg/kg TCE. However, the core treated with EK for six days after saturation had TCE concentrations below quantification. Two validation tests were conducted with this column design, using stock KB-1 solution as the tracer for both EM and EO migration in two setups with two separate cores (**Figure 13** and **Figure 14**). Details of the setup are presented in **Appendix E**. Breakthrough of the VC reductase gene was only detected in the EO test, suggesting that EO was an effective transport mechanism for bacteria. Bacterial transport by EM may not be efficient or may require a longer transport time compared to EO.

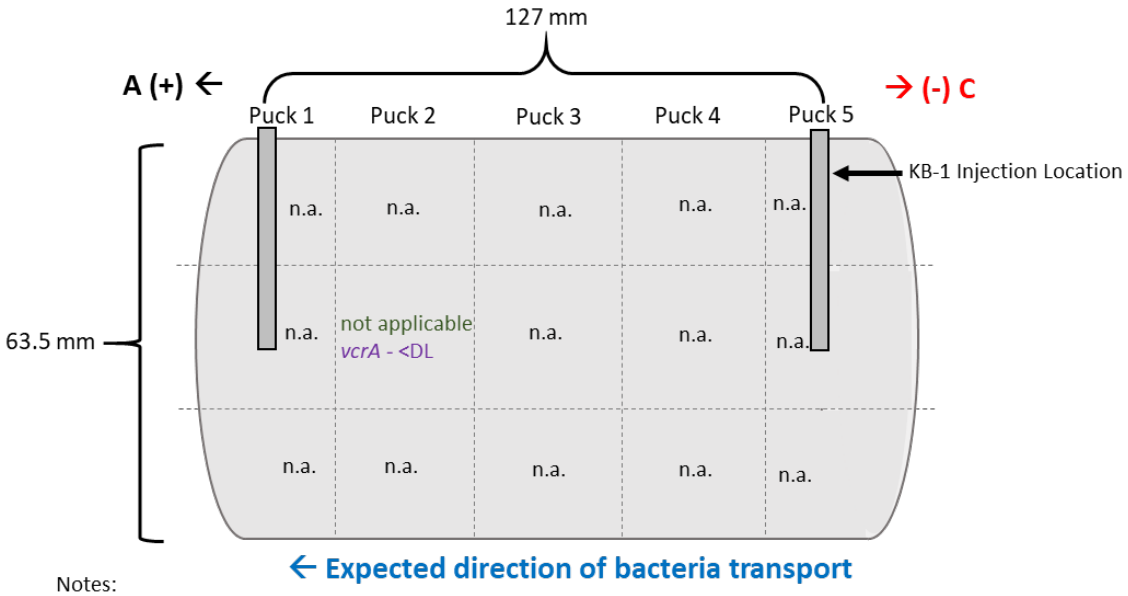


Figure 13 Analysis of reductase gene with KB-1 injected near the cathode end of the core and with bacteria expected to transport via EM.

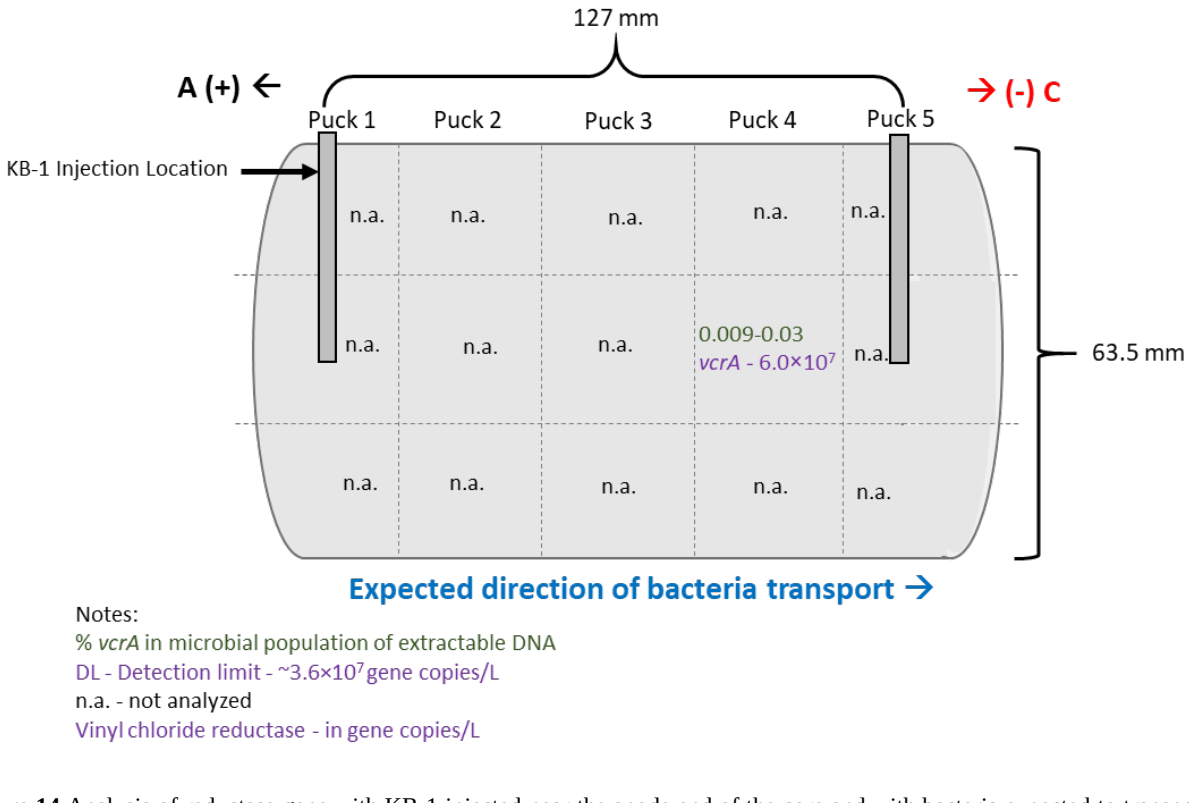


Figure 14 Analysis of reductase gene with KB-1 injected near the anode end of the core and with bacteria expected to transport via EO.

3.4.3 Column Design 3 – Sandstone Core with Clay Caps and Sand Wells

To improve the uniform delivery of multiple amendments to the sandstone core, including TCE, to allow sampling of porewater at each end of the core, and to overcome volume limitations in the supply wells, a third design was prepared where a sand unit was inserted at each end of the column. In this design version, a clay cap bounded each end of the sandstone core, as in Column Design 2, but this was followed by a sand unit, held in place with another clay cap (**Figure 15**). The silica sand (US Silica, Grade 3 Q-ROK) had a grain size of medium sand (**Figure 16**), and an average K of 2.65 cm/s (**Table 5**). The sand was washed in a 10% acid bath (either nitric or hydrochloric acid, as available) and rinsed at least ten times with RO water until pH was neutral, then rinsed a final time with Milli-Q water before use to ensure it was clean.

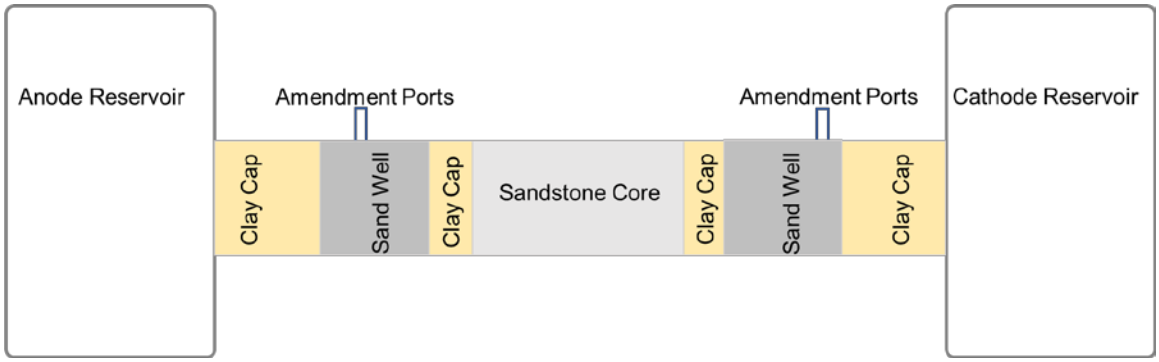


Figure 15 Schematic of final EK column design used for validation and EK-Bio tests (not to scale).

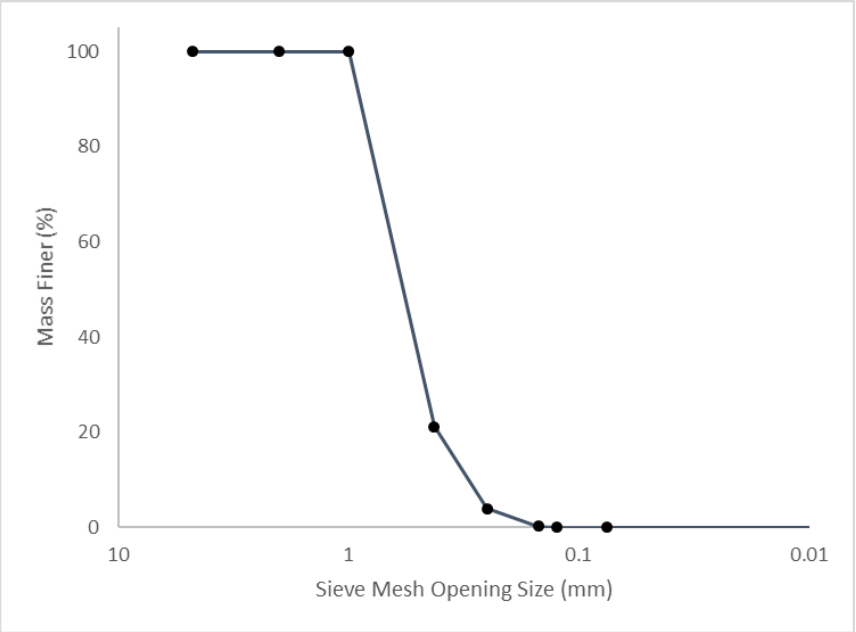


Figure 16 Grain size distribution of silica sand.

Table 5 Permeability of silica sand determined experimentally.

Trial	L (cm)	H_o (cm)	H_t (cm)	t (s)	k (cm/s)
1	3.8	145	84	8.89	3.15
2	3.8	145	85	8.84	3.07
3	3.8	145	84.7	8.82	3.08
4	2.8	144	83.5	7.99	2.08
5	2.8	145	85	8.83	2.26
6	2.8	145	85	8.84	2.26
				Avg. k	**2.65**

$$\text{Falling Head Permeability } k = \frac{aL}{At}\ln\left(\frac{H_0}{H_t}\right)$$

$$where\ a = area\ of\ water\ column, 1.948\ cm^2$$
$$L = length\ of\ soil\ column$$
$$A = area\ of\ soil\ column, 11.40\ cm^2$$
$$t = elapsed\ time\ from\ H_o$$

With the introduction of sand zones in the column design, the cores could be saturated with TCE using elements of both pressure saturation and peristaltic pumping techniques. TCE solution was prepared in advance in a pressure vessel to allow for equilibration, and sampled over time to verify that the concentration was close to the target. Once the concentration was within 10% of the target TCE concentration, the solution was transferred to a Tedlar bag and pumped at a constant rate via the peristaltic pump into the anode sand well. Applied EK would then transport TCE from the anode sand well into the core via EO. Continuous delivery of TCE solution ensured the cores had consistent TCE concentration; as TCE was flushed out of the cores, fresh solvent would be pumped in. The issues that were overcome using this saturation method included: volume limitations of the supply wells in the cores; ability to continuously add solution into the cores; advection influences due to EO; amendment transport distance; and dilution influences, which would have been problematic if amendments were added to reservoir chambers instead. All the specific tasks that were outlined to address apparatus development were accomplished at this stage (**Table 6**).

Table 6 Summary of tasks to address the first goal of apparatus development.

Goal	Task	Achieved?	How?
Apparatus Development	1. Seal and extract cores from column	Yes	PVC pipe and concrete silicone; sawing
	2. Prevent advective flow	Yes	Clay caps
	3. Deliver constant input of TCE	Yes	Peristaltic pump and sand wells
	4. Sample porewater	Yes	Sand wells
	5. Deliver amendment to cores	Yes	Peristaltic pump and sand wells

For the actual EK-Bio experiments using column design 3, two additional PVC pipe sections, of similar length as the main core pipe section, were required to house the additional clay and sand units. These additional pipe sections were connected to the main PVC pipe that contained the cores. One end of each additional PVC pipe was softened with a heat gun to flare and fit over one side of the central core pipe. Plastalina modelling clay (Craftsmart, Michaels) was applied at the two joints to prevent leakage once the column was fully assembled. This non-permanent method of joining the pipe sections allowed for easy assembly and disassembly of the column, and allowed the outer pipe sections to be reused; only the clay and sand units would need to be repacked for subsequent columns.

To pack the columns, one end of the column was filled with the clay and sand units, then repeated on the other side of the core. Filter paper (Whatman #42; 2.5 μm particle retention) was cut to size, placed adjacent to the core, then 1 cm of prepared kaolinite clay, prepared according to Section 3.4.2, tamped down, followed by another sheet of filter paper. A five cm thick unit of sand was added to the column, wetted, then tamped flush to minimize void space, followed by filter paper, and the final 5 cm layer of kaolinite. One final sheet of filter paper was placed on the outer surface of the clay, then a 3.2 mm thick, porous polyethylene (PE) filter, and a porous, 37 mm thick, mesh polytetrafluoroethylene (PTFE) cap was inserted into the pipe to prevent the clay and sand from slumping out of the column (**Figure 17**). Within each sand well, three sampling ports were installed to allow for input of amendment solutions and sampling (**Figure 18**).

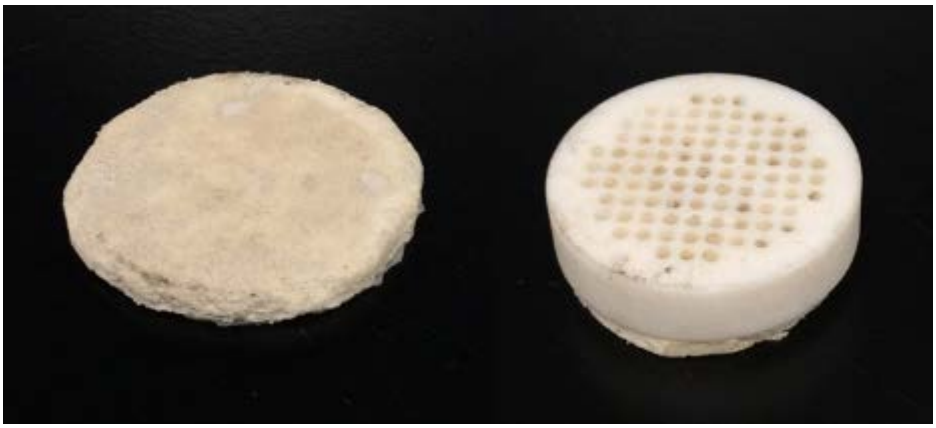


Figure 17 Materials inserted into the column to prevent clay slumping during assembly. Left: Porous PE filter placed adjacent to the outer clay cap. Right: Mesh PTFE cap placed on the outside of the PE filter (directly exposed to electrolyte buffer).

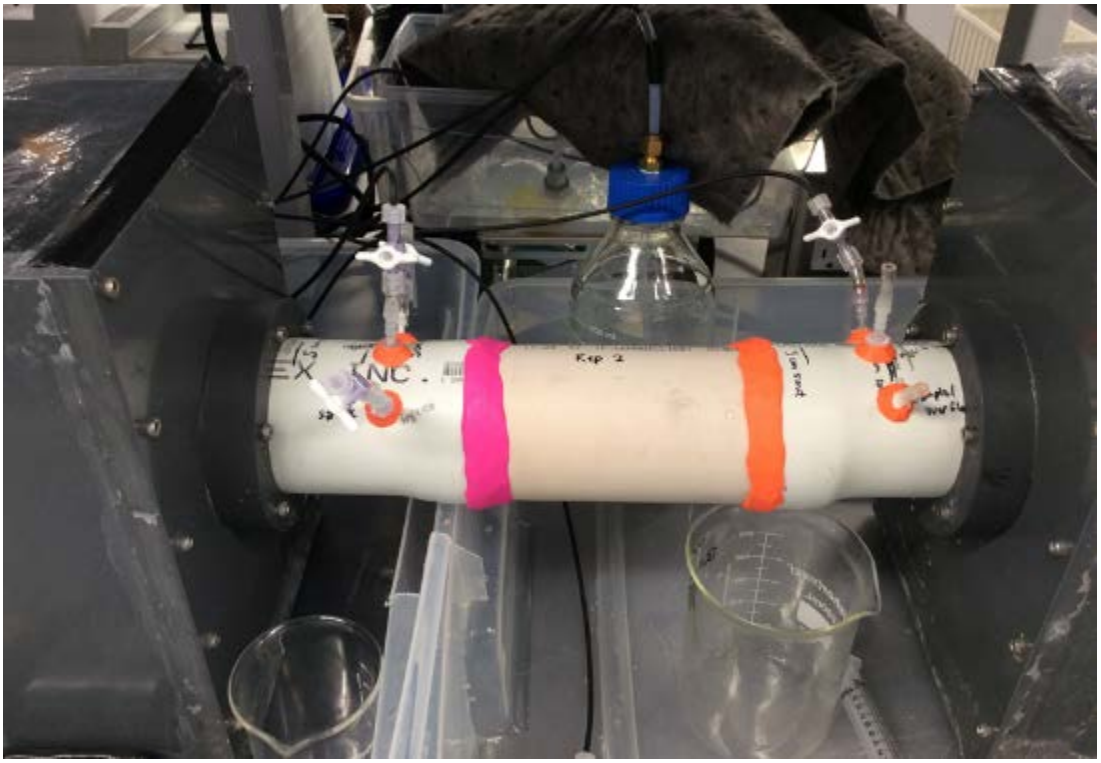


Figure 18 A column setup showing the three ports in both sand packs and the three sections of PVC pipe joined together.

3.5 EK Apparatus Validation – Column Design 3

3.5.1 EK Apparatus Validation

Two validation experiments were conducted to ensure the finalized column design was appropriate to migrate amendments. Chloride was chosen as the EM tracer because it could be migrated from cathode to anode. TCE was chosen as the EO tracer because as a non-charged molecule, it could be migrated from anode to cathode and would not be subject to EM. One column was assembled according to Section 3.4.3, with EM validation tested first, followed by EO validation on the same core. The column components were not replaced between tests.

3.5.2 EM Validation

In the EM test, 526 mg/L of potassium chloride (BioShop) tracer solution was continually pumped into the cathode sand well, while direct current was applied. The anion tracer was sampled at the anode sand well twice daily until Day 3; thereafter sampled periodically to assess breakthrough and steady state concentration. Breakthrough occurred around Day 5 and steady state began around Day 7 (**Figure 19**). The input solution entering the cathode sand well was also sampled periodically to monitor whether the tracer concentration dropped over time. Excess volume in the column was manually discharged into waste vessels intermittently, since the sand wells would not automatically purge excess volume once full. Diffusion had a negligible role in transporting chloride through the core, since transport of a chloride ion by diffusion only was calculated to reach between 1 and 2 cm from the cathode end into the core after five days, assuming $D_{molecule}$, or the diffusion coefficient, of chloride in water at 25°C was 2.03×10^{-5} cm/s (**Equation 3** and **Equation 4**). A molecule transported by advection only would reach just 1 cm into the core from the cathode end in five days.

$$\textbf{(3)}\ D_s = \frac{D_{molecule}}{\tau^2}$$

$$where\ D_{molecule} = diffusion\ coefficient_{molecule}, cm^2/s$$

$$\tau = tortuosity\ (estimated\ values\ ranging\ from\ 1.3\ to\ 2.4)$$

$$D_s = diffusion\ in\ porous\ media, cm^2/s$$

$$\textbf{(4)}\ x = \sqrt{D_s t}$$

$$t = time, s$$

$$x = distance, cm$$

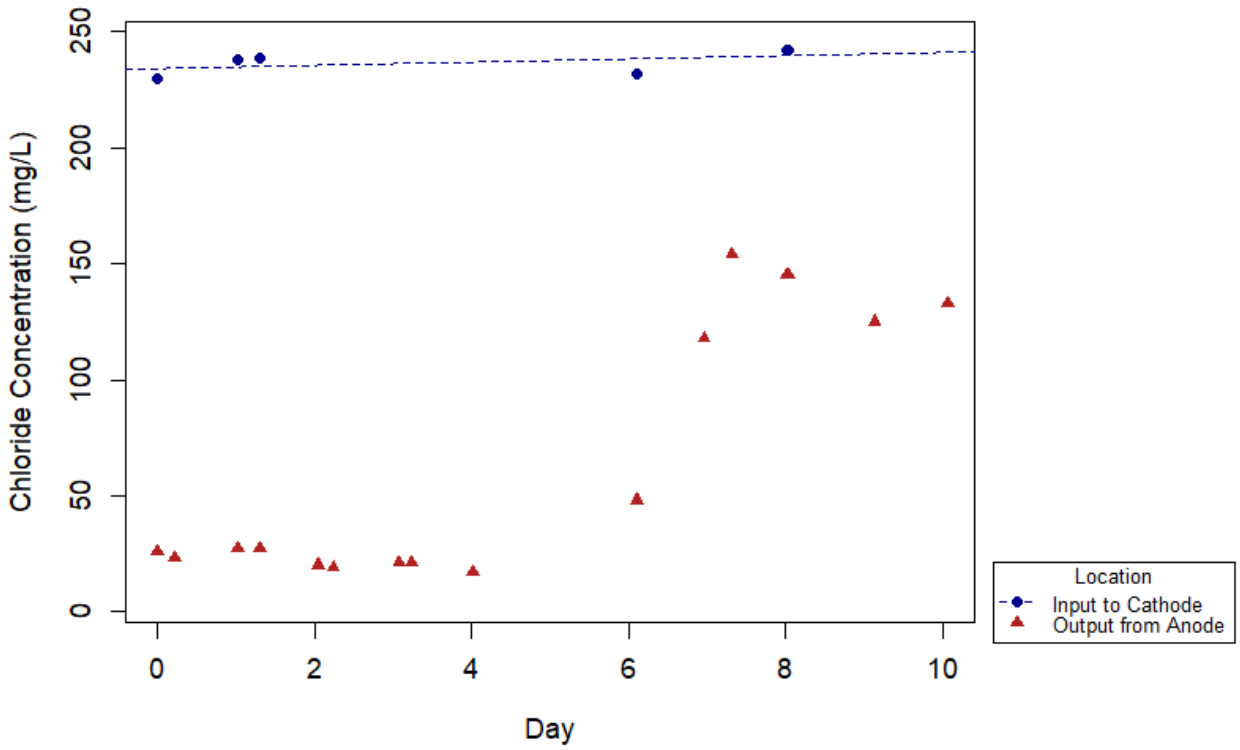


Figure 19 Chloride concentrations in the EM validation test, using column design 3. Breakthrough at the anode sand well occurred around Day 5 (no data available), and steady state concentration was reached around Day 7.

3.5.3 EO Validation

A 6 L batch of 186 mg/L TCE solution was made in advance, as described in Section 3.4.3. The TCE solution was continually pumped into the anode sand well, while direct current was applied, and sampled at the cathode sand well. Due to the analytical costs associated with analyzing VOCs, and the uncertainty of solvent breakthrough time, this test was sampled less frequently, compared to the EM test. This validation test was run for 26 days with power on (after factoring out power downtime and mechanical troubleshooting). Breakthrough of TCE at the cathode sand well occurred at, or before, Day 4 (earlier days not sampled), with steady state reached around Day 7 (**Figure 20**). Excess volume in the column was manually discharged into waste vessels intermittently.

The hydraulic heads of both reservoirs were measured daily to ensure there was no influence on transport by advection. The maximum head differential was 5 mm, but corrected as soon as there was a difference of 1 mm between the reservoirs. The differential was corrected by reducing the volume in the cathode reservoir, or adding fresh buffer to the anode reservoir to bring the heads back to the same height. Although the breakthrough time appeared to be sooner for TCE via EO compared to chloride via EM, which could

be due to faster transport by EO, it took longer for TCE to reach steady state compared to chloride, likely due to a greater retardation factor for TCE.

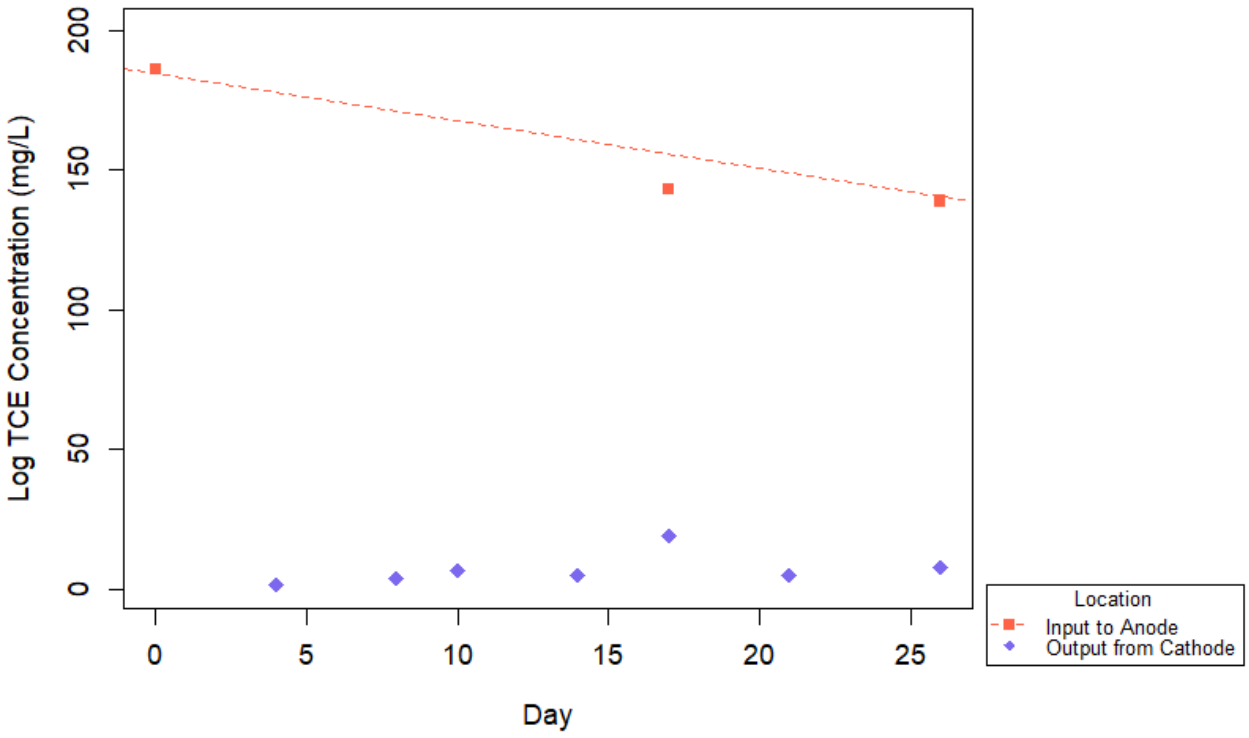


Figure 20 TCE concentrations in the EO validation test, using column design 3. Breakthrough at the cathode sand well occurred at, or around, Day 4, and steady state concentration was reached around Day 7.

3.5.4 Core Sampling

After EM and EO transport capabilities were proven by observing breakthrough of the target tracer, the column was disassembled. The core was extracted from the PVC sleeve, and sent to the analytical laboratory (Cascade) to sample and analyze the VOCs in the core. Due to the heterogeneous nature of the samples and the imprecise sampling process (chisel was used to section the core), the exact thickness of each puck was only approximately known; each section was consequently referred to according to relative distance from anode. Pucks 2, 3, and 4, which represented anode, centre, and cathode sections, were sampled by the laboratory to evaluate uniformity of tracer distribution in the core (**Figure 21**).

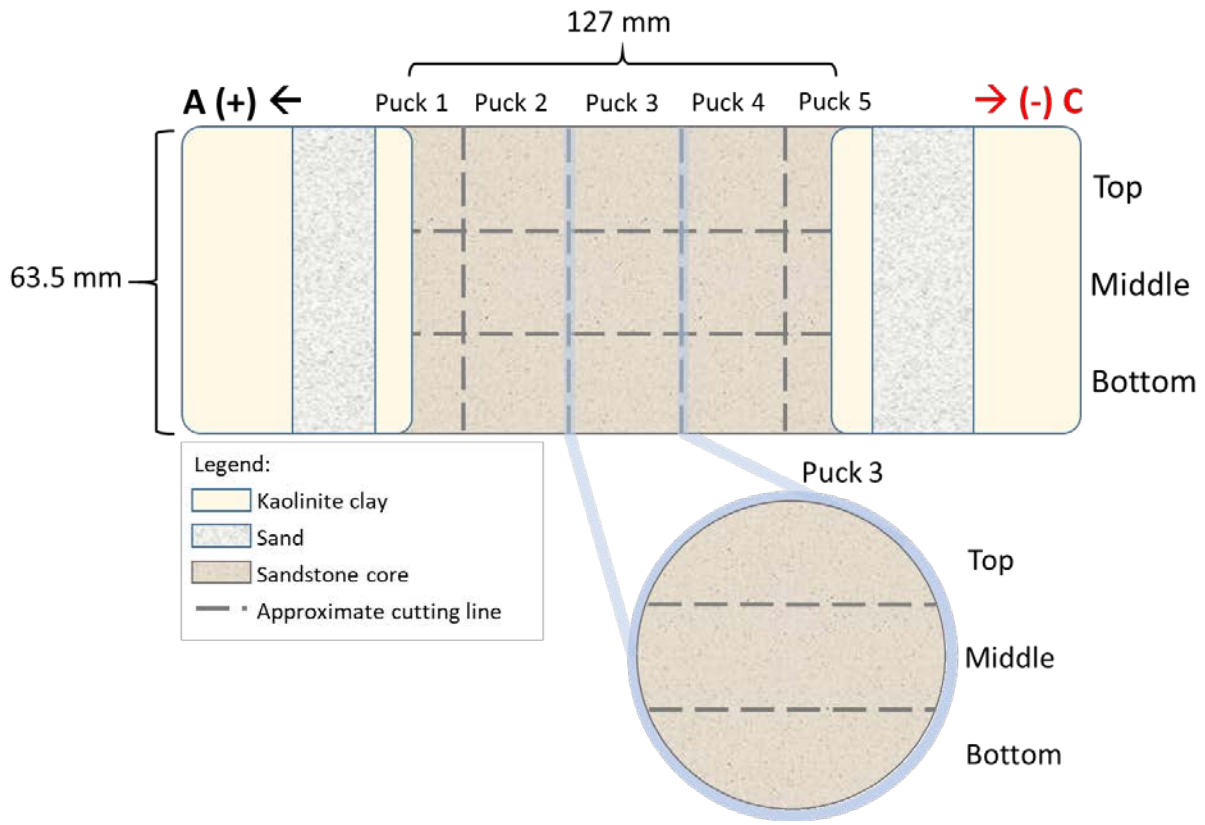


Figure 21 *Top:* Sectioning of cores into pucks, with Puck 1 closest to the anode and Puck 5 closest to the cathode. Only Pucks 2 to 4 were sampled. *Inset:* Example of how pucks were further divided into top, middle, and bottom pieces. For VOC analyses, the centre of each subsection was collected for analyses; remaining crushed material was used for all other analyses. Puck 3 was sampled in the top, middle, and bottom subsections.

The VOC results confirmed that the solvent had been effectively transported into the core via EO, and that lateral concentration distribution was sufficient to proceed with EK-Bio tests (**Figure 22**). The concentrations of TCE in the clay caps were higher than in the core, likely due to higher organic carbon content or sorption sites in the clay (geochemical analyses not conducted). Although two of the objectives for the amendment transport and distribution phase were to evaluate electron donor and bacterial transport by EK, given the positive results from the EM test with chloride tracer, there was sufficient evidence to believe that the donor would also behave similarly to the tracers. The concentration of bacteria to be dosed into the columns for the EK-Bio tests were expected to be high, such that transport by EK would not be hindered by retardation factors like attachment. Hence, three of the five tasks for amendment transport were addressed (**Table 7**).

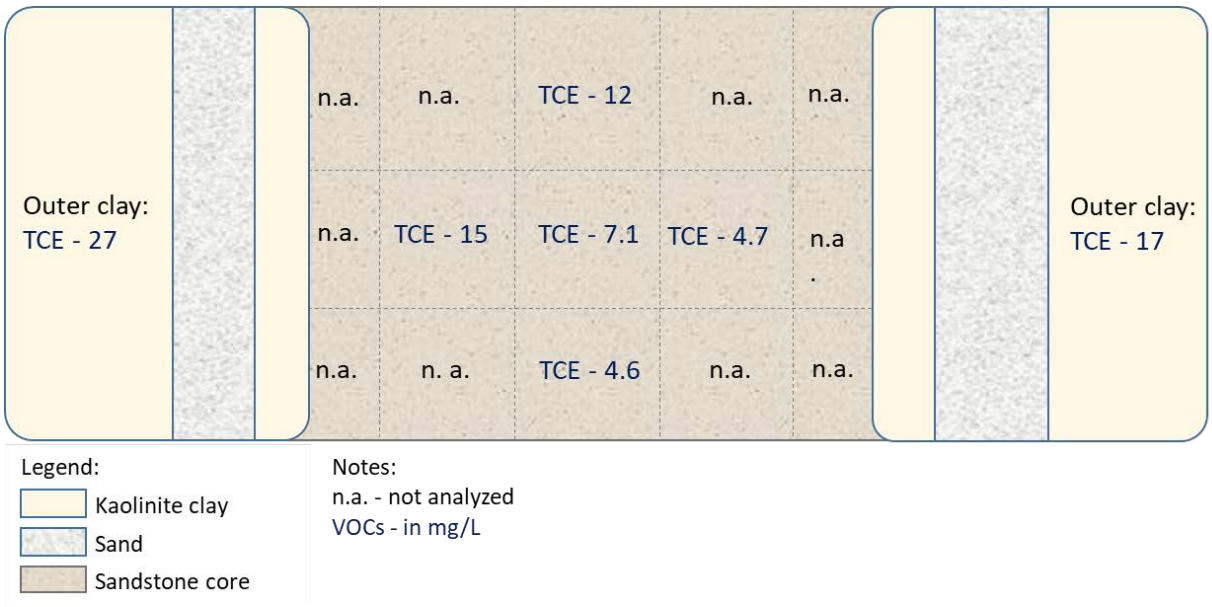


Figure 22 Distribution of TCE within the column. Inner clay caps not analyzed due to insufficient mass.

Table 7 Summary of tasks to address for the second goal of amendment and bacterial distribution.

Goal	Task	Achieved?
Amendment and Bacterial Distribution	1. Identify suitable tracers to distinguish between EM and EO	Yes – Chloride for EM, TCE for EO
	2. Migrate charged tracer via EM	Yes
	3. Migrate lactate via EM	No – addressed during EK-Bio
	4. Migrate TCE via EO	Yes
	5. Migrate KB-1 via EM and EO	No – addressed during EK-Bio

Chapter 4 EK-Bio Methodology – Column Design 3

Two sets of EK-Bio experiments were conducted, in which two cores were treated simultaneously in each set for a total of four experimental cores. The first set consisted of one core for baseline assessment immediately after EK treatment (column/core 1), and one core incubated for five weeks (column/core 2). The second set was comprised of two cores incubated for nine weeks (columns/cores 3 and 4). The add-on PVC pipe sections that housed the clay and sand units were reused for each subsequent test, and sand was cleaned and reused after each test according to Section 3.4. The sandstone cores were sacrificed after each test for post-EK (core 1) or post-incubation (cores 2, 3, and 4) analyses.

Columns used for EK-Bio testing were assembled according to column design 3 (Section 3.4). The columns were set in the electrolyte tanks, which were filled with buffer solution. The wetted sand wells were vacuumed under low negative pressure (approximately -1 psi) to remove excess water, then purged with CO_2 for 30 seconds to remove any residual void space prior to amendment introduction. A peristaltic pump delivered a continuous supply of KB-1 culture into both anode and cathode sand wells for 24 to 48 hrs while applying EK to ensure bacteria were well distributed by EO and EM, respectively, in the sandstone primary porosity prior to electron donor introduction. Both transport directions were utilized to maximize bacterial distribution within the primary porosity. Thereafter, 200 mg/L TCE and KB-1 were continuously pumped into the anode sand wells, while 767 mg/L sodium lactate (Wilclear, JRW Bioremediation) and KB-1 were continuously pumped into the cathode sand well, along with KB-1, with EK applied for ten or eleven days. The concentration of lactate was chosen based on the electron donor demand required by the KB-1 bacteria to completely dechlorinate the concentration of TCE, plus a built-in safety factor of 10 times the estimated electron donor demand (SiREM calculations/standard operating procedure; proprietary information). Two batches of TCE solution were prepared for the two sets of EK-Bio experiments. The TCE concentrations were measured prior to starting each set, and the lactate concentration was adjusted for the resulting TCE concentration.

Porewater samples from the anode sand wells were analyzed on the second, fifth, and tenth days after all three amendments were introduced into the columns. For the second experimental set, the anode sand well porewater was also sampled on the seventh day after all three amendments were introduced. The cathode sand wells were only sampled on the fifth day after all amendments were introduced (columns 3 and 4 only) and on the last day of EK prior to shutdown. Anions, VFAs, and VOCs were sampled at the port where breakthrough was monitored (i.e. opposite of injection location), except on the final day, when all ports were sampled. Otherwise, the electrolyte tanks were monitored daily for pH, ORP, and EC. The hydraulic head of both tanks were also measured occasionally using a measuring tape, and the head height readjusted

as necessary (e.g. some solution at the cathode tank was removed to drop the head or fresh buffer was added to the anode tank to raise the head) to counter the effects of EO. Both reservoirs were maintained at neutral pH to ensure continuity of ion flow, according to Hodges et al. (2013). The presence of an acid and base front could inadvertently prevent migration of desired ions. For instance, an acid front could solubilize certain ions in the matrix (Hodges et al., 2013). In addition, neutral pH conditions, between pH 6.6 and 8.0, are ideal for KB-1 viability (J. Webb, personal communication, March 1, 2017). The phosphate buffer solution in the electrode reservoirs maintained pH neutrality for eight days of EK before the reservoirs needed adjustment to return the electrolyte pH to near-neutral. To adjust the pH, dibasic potassium phosphate was added to the anode electrolyte; monobasic potassium phosphate was added to the cathode electrolyte. Alkalinity was not measured in the porewaters or in the core samples because the pH of the reservoirs were monitored and adjusted if the pH started to exceed the buffering capacity of the phosphate buffers.

After the EK treatment had concluded, the column was disassembled, and the clay caps collected for VOC analyses (ALS Waterloo). The baseline core was frozen for several hours at -80°C to minimize VOC loss and to aid the extraction process, then the PVC sleeve was sawed off. The silicone was also removed from the core. The core was then wrapped in foil, placed in a vacuum sealed bag, and shipped overnight on dry ice to Cascade laboratory for VOC extraction. Remaining crushed rock material was returned by Cascade to SiREM for anion, VFA, and reductase gene analyses.

For the cores that required incubation, the outer clay caps were removed, and the inner clay caps were left in place, adjacent to the sandstone core. The PVC sleeves were capped with PVC caps to minimize diffusion of VOCs out of the core, then wrapped in foil, placed in a vacuum sealed bag, and stored in an anaerobic glove box until the incubation period had completed (**Figure 23**). As the cores were incubated for long durations, it was expected that mass balance would not be achieved, due to loss through volatilization within the sleeve. However, critical lines of evidence for successful bacterial distribution into the primary porosity of sandstone included observation of degradation products and increases in gene counts within the core samples, not explicit mass balance.

The same process used to prepare the baseline core for shipping was followed for the incubated cores. Throughout the disassembly, extraction, incubation, and/or shipping processes, directional orientation was maintained (top of the core during EK treatment always remained at the top during all handling). External laboratory analysts also maintained directional orientation during sampling.

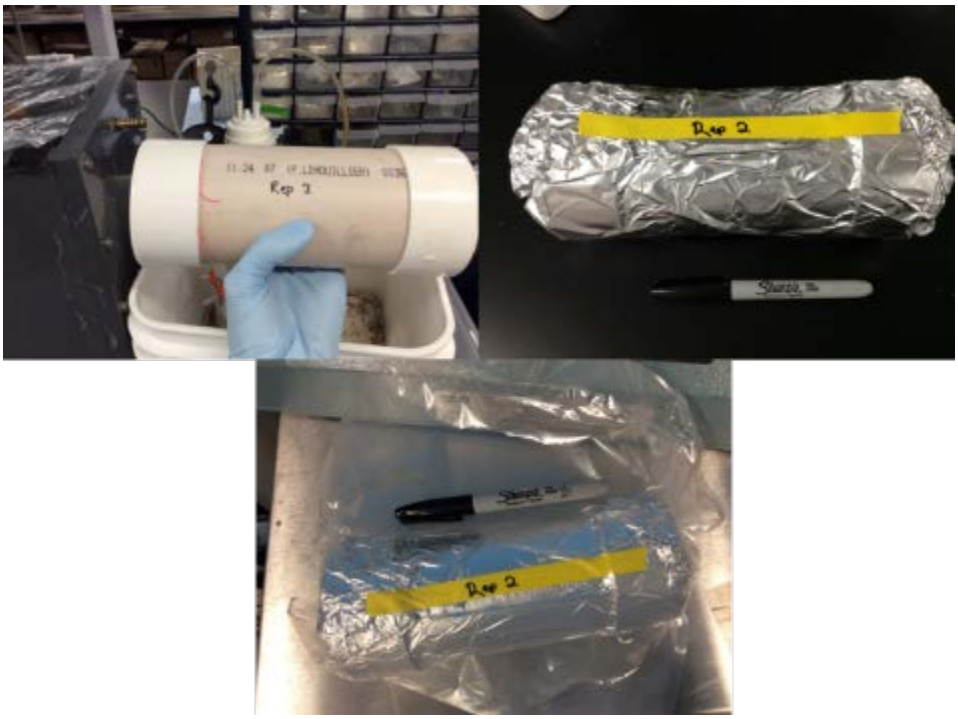


Figure 23 Preparation for incubation. *Top left:* PVC end caps to minimize VOC diffusion from the core. Inner clay caps left inside the sleeve. *Top right:* Core wrapped in foil. *Bottom:* Core vacuum sealed in a plastic bag for long-term storage in an anaerobic chamber.

Chapter 5 Results and Discussion

In the core samples taken after EK treatment, the following observations were hypothesized:

- Increase in *vcrA* and *bvcA* concentrations;
- Decrease in chlorinated ethene concentrations;
- Increase in chloride concentration as chlorinated ethenes were reduced; and
- Decrease in fatty acid concentrations via metabolism of lactate.

However, due to multiple design factors, such as: the open column system during EK; absence of samples taken from the sandstone porewater; arbitrarily chosen incubation time points for the cores, which may have overshot the time frame ideal for observing dechlorination activity; and multiple handlers/transfer locations of the cores, primarily to a laboratory in the United States for crushing and sampling, who also sent extracted samples back to SiREM for analysis of dissolved hydrocarbon gases (DHGs – ethene, ethane, and methane), evidence of TCE dechlorination was not defensible. VOC mass balance could not be achieved, as VC and ETH were not detected in any of the core samples, despite TCE concentrations decreasing with increasing incubation time, which may have been an artefact of volatilization or biotic reduction. Other observations did indicate that EK-Bio was effective for amendment transport into the core, mainly that *Dhc* were transported into the primary porosity and the populations increased with increasing incubation time (**Figure 24**).

Given that cores 1 and 2 were conducted separately from cores 3 and 4, some variability is expected. As such, the results for all four cores are presented as separate entities, with general observations made for each sequential time point. This section will conclude with an overall assessment of all the cores, based on common trends observed.

Tables 8 – 15 summarize the electrolyte solution chemistry, input amendment concentrations, and associated porewater concentrations during EK operation for each column. During EK operation, all anode reservoirs remained oxic and all cathode reservoirs remained anoxic.

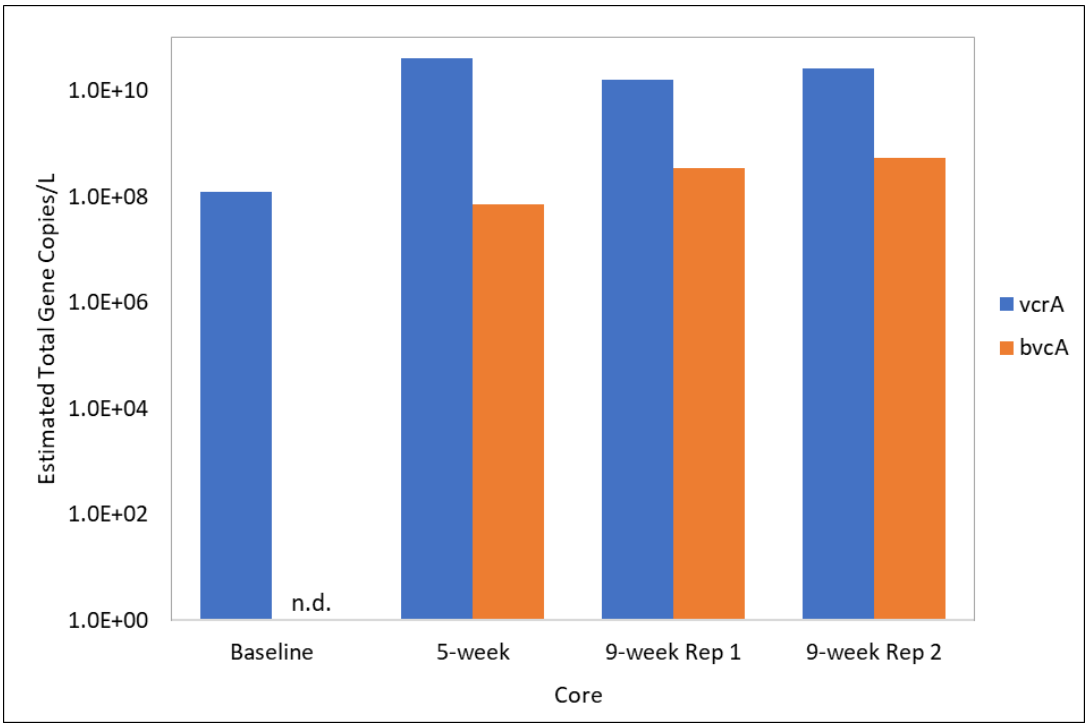


Figure 24 Estimated total reductase gene copy concentrations extracted from the four cores, based on data from **Figures 25-28**. Estimated or J-qualified detections not included in the total. *n.d. = not detected.*

5.1 Core 1 (Baseline)

The following were observed in the porewater collected from the sand wells of the baseline core during EK treatment:

- Production of low concentrations of cDCE, VC, and ETH in porewaters of both sand wells. However, cDCE concentrations were highest of the chlorinated ethenes in both sand wells;
- Low concentrations of acetate were produced from the metabolism of lactate in the anode sand well at the end of EK operation, but lactate was not detected; and
- High concentrations of acetate and propionate, low concentrations of lactate and butyrate in the cathode sand well at the end of EK operation.

The following data were obtained from the baseline core (**Figure 25**):

- *vcrA* was only detected above the quantitation limit in puck 2 (1.2×10^{8} *vcrA* gene copies/L), close to the anode. In all other locations, both *vcrA* and *bvcA* were non-detect, or between method detection limit and quantitation limit (1.2×10^{7} J *vcrA* gene copies/L in puck 4);

- Dechlorination of TCE to cDCE was observed in all sandstone core samples, and both cDCE and VC were detected in the inner clay caps. In all five core samples and both clay caps, cDCE concentrations were the highest of the chlorinated ethenes. DHGs, ETH, ethane, and methane, were not detected in the core, and were not analyzed in the clay; and
- Formate was detected in pucks 2 and 3-bottom; acetate in pucks 3-middle and 4; propionate in puck 4. Lactate was not detected in any of the samples.

The VC reductase gene concentrations, chlorinated ethene degradation, and VFAs evolution were as expected. The relatively short exposure time of KB-1 to the rock core environment and chlorinated solvent meant that the bacterial community had only a short time to acclimatize to the environment, and concentrations of reductase genes were not expected to be as high as input concentrations. Although KB-1 typically has 10^{11} *Dhc* gene copy cells/L, which roughly equates to 10^{11} *vcrA* gene copies/L (**Appendix D** – Interpretation Technical Note), the concentration of *vcrA* that was detected in puck 2 was three orders of magnitude lower than what was inputted into the core. The lower *vcrA* concentration can be explained by the acclimatization period required by *Dhc* and other bacteria in the bedrock environment, or attachment effects that may retard the transport of bacteria into the core. The presence of cDCE in all pucks suggested that *Dhc* bacteria had migrated into the core and were actively dechlorinating TCE, but since the *vcrA* concentrations were lower than the ideal 10^7 gene copies/L (**Appendix D** – Interpretation Technical Note), complete dechlorination would not occur, as was observed. The detection of *vcrA* in puck 2 and slight detection in puck 4 indicated that EO and EM, respectively, were effective in transporting *Dhc* bacteria past the clay caps into the core, and that EO was potentially the dominant transport mechanism for bacteria. The gene concentrations were as expected, given that the bacteria needed to migrate out of the sand wells, beyond the clay caps to reach the core. The potential for retardation via attachment could be high, given the multiple permeability units of the different geological materials.

Assuming that the bacterial community did not have sufficient time to acclimatize in the core, since doubling time in the field in unconsolidated materials may take up to two weeks for *Dhc* to reach 10^8 cells/L before complete dechlorination begins (J. Webb, personal communication, March 17, 2016), it was not surprising that dechlorination only proceeded to cDCE and that no ETH was detected, even though electron donor was readily available. The TCE and cDCE concentrations in the sandstone were within one or two orders of magnitude of that detected in the clay caps, whereas the concentrations of VC in the clay caps were around the same order of magnitude as the detection limit for the sandstone.

The absence of lactate in the core samples suggested that the bacteria were active during EK treatment, since both acetate and propionate were detected in the porewaters and extracted from the core. Acetate and

propionate are direct lactate fermentation products (Schaefer et al., 2010a). Total VFA concentrations were lower than expected, given the consistent high concentration of lactate that was amended to the core.

Table 8 Column 1 (baseline) electrode reservoir chemistry during EK operation.

Day	Voltage (V)	Anode Electrolyte			Cathode Electrolyte		
		pH	ORP (mV)	EC (mS/cm)	pH	ORP (mV)	EC (mS/cm)
0	48.9	7.15	203	5.68	7.13	197	5.65
1	68.0	7.06	579	4.83	7.32	-298	5.08
2	79.3	7.03	672	4.69	7.31	-398	5.08
3	69.0	--	--	--	--	--	--
4	66.7	6.89	845	4.74	7.48	-550	5.29
5	48.5	6.84	867	4.84	7.65	-626	5.35
6	49.8	6.67	878	4.45	7.72	-629	5.32
7	50.0	6.67	882	4.63	7.93	-643	5.53
8	49.3	6.64	851	4.45	8.28	-392	5.58
10	48.5	6.83	867	7.12	7.39	-576	6.52
11	45.4	6.83	864	6.92	7.39	-616	6.51

Notes:

-- not analyzed

Table 9 Column 1 (baseline) amendment input concentrations and analytes measured from sand well porewaters during EK treatment.

	Sample Type	Day	Chloride mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE mg/L	cDCE mg/L	VC mg/L	Ethene mg/L
Anode	Input	0	<1.5 U	<1.4 U	<1.4 U	<1.7 U	<7.8 U	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	253	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	181	5.5	3,690	346	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	6	441	19	304	303	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	7	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	8	476	26	914	231	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	11	--	--	--	--	--	--	--	--	--	--	131	<0.01 U	<0.01 U	<0.01 U
	PW	11	399	26	1,223	83	<0.4 U	3.6	<0.3 U	<0.2 U	<0.4 U	<0.7 U	9.88	20	0.07	0.02
Cathode	Input	0	22	<1.4 U	<1.4 U	5.5	752	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	<0.10 U	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	3	--	--	--	--	894	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	6	--	--	--	--	770	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	7	--	--	--	--	--	--	--	--	--	--	0.03	0.57	0.14	0.11
	Input	8	--	--	--	--	591	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	11	--	--	--	--	532	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	11	58	0.8	225	4.5	0.8	289	157	<0.2 U	1.2	<0.7 U	0.63	3.25	0.81	0.07

Notes:

-- not analyzed

PW porewater

U below reporting limit

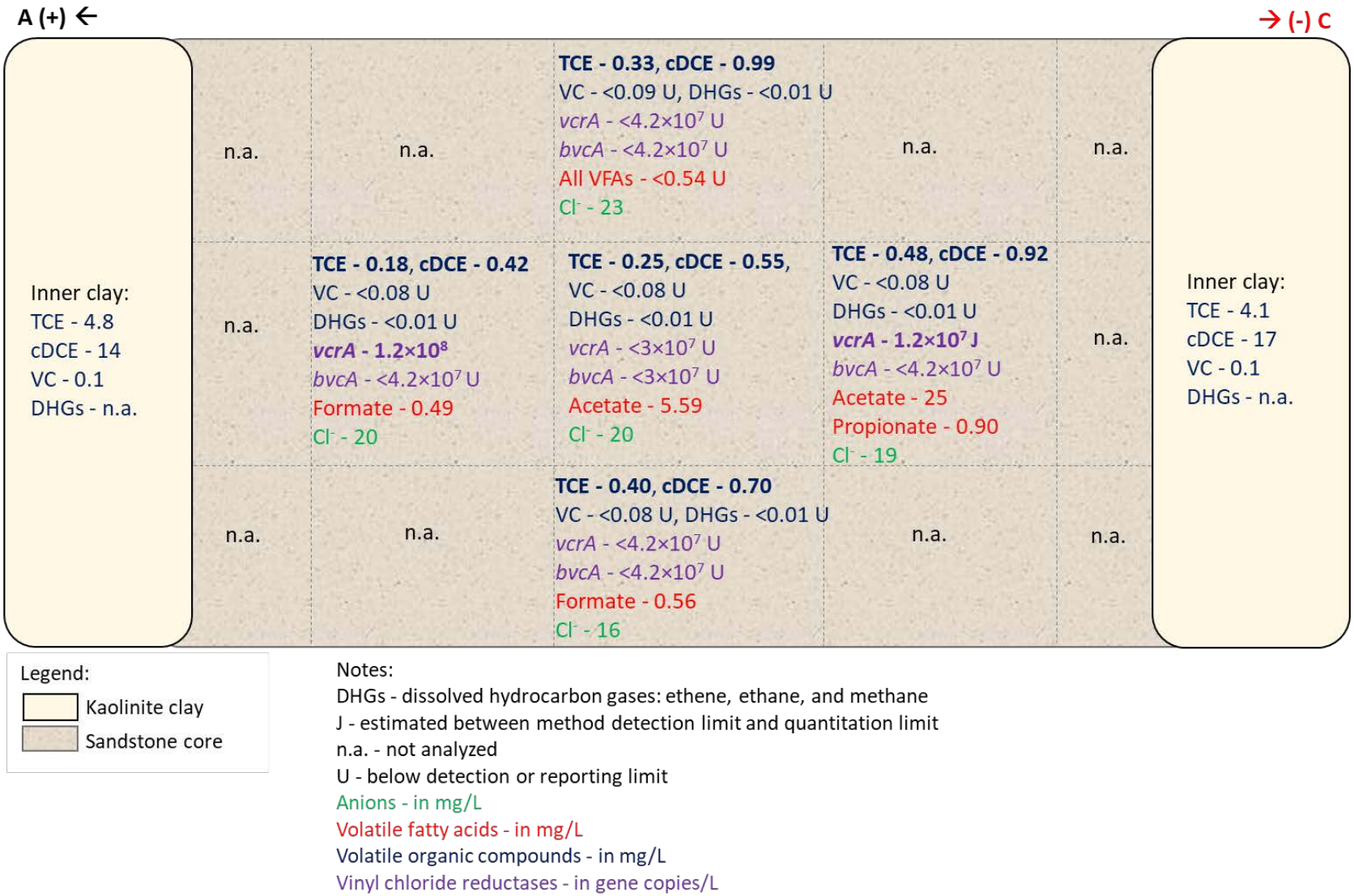


Figure 25 Distribution of anions, VFAs, VOCs, and VC reductases in column 1 (baseline) immediately after EK treatment. Bolded analytes for emphasis.

5.2 Core 2 (5-Week Incubation)

The following were observed in the porewaters collected from the sand wells of the 5-week incubated core during EK treatment:

- Low concentrations of cDCE, VC, and ETH were measured in the porewaters of the cathode sand well. No VC was detected in the anode sand well at the end of EK operation, but ETH was detected. Other than residual TCE in the anode sand pack, cDCE concentrations were highest in both porewaters;
- Low concentrations of acetate in the anode sand well at the end of EK operation; and
- Low concentrations of acetate and propionate in the cathode at the end of EK operation.

After the 5-week incubation period, the following data were obtained from the core (**Figure 26**):

- Both reductase genes were detected in the core, with *vcrA* concentrations between 10^8 and 10^{10} gene copies/L. In puck locations 3-middle and 3-bottom, *bvcA* concentrations were slightly above the quantitation limit at 10^7 gene copies/L porewater. In puck 3-top and puck 4, *bvcA* concentrations were between detection and quantitation limits, and *bvcA* was non-detect in puck 2, which correlated with the trend observed with *vcrA* detections, where *vcrA* was 10^8 gene copies/L in puck 2, but 10^9 and 10^{10} gene copies/L in all other locations;
- VOC concentrations were below detection (or between the method detection and quantitation limits) in all core samples except for TCE near the reporting limit in puck 4. There were detections of both TCE and cDCE near the reporting limit in the clay caps. The decrease in VOC concentrations correlated with the increase in reductase gene concentrations; as *Dhc* increased, dechlorination activity would be expected to increase as well. Concentrations of cDCE were higher than TCE in the clay, as was observed in the baseline column, and VC was not detected. DHGs were not detected in the core, and were not analyzed in the clay; and
- Acetate and formate were detected at low concentrations in pucks 3-middle, 3-bottom, and 4. As with the baseline core, lactate was not detected in any of the core samples.

The incubation period promoted bacteria viability, or at least *Dhc*, as indicated by the increase in *vcrA* concentrations in all samples, and the detection of *bvcA* in some of the samples, compared to the results of the baseline core. It was likely that the incubation period helped with the acclimatization process of KB-1 to the sandstone core, especially since the concentration of chlorinated ethenes decreased to nearly or complete non-detection in all core samples. The detection of VC and ETH in the porewater during EK

operation indicated that *Dhc* were alive and actively dechlorinating before incubation began. In order for ETH to be produced, other bacterial species besides *Dhc* also had to be viable, for instance, to ferment fatty acids to produce molecular hydrogen that could be used by *Dhc* to reduce chlorinated ethenes. Although concentrations of *bvcA* were 10^7 gene copies/L or less in all sampled locations, this was not surprising, since *bvcA* concentrations would only be expected to dominate in less reduced conditions (Van Der Zaan et al., 2010).

The absence of VOCs in all core samples, except for TCE in puck 4, in which the concentration was marginally above the detection limit, as well as low detections of TCE and cDCE in the clay caps, suggested that VOCs were either reduced biotically during the incubation period, lost through volatilization, or lost through sorption to the silicone coating in the column. The absence of DHGs challenged the hypothesis that only reductive dechlorination occurred to account for the decrease in VOC concentrations. Near-complete dechlorination was expected over the incubation period, despite not knowing the ideal incubation time for the given conditions to observe complete reduction of TCE (e.g. on the order of a couple weeks, or several months). Although DHGs were not detected in the core, they may have been produced, but volatilized sooner than they could have been captured for analysis.

Incubation also appeared to have promoted metabolism of the electron donor, which would explain the low concentrations of fatty acids extracted from the core samples. Since the lactate input into the column was consistently high, the resulting low VFA concentrations in the pucks after incubation could only be due to metabolism of fatty acids during EK treatment and incubation. The bacteria residing in the primary porosity were not dosed with additional electron donor during incubation, and thus it would be expected that the available electron donor would be consumed in that time.

Table 10 Column 2 (5-week incubation) electrode reservoir chemistry during EK operation.

Day	Voltage (V)	Anode Electrolyte			Cathode Electrolyte		
		pH	ORP (mV)	EC (mS/cm)	pH	ORP (mV)	EC (mS/cm)
0	30.2	7.32	188	5.13	7.39	185	5.19
1	26.9	7.13	457	5.01	7.34	-320	5.12
2	26.2	7.02	225	4.89	7.27	-416	5.12
3	26.7	--	--	--	--	--	--
4	27.9	6.84	735	4.81	7.34	-605	5.34
5	28.5	6.73	778	4.64	7.76	-636	5.10
6	30.3	6.77	709	4.60	7.78	-544	5.33
7	29.7	6.60	798	4.58	8.34	-519	5.53
8	27.6	6.56	807	4.50	8.34	-519	5.53
10	24.3	6.76	505	6.84	7.75	-663	6.19
11	23.9	6.76	805	6.86	7.47	-639	6.44

Notes:

-- not analyzed

Table 11 Column 2 (5-week incubation) amendment input concentrations and analytes measured from sand well porewaters during EK treatment.

	Sample Type	Day	Chloride mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE mg/L	cDCE mg/L	VC mg/L	Ethene mg/L
Anode	Input	0	<1.5 U	<1.4 U	<1.4 U	<1.7 U	<7.8 U	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	253	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	732	6.6	<0.1 U	250	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	6	740	33	141	223	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	7	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	8	683	11	230	36	<0.4 U	2.4	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	11	--	--	--	--	--	--	--	--	--	--	131	<0.01 U	<0.01 U	<0.01 U
	PW	11	651	9.5	317	20	<0.4 U	1.5	<0.3 U	<0.2 U	<0.4 U	<0.7 U	32	5.22	<0.01 U	0.02
Cathode	Input	0	22	<1.4 U	<1.4 U	5.5	752	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	<0.10 U	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	3	--	--	--	--	894	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	5	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	6	--	--	--	--	770	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	7	--	--	--	--	--	--	--	--	--	--	0.08	0.78	0.01	0.08
	Input	8	--	--	--	--	591	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	11					532	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	11	455	1.5	2,457	2.7	<0.4 U	18	4.9	<0.2 U	<0.4 U	<0.7 U	0.09	1.10	0.03	0.05

Notes:

-- not analyzed

PW porewater

U below reporting limit

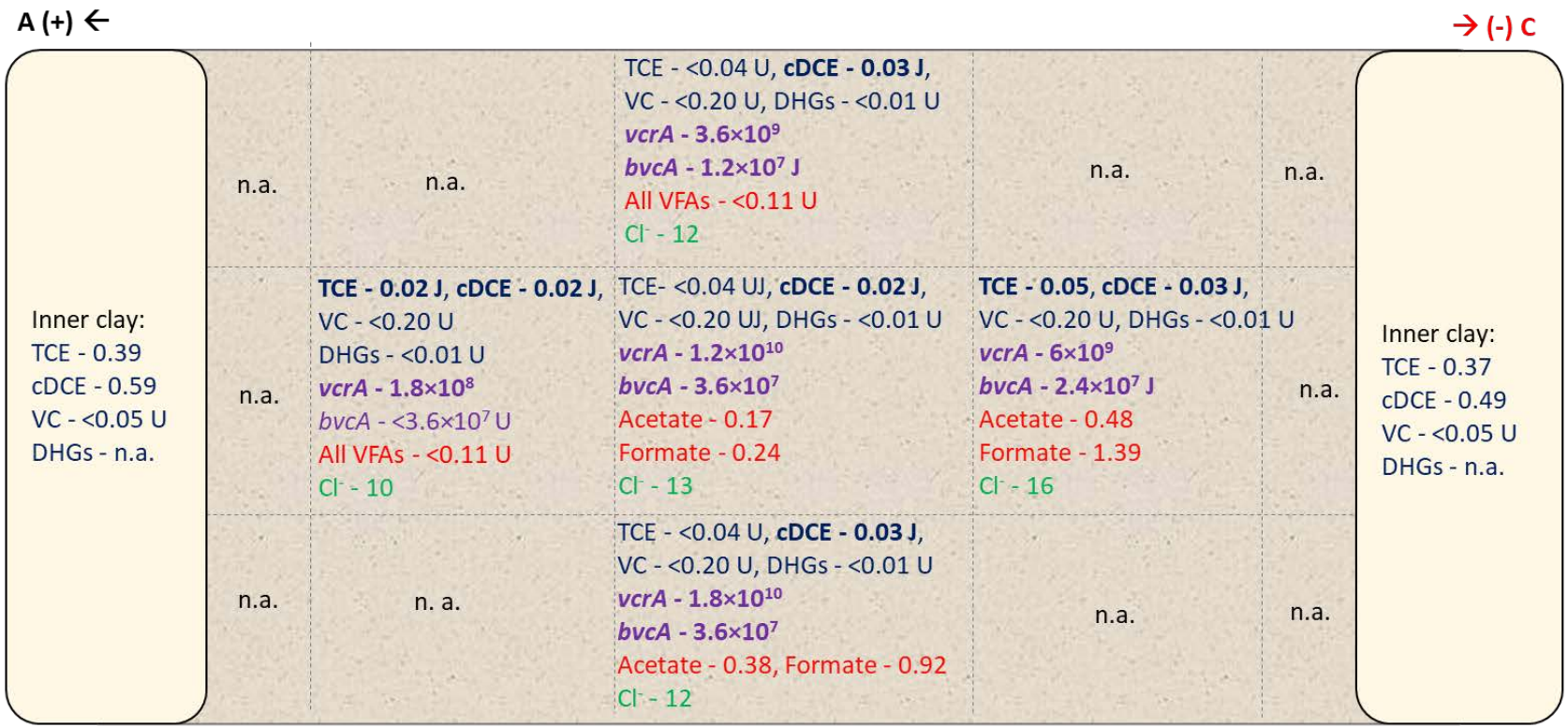


Figure 26 Distribution of anions, VFAs, VOCs, and VC reductases in column 2 after incubating in anaerobic conditions for five weeks. Bolded analytes for emphasis.

5.3 Core 3 (9-Week Incubation, Replicate 1)

The following were observed in the porewaters collected from the sand wells of the first 9-week incubated core during EK treatment:

- Concentrations of TCE and cDCE were low in the sand well near the anode at the end of EK operation, and there may have been unintentional biotic or abiotic degradation in the input solution, since cDCE was detected in the input solution at the end of the EK treatment;
- TCE, cDCE, and ETH were low in the sand well near the cathode, and ETH was below the detection limit at the end of EK treatment;
- Lactate concentrations in the anode sand well were moderate during EK operation, but were below detection at the end of EK operation; and
- Lactate concentration was sustained in the cathode sand well. There were low concentrations of propionate and pyruvate during EK operation, followed by a decrease in propionate below detection and a decrease in pyruvate to near the detection limit at the end of EK operation.

After the 9-week incubation period, the following data were observed in the core (**Figure 27**):

- Dechlorinating bacterial counts were high, with *vcrA* concentrations between 10^8 and 10^9 gene copies/L, and *bvcA* concentrations of 10^7 to 10^8 gene copies/L in all puck samples;
- No VOCs were measured above detection limit in any of the core samples, or in either of the clays. No DHGs were detected in any of the core samples; and
- Low concentrations of acetate were extracted from all five puck samples and formate was extracted from all puck locations except puck 3-top. As with the baseline and 5-week incubated cores, lactate was not recovered in this core.

Bacterial concentrations increased in core 3 as expected. Both *vcrA* and *bvcA* concentrations were higher than in the first two cores, which provided further indication that incubation encouraged bacterial viability, especially of the *Dhc* species. It was not surprising that there were no VOCs detected in the core samples after the incubation period ended. Based on the resulting reductase gene concentrations of 10^8 and 10^9 *vcrA* gene copies/L, it was expected that complete dechlorination would occur, and that the bacterial community would effectively dechlorinate whatever chlorinated ethenes remained in the core after EK operation ended. However, the evidence for VOC loss via biotic reductive dechlorination could not be confirmed because of the lack of DHG production captured in the core samples. Although ETH and other DHGs were not detected, it may be possible that ETH was produced by dehalorespiring bacteria, but was further reduced to other DHGs or CO_2 over time, which were not captured by the sampling process.

Based on the limited concentrations of organic acids extracted from the core samples, but the consistently high lactate input concentrations, it appeared that most of the electron donor that was transported into the core during EK operation were metabolized during the incubation period. It appeared that the electron donor concentration that had migrated into the column during EK operation was sufficient to support dechlorination during and after EK treatment, since there were no chlorinated ethenes in the core samples.

Table 12 Column 3 (9-week incubation, replicate 1) electrode reservoir chemistry during EK operation.

Day	Voltage (V)	Anode Electrolyte			Cathode Electrolyte		
		pH	ORP (mV)	EC (mS/cm)	pH	ORP (mV)	EC (mS/cm)
0	41.3	7.15	203	5.68	7.13	197	5.65
1	67.9	7.01	650	5.11	7.21	-405	5.34
2	44.7	7.02	760	4.85	7.33	-476	5.33
3	40.5	7.01	768	4.82	7.40	-625	5.20
4	35.4	6.89	783	4.92	7.49	-619	5.43
7	32.7	6.75	879	4.37	7.87	-636	5.38
8	30.4	6.63	873	4.44	8.03	-660	5.62
9	26.6	7.03	853	7.94	7.21	-617	6.51
10	23.8	6.98	819	7.83	7.24	-602	6.53
11	21.4	7.01	840	7.80	7.38	-623	6.70
12	19.8	6.92	846	7.69	7.40	-632	6.70

Table 13 Column 3 (9-week incubation, replicate 1) amendment input concentrations and analytes measured from sand well porewaters during EK treatment.

	Sample Type	Day	Chloride mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE mg/L	cDCE mg/L	VC mg/L	Ethene mg/L
Anode	Input	0	<0.1 U	<0.1 U	<0.1 U	<0.1 U	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	160	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	4	76	33	2,723	715	66	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	7	191	27	1,341	335	54	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	8	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	9	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	11	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	12	--	--	--	--	--	--	--	--	--	--	55	5.0	<0.1 U	<0.1 U
	PW	12	132	7.8	5,706	54	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	0.15	0.04	<0.02 U	<0.02 U
Cathode	Input	0	<0.1 U	<0.1 U	<0.1 U	<0.1 U	966	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	<0.10 U	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	7	107	<0.1 U	1,067	<0.1 U	365	<0.5 U	5.2	<0.2 U	<0.4 U	17	0.04	0.05	<0.01 U	0.02
	PW	8	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	9	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	11	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	12	--	--	--	--	976.2	<11 U	<6.2 U	< 4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	12	11	<0.1 U	3,184	1.6	261	<0.5 U	<0.3 U	<0.2 U	<0.4 U	1.4	0.05	0.04	<0.02 U	<0.02 U

Notes:

-- not analyzed

PW porewater

U below reporting limit

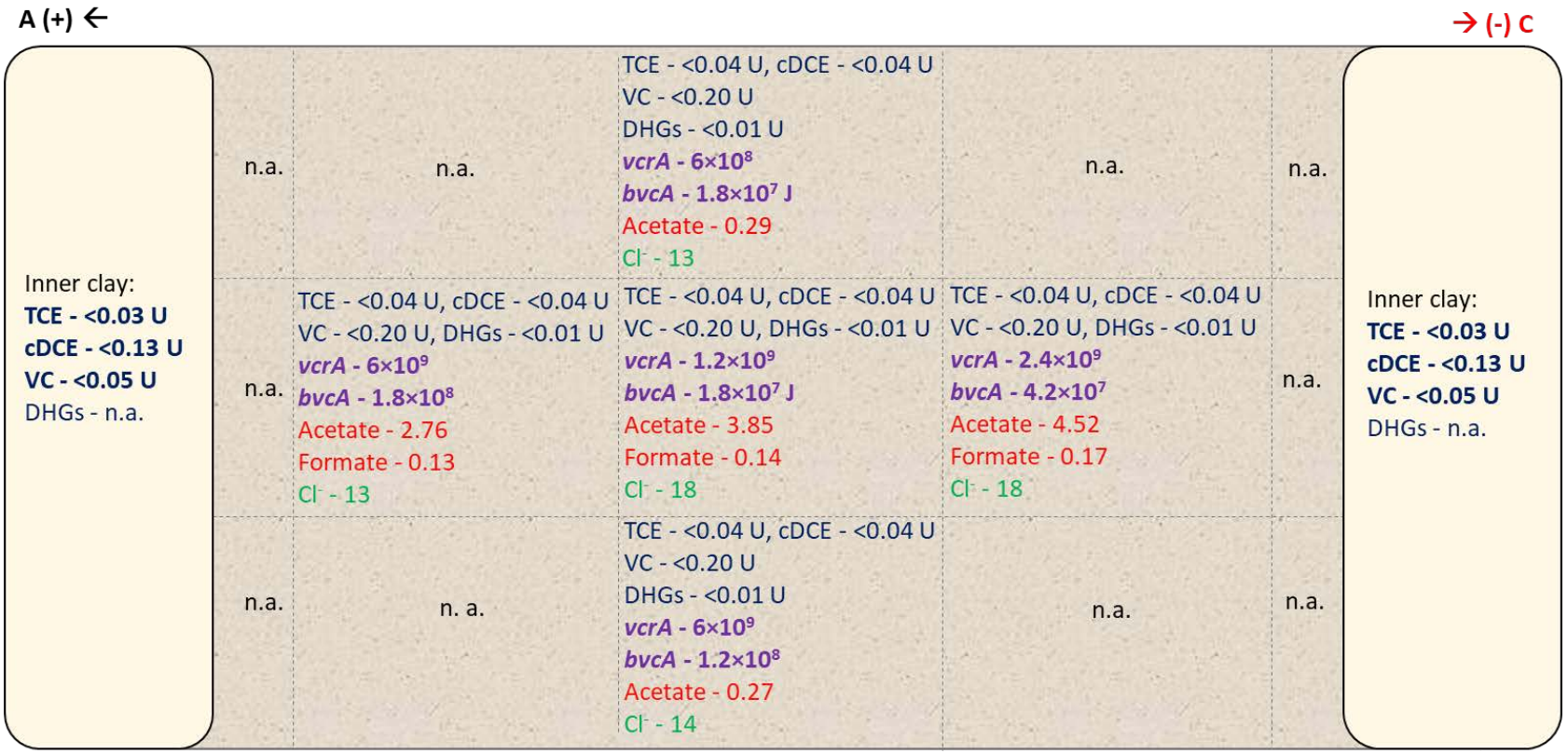


Figure 27 Distribution of anions, VFAs, VOCs, and VC reductases in column 3 after incubating for nine weeks (replicate 1). Bolded analytes for emphasis.

5.4 Core 4 (9-Week Incubation, Replicate 2)

The following were observed in the porewater collected from the sand wells of the second 9-week incubated core during EK treatment:

- Low concentrations of TCE and cDCE were detected in the anode sand well at the end of EK operation, with TCE higher than cDCE;
- TCE, cDCE, and VC were detected in the cathode sand well during EK operation, with cDCE having the highest concentration of the chlorinated ethenes. The cathode sand well was dry at the end of the EK treatment, so porewater samples could not be obtained from the cathode sand well;
- Moderate concentrations of lactate were measured in the anode sand well during EK operation, but was below detection at the end of EK operation; and
- Lactate concentration was high, and acetate and propionate concentrations were low in the cathode sand well during EK operation.

After the 9-week incubation period, the following data were observed (**Figure 28**):

- Dechlorinating reductive gene counts were high, with *vcrA* concentrations of 10^9 gene copies/L in all core samples, and *bvcA* concentrations of 10^7 to 10^8 gene copies/L in all core samples;
- No VOCs were measured above detection limit in any of the core or clay samples. DHGs were not detected in the cores samples;
- Moderate to high concentrations of acetate were detected in all core samples, low concentrations of propionate were measured in all core samples, and very low concentrations of formate was detected in only the puck 3 sample; and
- Chloride concentrations were highest in the pucks of this core compared to all other cores, but at concentrations that were not significant, relative to dechlorination of chlorinated ethenes.

Similar to the first 9-week incubation replicate core, *vcrA* and *bvcA* concentrations were elevated by at least an order of magnitude, compared to the baseline core. The incubation period likely promoted *Dhc* population growth, as observed by the increase in gene copies. It is unknown whether the bacteria were responsible for complete reductive dechlorination of TCE, or if a combination of factors were responsible for chlorinated ethene loss, as described in Section 5.2. However, *vcrA* concentrations greater than 10^7 gene copies/L do support the hypothesis that VOCs were biotically reduced.

The high concentrations of fermentation products, especially of acetate, through the whole core provided further evidence that bacteria were viable and active. As lactate is metabolized to acetate, molecular hydrogen is released, which is required by dehalogenating species to reduce chlorinated ethenes to non-

toxic end products. The presence of acetate suggested that chlorinated ethenes could be reduced, if the molecules were accessible to the appropriate bacteria. It was possible that migration of lactate in this column was the most effective of all the columns, or that the concentration that effectively migrated into core 4 was the most effective of all the cores, given the high concentrations of acetate in this core relative to cores 1 to 3. The concentration of electron donor that was transported into core 4 appeared to be sufficient for complete dechlorination of TCE. The concentrations of chloride in the core samples were higher than all other cores, and may suggest dechlorination. However, the concentrations may be due to experimental anomalies, and have no implications on the results of this study.

Table 14 Column 4 (9-week incubation, replicate 2) electrode reservoir chemistry during EK operation.

Day	Voltage (V)	Anode Electrolyte			Cathode Electrolyte		
		pH	ORP (mV)	EC (mS/cm)	pH	ORP (mV)	EC (mS/cm)
0	31.7	7.18	197	5.57	--	--	--
1	31.6	7.04	149	5.09	7.22	-388	5.33
2	36.5	7.05	190	4.85	7.35	-470	5.12
3	26.4	6.99	201	4.87	7.46	-610	5.30
4	28.9	6.89	646	4.87	7.60	-618	5.40
7	39.9	6.76	798	4.58	7.90	-636	5.38
8	28.5	6.67	787	4.57	8.25	-657	5.76
9	35.1	7.02	814	7.43	7.25	-610	6.61
10	24.6	7.00	758	7.48	7.32	-588	6.66
11	35.4	7.06	786	7.64	7.41	-647	6.54
12	31.3	7.03	815	7.94	7.45	-625	6.82

Notes:

-- not analyzed

Table 15 Column 4 (9-week incubation, replicate 2) amendment input concentrations and analytes measured from sand well porewaters during EK treatment.

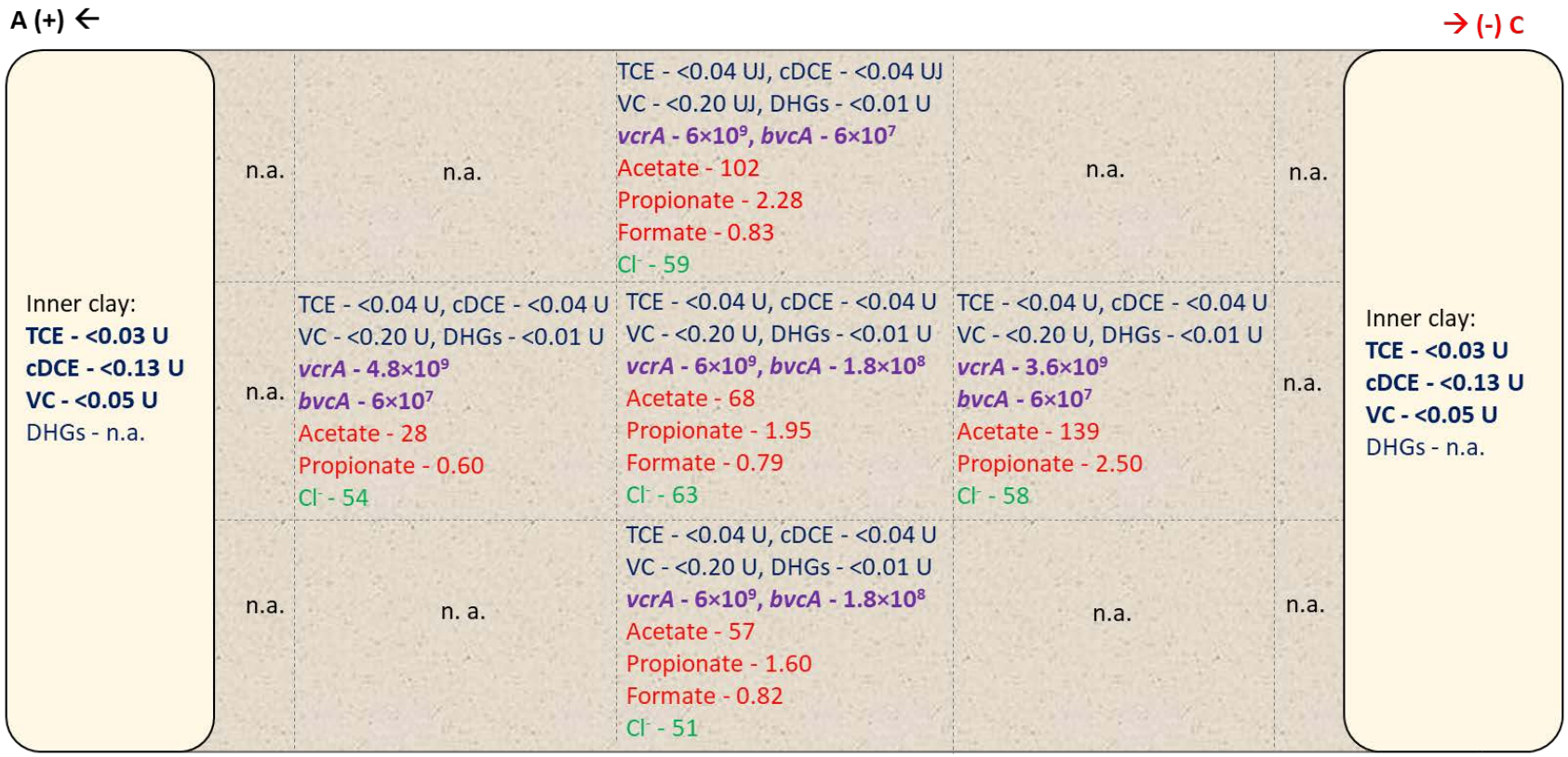
	Sample Type	Day	Chloride mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE mg/L	cDCE mg/L	VC mg/L	Ethene mg/L
Anode	Input	0	<0.1 U	<0.1 U	<0.1 U	<0.1 U	<0.4 U	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	160	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	4	107	3.8	3,282	114	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	7	273	14	2,885	449	121	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	--	--	--	--
	PW	8	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	9	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	11	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	12	--	--	--	--	--	--	--	--	--	--	55	5.0	<0.1 U	<0.1 U
	PW	12	189	7.7	7,217	98	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	0.84	0.07	<0.02 U	<0.02 U
Cathode	Input	0	<0.1 U	<0.1 U	<0.1 U	<0.1 U	966	<11 U	<6.2 U	<4.4 U	<8.2 U	<14 U	<0.10 U	<0.10 U	<0.10 U	<0.10 U
	PW	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	4	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	7	290	<0.1 U	454	12	179	32	9.3	<0.2 U	<0.4 U	<0.7 U	0.45	1.00	0.10	<0.01 U
	PW	8	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	9	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	10	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	PW	11	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Input	12	--	--	--	--	976.2	<11 U	<6.2 U	< 4.4 U	<8.2 U	<14 U	--	--	--	--
	PW	12	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:

-- not analyzed

PW porewater

U below reporting limit



Legend:
- Kaolinite clay
- Sandstone core

Notes:
DHGs - dissolved hydrocarbon gases: ethene, ethane, and methane
J - estimated between method detection limit and quantitation limit
n.a. - not analyzed
U - below detection or reporting limit
Anions - in mg/L
Volatile fatty acids - in mg/L
Volatile organic compounds - in mg/L
Vinyl chloride reductases - in gene copies/L

Figure 28 Distribution of anions, VFAs, VOCs, and VC reductases in column 4 after incubating for nine weeks (replicate 2).

5.5 Overall Discussion

The redox conditions created by hydrolysis at the cathode promoted an anoxic environment suitable for KB-1 bacteria. Within 24 hours of EK operation, reducing conditions developed. Based on rRNA extractions from the cores, there was increasing *Dhc* concentrations in the cores with increasing incubation time. The initial saturation treatment of the cores with sodium lactate solution, along with the reducing conditions, promoted bacterial viability, which was also observed in Mao et al. (2012). Comparison of reductase gene concentrations, especially of *bvcA*, between the 5-week incubated core and the 9-week incubated cores demonstrated that bacteria could be transported through primary porosity of Idaho Gray sandstone, and that incubation resulted in population growth. Conditions that negatively impacted bacterial viability would have resulted in lower gene copy concentrations than were observed, and the increase in concentrations in cores 3 and 4 would not have been observed. The Gene-Trac analyses only detect for the presence of functional genes that reduce VC to ETH; it is not able to distinguish between cells that were alive at the time of extraction and positive for the respective genes, and cells that were dead, but positive for the respective genes. Although whole community sequencing of each core was not conducted, which would have revealed more specific information of the species present in each core, and possibly of the role of each species in degradation, the resulting *vcrA* gene copy concentrations of 10^9 and 10^{10} per L of porewater in the incubated cores indicated that over the duration of the incubation period, reproduction did occur, which increased concentrations by at least one order of magnitude, compared to the baseline core.

Although large pores may be surrounded by clay matrix and constrict overall pore size and connectivity (Lu et al., 2015), based on the average pore diameter of the sandstone, 13 μm as determined by MIP, and *Dhc* approximately 1 μm in diameter (Maymó-Gatell et al., 1999), bacteria were hypothesized to be able to enter into the primary porosity. The gene copy detections in the EK-Bio experiments confirmed bacteria presence detected by Lima et al. (2012) within the matrix of the Lone Rock Formation sandstone, which had a pore throat radii estimated between 0.025 to 6.3 μm. It is possible that although KB-1 bacteria were effectively transported into the primary porosity, they may have encountered dead end pores, which were not connected to effective porosity. However, given the increases in reductase genes, the effect of dead end pores, pore connectivity, and pore diameters of the sandstone did not appear to significantly affect population growth of *Dhc*. Likewise, if most dechlorinating bacteria within the columns were trapped in the sand wells, instead of transported into the sandstone, reductase gene copy concentrations would have been highest near the sand wells. Instead, there were consistent concentrations and distribution of reductive genes throughout all cores, within an order of magnitude in individual cores, for all incubated cores.

Although stock KB-1 was injected in the sand wells and migrated via EO and EM into the cores with the expectation that more bacteria would promote faster dechlorination rates, the chosen incubation time points

of five and nine weeks may have been too long, and/or the KB-1 bacterial concentration may have been too high, to adequately capture the peak of dechlorination activity. By the time the 5-week incubated core was sampled, VOC concentrations were mostly below detection. By the time of sampling the 9-week incubated cores, all VOC concentrations were below detection. In all cores, VC and ETH were not detected, which are critical indicators of biotic reductive dechlorination. It was unclear whether DHGs were not produced, were produced and then transformed, were lost to volatilization, or whether the analytical method was unable to capture and detect gases. However, given the unknowns inherent in the experimental procedures, the ideal KB-1 concentration to incubation time relationship could not have been predicted *a priori*. Increases of key dechlorinating species were observed as hypothesized, so despite the inability to attain mass balance with VOCs to conclude that reductive dechlorination occurred, the primary objective of bacterial viability was achieved.

It is possible that introducing multiple amendments at the same time diluted the overall concentrations of amendments that were transported into the cores via EM and EO from the sand wells. In the validation tests, only one tracer was introduced at a time into the column. However, injection of all amendment solutions into the sand wells at the same time was the only viable option to ensure the cores were artificially contaminated and received amendment to achieve the objectives for this research. Some loss of TCE in the cores other than through reductive dechlorination was possible via volatilization, sorption to the silicone sleeve, or electrochemical processes. TCE input solution sampled at the start and end of EK application showed that there was between 48 and 66% loss of TCE between the start and end of EK, indicating possible volatilization out of the Tedlar bag, which was not perfectly gas-tight. Although the silicone used to seal the cores to the PVC sleeves was tested for the release of VOCs once cured, it was not additionally tested for sorption ability of VOCs, which could account for loss of chlorinated ethenes. Removal of TCE via hydrochlorination has been documented, in which the abundance of atomic hydrogen produced at the anode by hydrolysis reduced TCE (Fallahpour et al., 2017; Rajic et al., 2015). It is possible that the hydrogen ions produced at the anode allowed bacteria to reductively dechlorinate TCE in the column. However, chlorinated ethenes detected in the clay caps near the cathode of the baseline and 5-week incubated cores suggested that TCE was not only present in the sand well near the anode, but was effectively transported through the core near the cathode end.

Periodic sampling of the input lactate solution revealed that the donor concentration remained stable over time. In all column setups, some form of fatty acid was measured in the anode and cathode sand well porewaters, which confirmed that lactate was continuously added to the columns. The lower concentrations of VFAs extracted from the cores could be indicative of bacterial fermentation processes since the bacteria were not dosed with additional donor during the incubation period.

Chloride mass balance did not appear to correlate with ethene dechlorination, as the chloride concentrations were lower than would be expected from TCE dechlorination. Sources that could lead to biased high chloride concentrations include the RO water used to make up the solutions and possible abiotic reactions within the cores during EK operation. EM could be responsible for transporting chloride out of the sandstone porewater, which would lead to low biased concentrations. The reservoir solutions and clay caps were not analyzed for reductase genes, VOCs, anions, or organic acids. It is possible that some analytes were transported beyond the sand wells into the reservoirs. Gill et al. (2015) assessed the impact of physical heterogeneity on amendment mass flux distribution in an artificial aquifer setup and found that in mixed hydraulic conductivity settings (low K and high K layers perpendicular to the voltage potential), the negatively charged nitrate amendment tended to accumulate at the interface between K layers. In addition, amendment mass transport was highest in the low K layer, where the effective ionic mobility was lowest (Gill et al., 2015). In the EK-Bio tests, it may be possible that EM and/or EO transported more ions to the electrode reservoirs than anticipated. The clay caps may potentially sorb more ions, due to higher sorption sites than the sandstone, or they could be a conduit for faster ion transport. The chloride concentrations that were extracted from the core samples cannot be explained through mass balance of dechlorination of TCE, since cores 1 to 3 had very similar concentrations around 18 ± 5 mg/L chloride (n=15), but concentrations were three times higher in core 4, which had an average chloride concentration of 64 ± 4 mg/L (n=5). The chloride concentrations extracted from the core samples did not correlate with the higher concentrations in the porewater during EK operation. Overall, the chloride concentrations did not affect the ability to demonstrate effective bacterial viability and weakly suggested biotic reductive dechlorination.

Chapter 6 Conclusions

In this thesis, the primary objectives were to evaluate the propensity for bacteria to survive, thrive, and reproduce within the primary porosity of intact Idaho Gray sandstone bedrock and to determine bacterial reductive dechlorination ability of TCE within the primary porosity. As this type of experiment has never been attempted previously, specific tasks that needed to be addressed included design of a system to migrate bacteria and amendments into intact sandstone cores at a rate faster than diffusion EK was paired with bioaugmentation to migrate aqueous TCE contaminant, electron donor, and KB-1 bacteria into the primary porosity of the sandstone cores to overcome slow diffusion rates that otherwise hinder experimentation with bedrock. The EK column used in these EK-Bio tests were first validated to prove EM and EO transport mechanisms could occur.

Despite the challenges of studying intact cores, EK validation testing confirmed that the column configuration of sand and clay units on the periphery of the sandstone cores was appropriate for promoting EK transport. KB-1 bacteria were transported into the primary porosity of intact sandstone cores, and key dehalogenating species were capable of acclimatizing and reproducing, given the increases in *vcrA* and *bvcA* gene copy concentrations that were observed in the 5- and 9-week incubated core samples. Despite maintaining the cores in anaerobic conditions, chlorinated ethene mass balance could not be achieved, thus complete biotic reductive dechlorination within the primary porosity of the sandstone could not be quantified. Evaluation of lactate at the beginning, during, and at the end of EK treatment showed that the donor concentration remained stable throughout EK operation, and consequently, lactate had successfully migrated through the core. The nature of testing intact cores, in which the setups were constructed and operated at different times, due to equipment availability limitations, made replication of treatments challenging. Overall, each task outlined for the evaluation of EK-Bio in sandstone were achieved or likely achieved (**Table 16**):

Table 16 Summary of goals and tasks accomplished for this thesis.

Goal	Task	Achieved?	Line(s) of Evidence
Dechlorination in Bedrock	1. Establish suitable geochemistry conditions	Yes	Negative ORP in cathode reservoirs
	2. Assess microbial activity	Yes	*vcrA* and *bvcA* increased with increasing incubation time
	3. Assess reductive dechlorination abilities	Likely	TCE decreased with increasing incubation time, but VC and DHGs not detected
	4. Evaluate organic acid end-product formation	Likely	Total VFAs decreased with increasing incubation time

The results of this proof-of-concept study are valuable to environmental practitioners working at sites where sandstone bedrock is contaminated with chlorinated solvents, and where remediation options are limited. Given the results from this study, EK may be a potential option to transport bacteria and electron donor into the primary porosity of chlorinated solvent impacted sandstone to promote bioaugmentation. Multiple lines of evidence, including reductase gene production, fatty acid fermentation, and the production of chlorinated solvent end-products should be monitored to evaluate the efficacy of dechlorination. The design of amendment injection wells would need consideration of geological conditions, such as well volume, well spacing, and concentrations of amendment to ensure solvents are effectively dechlorinated.

In future experiments, it would be useful to also include an assessment of live cells and community sequencing at the end of incubation periods, which would more accurately represent bacterial viability. An enclosed system may be required to capture the formation of gases in real time within the column, since measuring gases after sampling for VOCs was not feasible with the current design. Finally, testing EK-Bio in Idaho Gray sandstone cored parallel to the bedding planes, other sandstones of different porosities, or other bedrock types would help to assess whether the paired technology is feasible in rock types other than what was used in this study, and may provide further information regarding limitations of pore throat sizes for effective bacterial transport.

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Appendices

Appendix A: Pressure Saturation Data – Sandstone, Limestone, Shale

Figure A-1 Example pieces, from left to right, of Indiana carbonate limestone, Mancos shale, and Idaho Gray sandstone.

Table A-1 Reductase enzyme concentrations extracted from Idaho Gray sandstone, Indiana carbonate limestone, and Mancos shale saturated with amendment solution and KB-1, then incubated in anaerobic conditions for the specified period of time.

Incubation Period (d)	Rock Type	Replicate	Percent *vcrA* (%)	*vcrA* Gene Copies/L
13	Sandstone	1	0.3-0.9	1.2×10^{10}
13	Sandstone	2	0.001-0.004	1.2×10^{7} J
13	Carbonate	1	0.03-0.1	2.4×10^{8}
13	Carbonate	2	ND	3.0×10^{7} U
13	Shale	1	ND	3.0×10^{7} UE
13	Shale	2	ND	3.0×10^{7} UE
13	Shale	Control	ND	3.0×10^{7} UE
30	Sandstone	1	0.009-0.03	4.8×10^{7} J
30	Sandstone	2	ND	3.6×10^{7} U
30	Carbonate	1	ND	3.6×10^{7} U
30	Carbonate	2	ND	3.6×10^{7} U
30	Shale	1	ND	3.0×10^{7} UE
30	Shale	2	ND	3.0×10^{7} UE
30	Shale	Control	ND	3.6×10^{7} UE
60	Sandstone	1	0.001-0.004	6.0×10^{7} J
60	Sandstone	2	0.0005-0.001	6.0×10^{7} J
60	Sandstone	Control	ND	3.6×10^{7} UE
60	Carbonate	1	ND	3.0×10^{7} UE
60	Carbonate	2	0.0007-0.002	1.2×10^{7} J
60	Carbonate	Control	0.0008-0.002	3.0×10^{7} J
60	Shale	1	ND	3.0×10^{7} UE
60	Shale	2	ND	3.0×10^{7} UE
60	Shale	Control	ND	3.0×10^{7} UE

Notes

E	extracted genomic DNA not detected in the sample
J	estimated between method detection limit and quantitation limit
ND	non-calculable due to non-detect
U	below quantitation limit

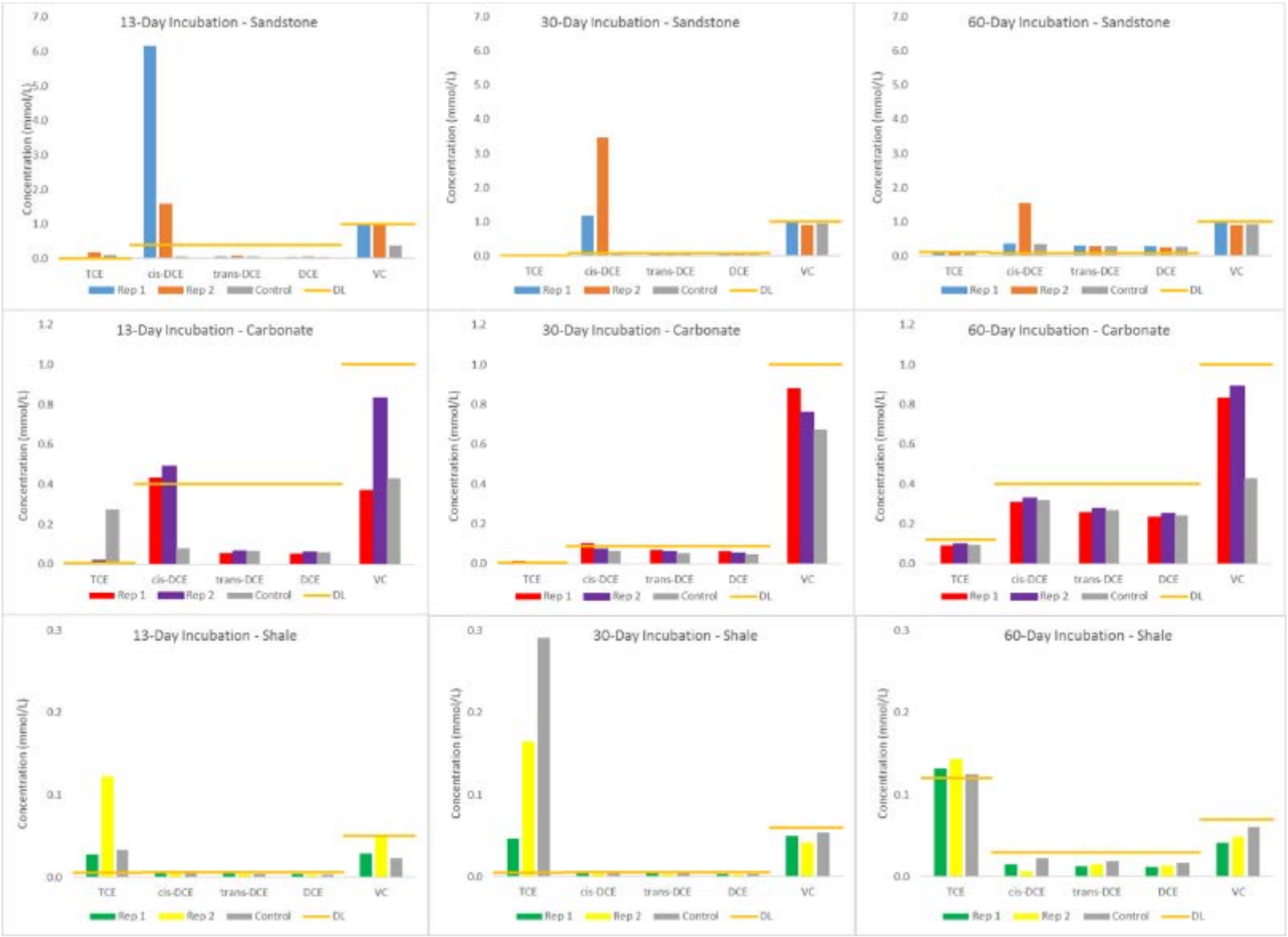

Figure A-2 VOC concentrations extracted from the three rock types in the preliminary pressure saturation tests, after incubation for the specified period of time.

Appendix B: Mercury Intrusion Porosimetry Data

Geotechnical Research Centre - Poresize Analysis

Sample ID: SANDSTONE
Operator: N. YASRI
Submitter: ELAINE SECORD
File: C:\9500\DATA\001-586.SMP

LP Analysis Time: 9/23/2016 10:43:08AM
HP Analysis Time: 9/23/2016 12:16:29PM
Report Time: 9/23/2016 12:16:29PM

Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Summary Report

Penetrometer parameters

Penetrometer:	13-0882 3 ml, 0.39, Solid		
Pen. Constant:	10.790 µL/pF	Pen. Weight:	61.5616 g
Stem Volume:	0.3870 mL	Max. Head Pressure:	4.6800 psia
Pen. Volume:	3.6300 mL	Assembly Weight:	96.7400 g

Hg Parameters

Adv. Contact Angle:	140.000 degrees	Rec. Contact Angle:	140.000 degrees
Hg Surface Tension:	480.000 dynes/cm	Hg Density:	13.5369 g/mL

User Parameters

Param 1: 0.000 Param 2: 0.000 Param 3: 0.000

Low Pressure:

Evacuation Pressure:	50	µmHg
Evacuation Time:	5	mins
Mercury Filling Pressure:	1.98	psia
Equilibration Time:	10	secs
Maximum Intrusion Volume:	0.010	mL/g

High Pressure:

Equilibration Time:	10	secs
Maximum Intrusion Volume:	0.010	mL/g

No Blank Correction

Intrusion Data Summary

Total Intrusion Volume =	0.0926	mL/g
Total Pore Area =	0.029	m²/g
Median Pore Diameter (Volume) =	58.4804	µm
Median Pore Diameter (Area) =	1.7188	µm
Average Pore Diameter (4V/A) =	12.9614	µm
Bulk Density at 1.98 psia =	1.8566	g/mL
Apparent (skeletal) Density =	2.2419	g/mL
Porosity =	17.1878	%
Stem Volume Used =	55	%

Pore Structure Summary

Threshold Pressure:	2.59	psia (Calculated)
Characteristic length =	82.3338	µm
Conductivity formation factor =	0.090	
Permeability constant =	0.00442	
Permeability =	2685.2490	mdarcy
BET Surface Area =	200.0000	m²/g
Pore shape exponent =	1.00	
Tortuosity factor =	2.036	
Tortuosity =	2.8390	
Percolation Fractal dimension =	2.939	
Backbone Fractal dimension =	N/A	

Mayer Stowe Summary

Interstitial porosity =	25.9500	%
Breakthrough pressure ratio =	9.4600	

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Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Material Compressibility

Linear Coefficient = -4.4225e-06 1/psia
Quadratic Coefficient = 6.2304e-11 1/psia²

Geotechnical Research Centre - Poresize Analysis

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Report Time: 9/23/2016 12:16:29PM

Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Tabular Report

Pore Diameter (µm)	Mean Diameter (µm)	Incremental Pore Volume (mL/g)	% Incremental Intrusion Volume	Mayer-Stowe Cumulative Volume finer % (%)	Cumulative Pore Area (m^2/g)
107.6600	107.6600	0.0000	0.0000	100.0000	0.000
86.9080	97.2840	0.0140	15.0786	84.9214	0.001
72.8927	79.9004	0.0175	18.8735	66.0480	0.001
71.0102	71.9515	0.0020	2.1226	63.9254	0.002
61.3927	66.2015	0.0102	11.0539	52.8714	0.002
53.5575	57.4751	0.0058	6.2850	46.5864	0.003
38.9967	46.2771	0.0081	8.7292	37.8572	0.003
35.6942	37.3455	0.0016	1.7458	36.1114	0.003
28.5723	32.1333	0.0034	3.6571	32.4543	0.004
25.2085	26.8904	0.0016	1.7367	30.7176	0.004
22.5469	23.8777	0.0013	1.4059	29.3118	0.004
20.3940	21.4704	0.0011	1.1853	28.1264	0.005
16.4686	18.4313	0.0022	2.3247	25.8017	0.005
13.3577	14.9131	0.0019	2.0766	23.7251	0.006
10.6880	12.0228	0.0018	1.9480	21.7771	0.006
8.5476	9.6178	0.0017	1.8561	19.9210	0.007
7.8291	8.1884	0.0010	1.0911	18.8299	0.007
5.1214	6.4753	0.0021	2.2655	16.5644	0.009
4.5709	4.8461	0.0005	0.5345	16.0299	0.009
3.8394	4.2052	0.0007	0.7959	15.2341	0.010
2.9743	3.4069	0.0010	1.0670	14.1671	0.011
2.4721	2.7232	0.0007	0.7460	13.4210	0.012
1.9045	2.1883	0.0008	0.8760	12.5450	0.013
1.5614	1.7329	0.0008	0.8738	11.6712	0.015
1.2339	1.3976	0.0010	1.0876	10.5835	0.018
0.9813	1.1076	0.0009	0.9898	9.5938	0.021
0.8009	0.8911	0.0007	0.8061	8.7877	0.025
0.6509	0.7259	0.0007	0.7838	8.0039	0.029
0.5099	0.5804	0.0008	0.8251	7.1787	0.034
0.4124	0.4611	0.0006	0.6841	6.4946	0.040
0.3346	0.3735	0.0005	0.5905	5.9041	0.045
0.2671	0.3008	0.0006	0.6273	5.2768	0.053
0.2155	0.2413	0.0005	0.5630	4.7139	0.062
0.1781	0.1968	0.0005	0.4940	4.2198	0.071
0.1425	0.1603	0.0005	0.5689	3.6509	0.084
0.1124	0.1275	0.0006	0.6002	3.0507	0.102
0.0909	0.1017	0.0005	0.4958	2.5550	0.120
0.0737	0.0823	0.0005	0.4920	2.0629	0.142
0.0594	0.0665	0.0005	0.4906	1.5724	0.169
0.0475	0.0535	0.0004	0.4852	1.0872	0.203
0.0382	0.0429	0.0004	0.4031	0.6841	0.238
0.0310	0.0346	0.0003	0.3239	0.3603	0.272
0.0249	0.0279	0.0003	0.2835	0.0768	0.310
0.0202	0.0225	0.0002	0.1685	-0.0918	0.338
0.0162	0.0182	0.0003	0.3297	-0.4214	0.405
0.0144	0.0153	-0.0000	-0.0091	-0.4123	0.403

Sample ID: SANDSTONE
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Correction Type: None
Show Neg. Int: Yes

Tabular Report

Pore Diameter (µm)	Mean Diameter (µm)	Incremental Pore Volume (mL/g)	% Incremental Intrusion Volume	Mayer-Stowe Cumulative Volume finer % (%)	Cumulative Pore Area (m^2/g)
0.0130	0.0137	0.0001	0.0593	-0.4716	0.419
0.0107	0.0119	-0.0000	-0.0192	-0.4524	0.413
0.0085	0.0096	0.0000	0.0435	-0.4959	0.429
0.0071	0.0078	-0.0000	-0.0113	-0.4846	0.424
0.0061	0.0066	0.0000	0.0094	-0.4941	0.429
0.0053	0.0057	0.0000	0.0029	-0.4970	0.431
0.0047	0.0050	-0.0003	-0.2913	-0.2057	0.217
0.0043	0.0045	-0.0001	-0.0581	-0.1476	0.169
0.0039	0.0041	-0.0001	-0.0704	-0.0772	0.105
0.0036	0.0037	-0.0001	-0.0772	0.0000	0.029
0.0046	0.0041	0.0005	0.4975	-0.4975	0.478
0.0060	0.0053	0.0007	0.7953	-1.2927	1.032
0.0078	0.0069	0.0005	0.5156	-1.8083	1.308
0.0102	0.0090	0.0004	0.3812	-2.1894	1.465
0.0133	0.0117	0.0003	0.2900	-2.4794	1.556
0.0172	0.0153	0.0002	0.2342	-2.7136	1.613
0.0222	0.0197	0.0001	0.1574	-2.8710	1.643
0.0292	0.0257	0.0001	0.1145	-2.9854	1.659
0.0373	0.0333	0.0001	0.0714	-3.0568	1.667
0.0495	0.0434	0.0000	0.0251	-3.0820	1.669
0.0646	0.0570	-0.0000	-0.0389	-3.0431	1.667
0.0820	0.0733	-0.0001	-0.0823	-2.9608	1.663
0.1066	0.0943	-0.0002	-0.1781	-2.7828	1.656
0.1421	0.1243	-0.0002	-0.1642	-2.6185	1.651
0.1776	0.1599	-0.0002	-0.1759	-2.4426	1.647
0.2367	0.2072	-0.0002	-0.2305	-2.2121	1.643
0.3048	0.2708	-0.0002	-0.2265	-1.9856	1.640
0.4251	0.3649	-0.0003	-0.2999	-1.6857	1.636
0.5309	0.4780	-0.0002	-0.2494	-1.4363	1.635
0.7066	0.6187	-0.0003	-0.2937	-1.1426	1.633
0.8793	0.7929	-0.0002	-0.2605	-0.8821	1.632
1.1140	0.9966	-0.0003	-0.2954	-0.5867	1.630
1.4501	1.2820	-0.0003	-0.3244	-0.2623	1.630
1.8767	1.6634	-0.0004	-0.3788	0.1165	1.629
2.4608	2.1687	-0.0004	-0.4161	0.5326	1.628
3.1201	2.7905	-0.0004	-0.4312	0.9638	1.627
4.0091	3.5646	-0.0005	-0.4894	1.4532	1.627
6.4933	5.2512	-0.0010	-1.1112	2.5644	1.626

Geotechnical Research Centre - Poresize Analysis

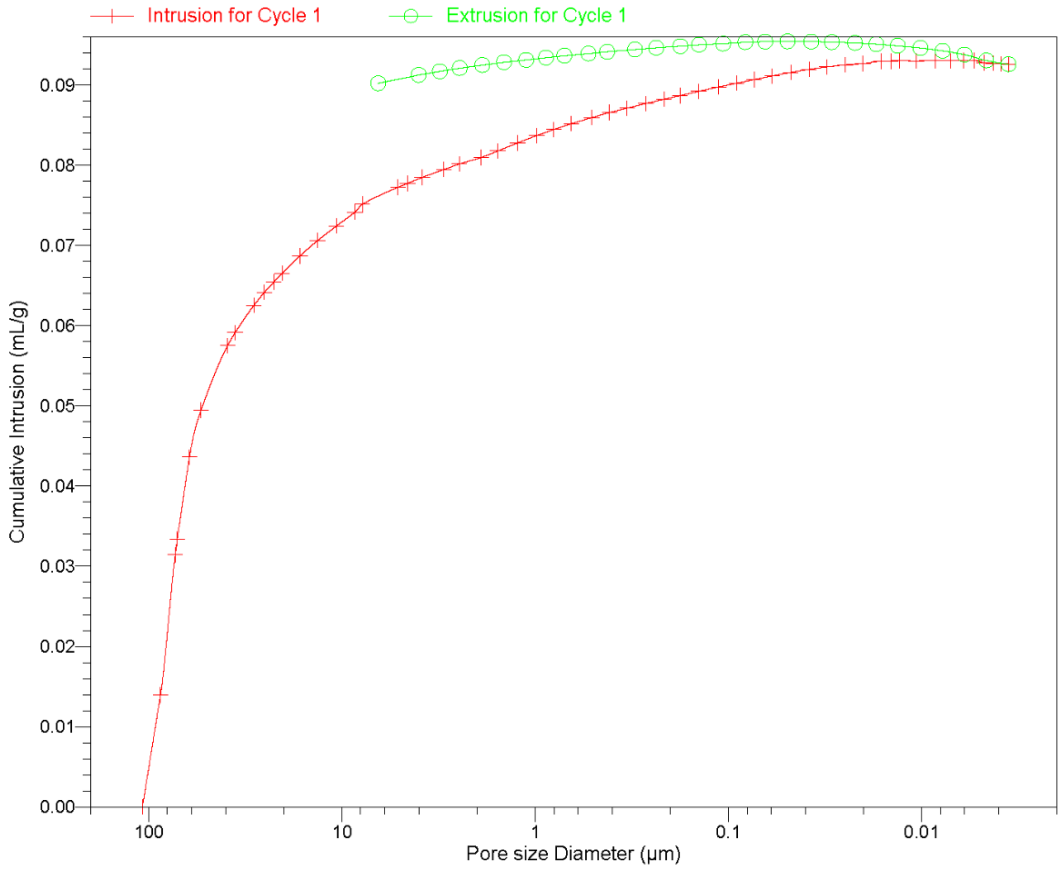
AutoPore IV 9500 V1.07 Serial: 689 Port: 1/1 Page 5

Sample ID: SANDSTONE
Operator: N. YASRI
Submitter: ELAINE SECORD
File: C:\9500\DATA\001-586.SMP

LP Analysis Time: 9/23/2016 10:43:08AM
HP Analysis Time: 9/23/2016 12:16:29PM
Report Time: 9/23/2016 12:16:29PM

Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Cumulative Intrusion vs Pore size



Geotechnical Research Centre - Poresize Analysis

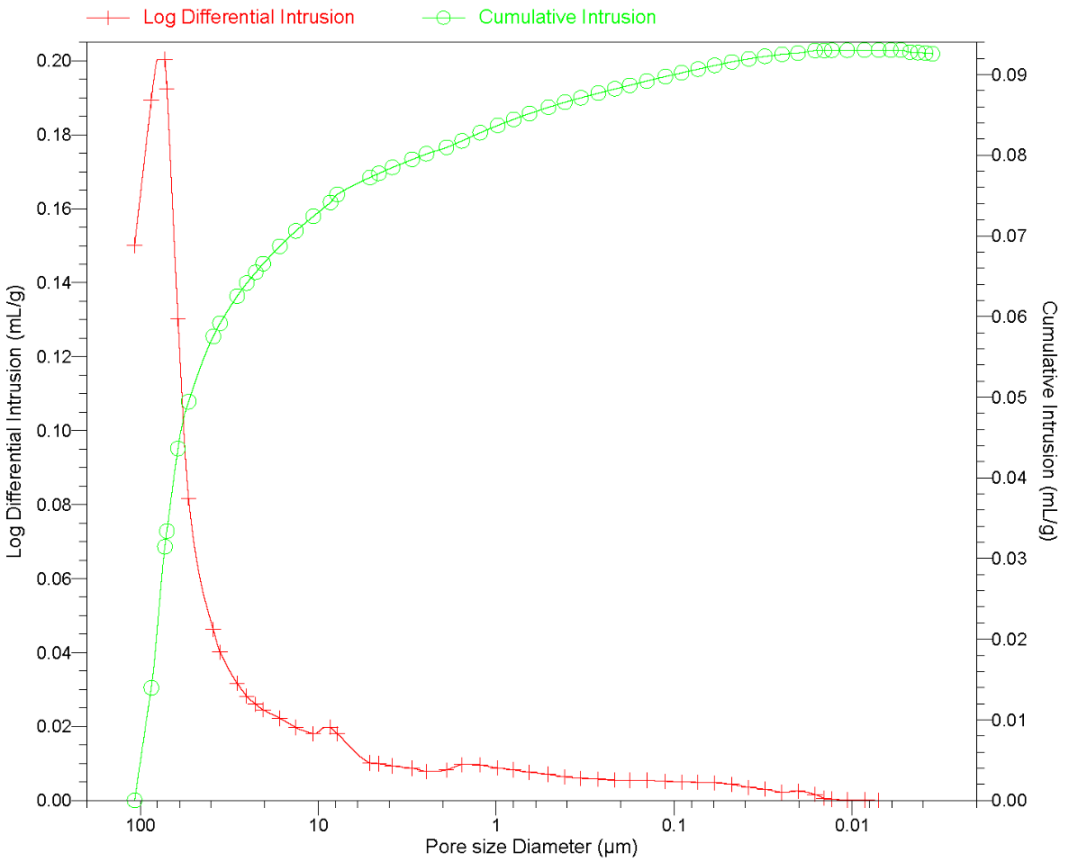
AutoPore IV 9500 V1.07 Serial: 689 Port: 1/1 Page 6

Sample ID: SANDSTONE
Operator: N. YASRI
Submitter: ELAINE SECORD
File: C:\9500\DATA\001-586.SMP

LP Analysis Time: 9/23/2016 10:43:08AM
HP Analysis Time: 9/23/2016 12:16:29PM
Report Time: 9/23/2016 12:16:29PM

Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Log Differential Intrusion vs Pore size



Geotechnical Research Centre - Poresize Analysis

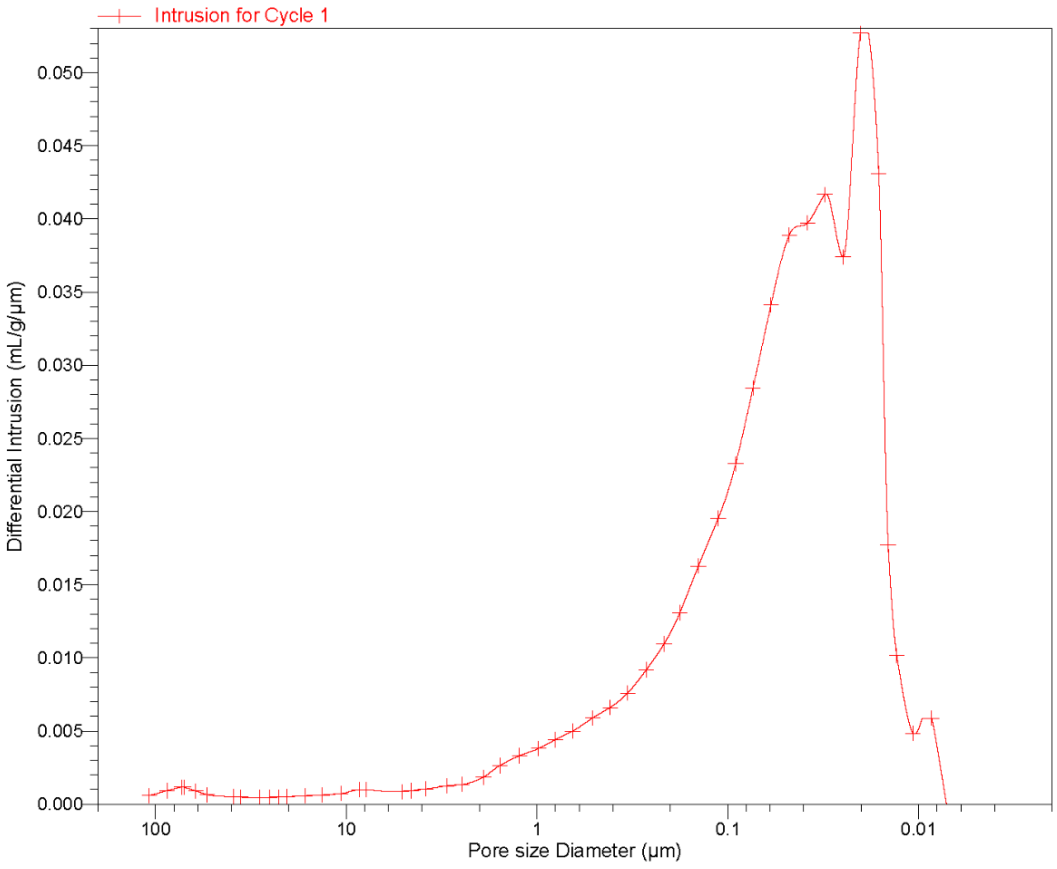
AutoPore IV 9500 V1.07 Serial: 689 Port: 1/1 Page 7

Sample ID: SANDSTONE
Operator: N. YASRI
Submitter: ELAINE SECORD
File: C:\9500\DATA\001-586.SMP

LP Analysis Time: 9/23/2016 10:43:08AM
HP Analysis Time: 9/23/2016 12:16:29PM
Report Time: 9/23/2016 12:16:29PM

Sample Weight: 2.2190 g
Correction Type: None
Show Neg. Int: Yes

Differential Intrusion vs Pore size



Appendix C: Zeta Potential Data

Brookhaven Instruments Corp.
PALS Zeta Potential Analyzer Ver. 3.57

Date: Oct 16, 2015
Time: 14:47:35
Batch: 0

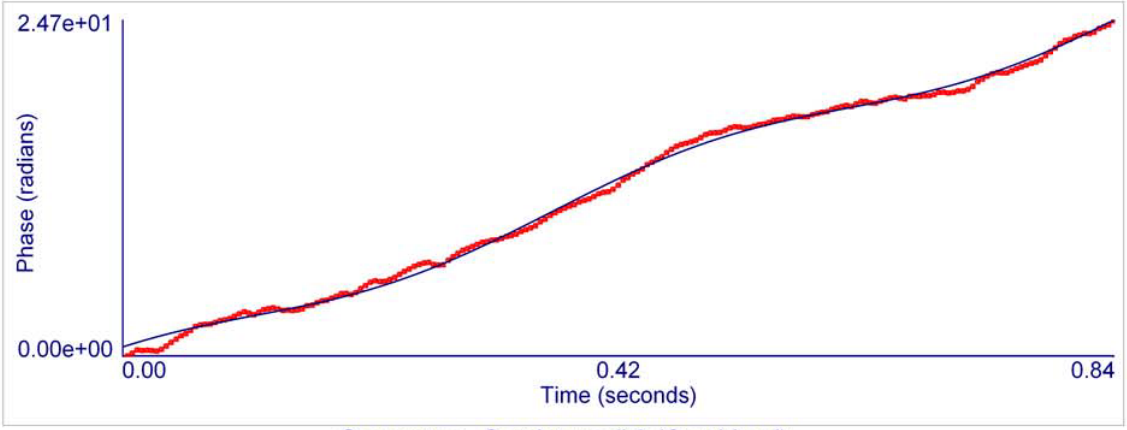
Sample ID **Geosyntec - Sandstone # 2 (Combined)**
Operator ID **Caitlin Marshall**
Notes **Suspension**

Measurement Parameters:

Mean Zeta Potential	= -12.80 mV	Liquid	= Water
Zeta Potential Model	= Smoluchowski	Temperature	= 23.5 °C
Mean Mobility	= -0.97 (μ/s) / (V/cm)	Viscosity	= 0.922 cP
pH	= 7.00	Refractive Index	= 1.331
Conductance	= 318 μS	Dielectric Constant	= 79.08
Concentration	= 0.10 mg/mL	Particle Size	= 1000.0 nm

Instrument Parameters:

Sample Count Rate	= 473 kcps	Voltage	= 4.00 volts
Ref. Count Rate	= 1540 kcps	Electric Field	= 8.03 V/cm
Wavelength	= 659.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00
Cycles Per Run	= 10		



Geosyntec - Sandstone # 2 (Combined)

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-0.76	-10.03	0.0704
2	-0.56	-7.33	0.0393
3	-0.98	-12.88	0.0601
4	-0.70	-9.21	0.0504
5	-1.86	-24.55	0.0520
Mean	-0.97	-12.80	0.0545
Std. Error	0.23	3.07	0.0052
Combined	-0.80	-10.52	0.0244

Brookhaven Instruments Corp.
PALS Zeta Potential Analyzer Ver. 3.57

Date: Oct 16, 2015
Time: 14:53:56
Batch: 0

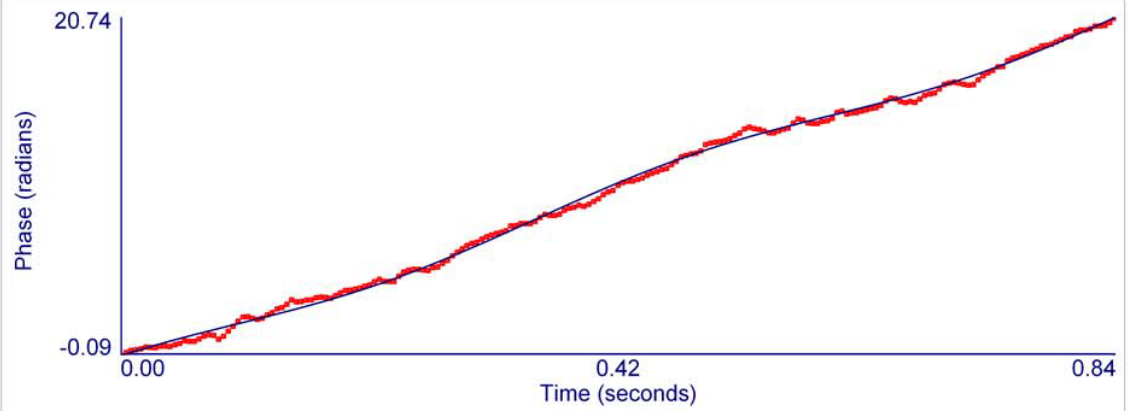
Sample ID **Geosyntec - Sandstone # 3 (Combined)**
Operator ID **Caitlin Marshall**
Notes **Suspension**

Measurement Parameters:

Mean Zeta Potential	= -12.86 mV	Liquid	= Water
Zeta Potential Model	= Smoluchowski	Temperature	= 23.5 °C
Mean Mobility	= -0.98 (μ/s) / (V/cm)	Viscosity	= 0.922 cP
pH	= 7.00	Refractive Index	= 1.331
Conductance	= 366 μS	Dielectric Constant	= 79.08
Concentration	= 0.10 mg/mL	Particle Size	= 1000.0 nm

Instrument Parameters:

Sample Count Rate	= 426 kcps	Voltage	= 4.00 volts
Ref. Count Rate	= 1388 kcps	Electric Field	= 7.34 V/cm
Wavelength	= 659.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00
Cycles Per Run	= 10		



Geosyntec - Sandstone # 3 (Combined)

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-0.48	-6.33	0.0686
2	-0.46	-6.00	0.0455
3	-1.28	-16.88	0.0933
4	-1.02	-13.44	0.0863
5	-1.65	-21.67	0.0998
Mean	-0.98	-12.86	0.0787
Std. Error	0.23	3.03	0.0098
Combined	-0.48	-6.30	0.0179

Brookhaven Instruments Corp.
PALS Zeta Potential Analyzer Ver. 3.57

Date: Oct 20, 2015
Time: 12:41:15
Batch: 0

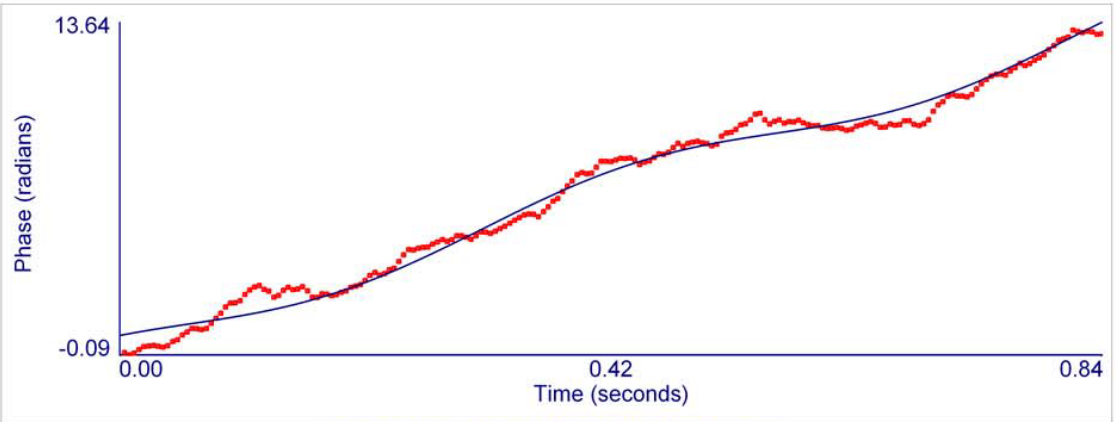
Sample ID **Geosyntec - Sandstone # 8 (Combined)**
Operator ID **Caitlin Marshall**
Notes **Suspension**

Measurement Parameters:

Mean Zeta Potential	= -12.41 mV	Liquid	= Water
Zeta Potential Model	= Smoluchowski	Temperature	= 23.5 °C
Mean Mobility	= -0.94 (μ/s) / (V/cm)	Viscosity	= 0.922 cP
pH	= 7.00	Refractive Index	= 1.331
Conductance	= 424 μS	Dielectric Constant	= 79.08
Concentration	= 0.10 mg/mL	Particle Size	= 1000.0 nm

Instrument Parameters:

Sample Count Rate	= 422 kcps	Voltage	= 4.00 volts
Ref. Count Rate	= 1404 kcps	Electric Field	= 7.68 V/cm
Wavelength	= 659.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00
Cycles Per Run	= 10		



Geosyntec - Sandstone # 8 (Combined)

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-1.01	-13.29	0.0678
2	-0.59	-7.77	0.0798
3	-0.68	-8.93	0.0570
4	-0.91	-12.01	0.0780
5	-1.52	-20.07	0.0468
Mean	-0.94	-12.41	0.0659
Std. Error	0.16	2.16	0.0063
Combined	-0.51	-6.69	0.0296

Brookhaven Instruments Corp.
PALS Zeta Potential Analyzer Ver. 3.57

Date: Oct 22, 2015
Time: 15:37:20
Batch: 0

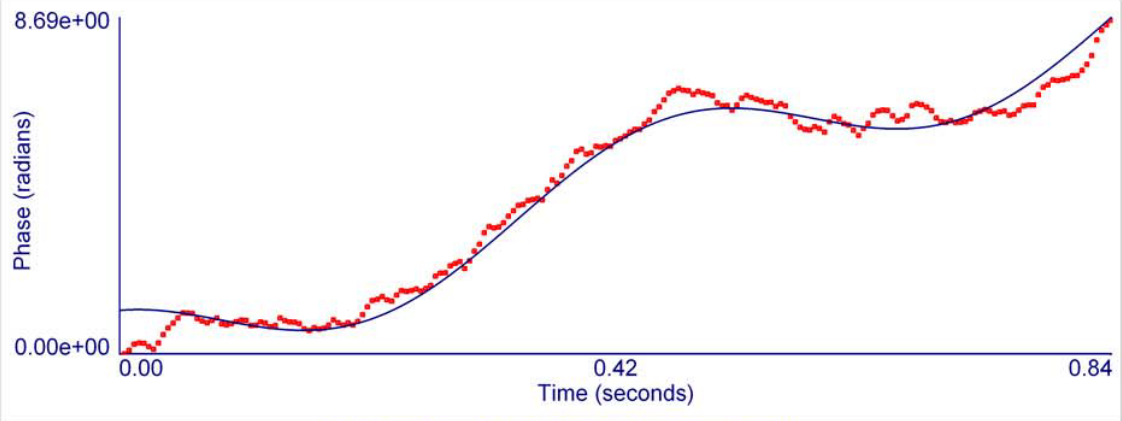
Sample ID **Geosyntec - Sandstone # 10 (Combined)**
Operator ID **Caitlin Marshall**
Notes **Suspension**

Measurement Parameters:

Mean Zeta Potential	= -13.09 mV	Liquid	= Water
Zeta Potential Model	= Smoluchowski	Temperature	= 23.5 °C
Mean Mobility	= -0.99 (μ/s) / (V/cm)	Viscosity	= 0.922 cP
pH	= 7.00	Refractive Index	= 1.331
Conductance	= 493 μS	Dielectric Constant	= 79.08
Concentration	= 0.10 mg/mL	Particle Size	= 1000.0 nm

Instrument Parameters:

Sample Count Rate	= 433 kcps	Voltage	= 4.00 volts
Ref. Count Rate	= 1537 kcps	Electric Field	= 7.61 V/cm
Wavelength	= 659.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00
Cycles Per Run	= 10		



Geosyntec - Sandstone # 10 (Combined)

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-0.81	-10.72	0.0494
2	-1.26	-16.63	0.0490
3	-0.84	-11.00	0.0378
4	-0.90	-11.82	0.0550
5	-1.16	-15.29	0.0988
Mean	-0.99	-13.09	0.0580
Std. Error	0.09	1.20	0.0106
Combined	-0.98	-12.95	0.0246

Brookhaven Instruments Corp.
PALS Zeta Potential Analyzer Ver. 3.57

Date: Oct 22, 2015
Time: 15:46:07
Batch: 0

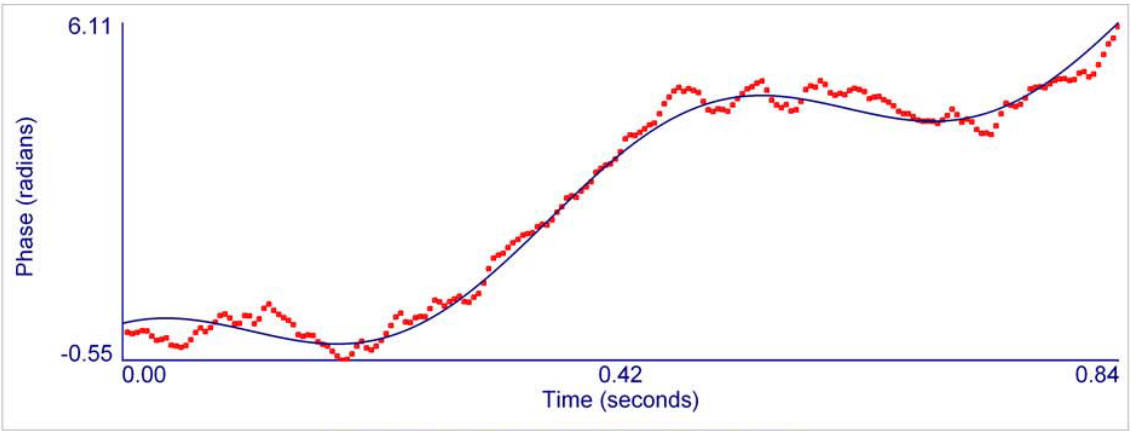
Sample ID **Geosyntec - Sandstone # 11 (Combined)**
Operator ID **Caitlin Marshall**
Notes **Suspension**

Measurement Parameters:

Mean Zeta Potential	= -11.62 mV	Liquid	= Water
Zeta Potential Model	= Smoluchowski	Temperature	= 23.5 °C
Mean Mobility	= -0.88 (μ/s) / (V/cm)	Viscosity	= 0.922 cP
pH	= 7.00	Refractive Index	= 1.331
Conductance	= 491 μS	Dielectric Constant	= 79.08
Concentration	= 0.10 mg/mL	Particle Size	= 1000.0 nm

Instrument Parameters:

Sample Count Rate	= 583 kcps	Voltage	= 4.00 volts
Ref. Count Rate	= 1528 kcps	Electric Field	= 7.63 V/cm
Wavelength	= 659.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00
Cycles Per Run	= 10		



Geosyntec - Sandstone # 11 (Combined)

Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-0.33	-4.40	0.0423
2	-1.13	-14.83	0.0370
3	-1.03	-13.53	0.0334
4	-1.15	-15.18	0.0411
5	-0.77	-10.15	0.0334
Mean	-0.88	-11.62	0.0374
Std. Error	0.15	2.01	0.0019
Combined	-0.86	-11.28	0.0175

Appendix D: Gene-Trac® Functional Gene Assay Report – Untreated Sandstone

SiREM
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130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: David Reynolds & Matt Vanderkooy, Geosyntec
Project: EK-TAC
Customer Reference: NCRESDEV-TAC15-P05

SiREM Reference: S-4017
Report Date: 15-Aug-16
Data Files: iQ5-FGA-QPCR-0895
iQ5-DB-FGA-QPCR-0603

Table 1b: Test Results

Sample ID	VC Reductase (*vcrA*)		BAV1 VC Reductase (*bvcA*)		TCE Reductase (*tceA*)	
	Percent *vcrA* (3)	Gene Copies/Liter	Percent *bvcA* (3)	Gene Copies/Liter	Percent *tceA* (3)	Gene Copies/Liter
Sandstone -ve control	NA	5×10^3 U	NA	5×10^3 U	NA	5×10^3 U

See final page for notes.

Analyst: Kela Bartle
Kela Bartle, B.Sc.
Laboratory Technician

Approved: J. Wilkinson
Jennifer Wilkinson
Senior Laboratory Technician

Table 2: Detailed Test Parameters, Test Reference S-4017

Customer Sample ID	Sandstone -ve control
SiREM FGA Sample ID	FGA-7142
Date Sampled[4]	4-Aug-16
Matrix	Crushed Rock
Date Received[4]	5-Aug-16
Sample Temperature	NA
Filtration Date[4]	NA
Volume Used for DNA Extraction	0.25 g
DNA Extraction Date	9-Aug-16
DNA Concentration in Sample (extractable)	215 ng/g (J)
PCR Amplifiable DNA	ND
FGA qPCR Date Analyzed	10-Aug-16
Laboratory Controls (see Tables 3 & 4)	Passed
Comments	- -

See final page for notes.

Table 3: Gene-Trac FGA Control Results, Test Reference S-4017

Laboratory Control	Analysis Date	Control Description	*vcrA*		*bvcA*		*tceA*		Comments
			Spiked Gene Copies per Liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	
Positive Control Low Concentration	10-Aug-16	Genomic DNA (CSLF-0763)	6.0×10^4	$9.1 \times 10^{4\,(3)}$	5.3×10^3	$1.1 \times 10^{4\,(3)}$	1.7×10^5	2.0×10^5	See Note 3
Positive Control High Concentration	10-Aug-16	Genomic DNA (CSHF-0763)	8.1×10^6	6.8×10^6	7.5×10^5	4.4×10^5	1.6×10^7	$7.7 \times 10^{6(3)}$	See Note 3
DNA Extraction Blank	10-Aug-16	Sterile Water (FB-2717)	0	5.2×10^3 U	0	5.2×10^3 U	0	5.2×10^3 U	Passed
Negative Control	10-Aug-16	Reagent Blank (TBF-0734)	0	5.2×10^3 U	0	5.2×10^3 U	0	5.2×10^3 U	Passed

See final page for notes.

Notes:
vcrA = VC reductase
bvcA = BAV1 VC reductase
tceA = TCE reductase
J The associated value is an estimated quantity between the method detection limit and quantitation limit.
U Not detected, associated value is the quantitation limit.
B Analyte was detected in the method blank within an order of magnitude of the test sample.
E Extracted genomic DNA was not detected in the sample.
I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers.
ng/g = nanograms per gram
g = grams
NA = not applicable
ND = not detected
DNA = deoxyribonucleic acid
16S rRNA = 16S ribosomal ribonucleic acid
PCR = polymerase chain reaction
qPCR = quantitative PCR
°C = degrees Celsius

[1] Percent of functional gene in microbial population. This value is calculated by dividing the functional gene copies quantified by the total number of estimated prokaryotes in the sample (based on the total quantity of DNA extracted from the sample). A value of 100% would suggest that all microbes in the sample contain the gene.

[2]Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

[3]Control results are deemed acceptable if one of two positive controls falls within the recovery limit guidelines (+/- 50%).

SiREM

Chain-of-Custody Form

siremlab.com

130 Stone Rd. W
Guelph, ON N1G 3Z2
(519) 822-2265

Lab # S-4017

*Project Name: EK-TAC	*Project #: NCRESDEV-TAC15-P05
*Project Manager: David Reynolds/Matt Vanderkooy	*Company: Geosyntec
*Email Address: elaine.secord@uwaterloo.ca	
Address (Street):	
City:	State/Province: Country:
*Phone #: 226-600-3561	
*Sampler's Signature:	*Sampler's Printed Name: Elaine Secord

Analysis

Preservative Key
0. None
1. HCL
2. Other
3. Other
4. Other
5. Other
6. Other

Client Sample ID	Sampling Date	Sampling Time	Matrix	# of Containers	Gene-Trac DHC	Gene-Trac VC	Gene-Trac DHB	Gene-Trac DHG	Gene-Trac tceA	Volatile Fatty Acids	Dissolved hydrocarbon gases	Treatability Study	Other Information
Sandstone -ive control	8/4/16	16:00	Rock	1		X							For microbial baseline

Billing Information
P.O. #:
*Bill To: NCRESDEV-TAC15-P05

Turnaround Time Requested
Normal ☑
Rush ☐

For Lab Use Only
Cooler Condition:
Cooler Temperature: N/A
Custody Seals: Yes ☐ No ☐

For Lab Use Only
Proposal #:

Relinquished By:	Received By:	Relinquished By:	Received By:	Relinquished By:	Received By:
Signature:	Signature: Kela Ashworth	Signature:	Signature:	Signature:	Signature:
Printed Name: Elaine Secord	Printed Name: Kela Ashworth	Printed Name:	Printed Name:	Printed Name:	Printed Name:
Firm: Geosyntec	Firm: SiREM	Firm:	Firm:	Firm:	Firm:
Date/Time: Aug 4, 2016	Date/Time: 5 Aug 2016 12:45	Date/Time:	Date/Time:	Date/Time:	Date/Time:

Distribution: White - return to Originator; Yellow - Lab Copy; Pink - Retained by Client
** Mandatory Fields*

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Technical Note 1.5:
Interpretation of Gene-Trac® Dhc, *vcrA*, *bvcA and tceA* Assays

This note provides technical background and guidelines for interpretation of the following Gene-Trac® assays:

(1) Gene-Trac® Dhc

(2) Gene-Trac® *vcrA*

(3) Gene-Trac® *bvcA*

(4) Gene-Trac® *tceA*

Gene-Trac® Dhc-Total *Dehalococcoides* Test

Background

Gene-Trac® Dhc is a quantitative polymerase chain reaction (qPCR) test for the microbial species *Dehalococcoides mccartyi* (i.e., *Dehalococcoides* [Dhc]). The Gene-Trac® Dhc test targets sequences of the 16S ribosomal ribonucleic acid (16S rRNA) gene unique to Dhc. Note the 16S rRNA gene does not directly participate in dechlorination, but is used as a molecular fingerprint in the identification and quantification of a wide variety of microbial groups. The detection of Dhc in environmental samples is significant as Dhc contain the greatest number of reductive dehalogenase genes of any microbial group (Tas et al., 2010). Dhc are capable of reductive dechlorination of a wide variety compounds/compound classes including:

- Chlorinated ethenes (tetrachloroethene [PCE], trichloroethene [TCE] , cis-1,2-dichloroethene [*c*DCE], 1,1-dichloroethene [1,1-DCE], trans-1,2-dichloroethene [*t*DCE, vinyl chloride [VC]) (Duhamel et al., 2002);
- 1,2-dichloroethane (1,2-DCA) to ethene (Grostern and Edwards, 2006);
- Selected polychlorinated biphenyl [PCB] congeners (Bedard et al., 2007);
- Selected chlorinated benzene compounds (Adrian et al., 2000; Fennell et al., 2004);
- Chlorophenols and polychlorinated dibenzo-*p*-dioxins (Fennell et al., 2004) and;
- 1,2-dibromoethane (Magnusson et al., 2000).

In addition to screening for diverse dechlorinating activities, Gene-Trac® Dhc can also be used to assess the *in situ* growth of Dhc containing bioaugmentation cultures such as KB-1® (Major et al., 2002).

Gene-Trac® Dhc Results Interpretation

Negative (Non-detect [ND]) Gene-Trac® Dhc Test Results

The absence of Dhc is associated with a lack of dechlorination or only partial reductive dechlorination of chlorinated ethenes. Where Dhc are absent the accumulation of cDCE is commonly observed, particularly after electron donor addition, often due to the presence of partial dechlorinators (e.g., *Dehalobacter, Geobacter*). Bioaugmentation with Dhc containing cultures (e.g., KB-1®) often improves bioremediation performance at sites lacking indigenous Dhc.

Positive (Detect) Gene-Trac® Dhc Test Results

The detection of Dhc is correlated with the complete biological dechlorination of chlorinated ethenes to non-toxic ethene at contaminated sites (Hendrickson et al., 2002). A positive Gene-Trac® Dhc test indicates that Dhc DNA was detected and is correlated with the occurrence of reductive dechlorination. Note, not all Dhc can convert vinyl chloride to ethene; this capability can be determined by quantifying the functional genes (*vcrA*, *bvcA*, *tceA*) (see following section). In most cases Dhc must be present at sufficient concentrations in order for significant dechlorination to be observed, guidelines for expected impacts on chlorinated ethenes at various Dhc concentrations in groundwater are indicated below.

- **10^4 Dhc gene copies per liter (or lower):** indicates low concentrations of Dhc which may indicate site conditions that are sub-optimal for high rates of dechlorination. Increases in Dhc concentrations at the site may be possible if conditions are optimized (e.g., electron donor addition/pH adjustment).

- **10^5-10^6 Dhc gene copies per liter:** indicates the sample contains moderate concentrations of Dhc which may, or may not, be associated with observable dechlorination activity.

- **1 x 10^7 Dhc gene copies per liter (or above):** indicates that the sample contains high concentrations of Dhc often associated with significant dechlorination rates (Lu et al., 2006).

- **10^9-10^{10} Dhc gene copies per liter:** are generally the highest observed for groundwater samples and are associated with very high rates of dechlorination

Interpretation of Functional Gene Assays for *vcrA*, *bvcA* and *tceA*

Background

Gene-Trac® *vcrA*, *bvcA* and *tceA* tests are provided combined as a functional gene assay package. These tests quantify genes that code for enzymes that dechlorinate chlorinated ethenes and other compounds. The *vcrA*, *bvcA* and *tceA* genes play specific roles in reductive dechlorination, specifically *tceA* converts TCE and cDCE to VC and *vcrA* and *bvcA* convert cDCE and VC to non-toxic ethene (Figure 1).

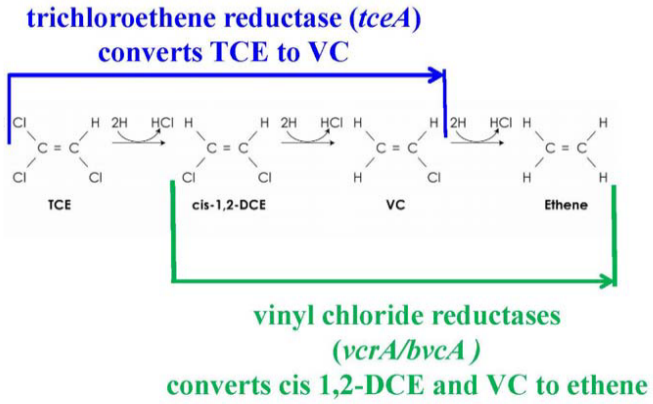
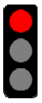


Figure 1: Major (energy yielding) activities against chlorinated ethene of enzymes coded for by the *tceA*, *vcrA* and *bvcA* genes.

Results Interpretation

Table 1 provides interpretation guidelines for different scenarios for Gene-Trac® Dhc, *vcrA*, *bvcA and tceA* tests. In general, accumulation of VC is more likely where Gene-Trac® *vcrA/bvcA* results are ND, or significantly lower than Gene-Trac® Dhc/*tceA*. Where abundance of *vcrA*/*bvcA* is similar to total Dhc the chances of VC accumulation are reduced.

Table 1: Interpretation of Gene-Trac® Dhc, *vcrA*, bvcA, *tceA* test results

Gene Copies/L				Summary	Interpretation	Remediation Implications	
Dhc	*vcrA*	*bvcA*	*tceA*				
ND	ND	ND	ND	ND for Dhc and functional genes	Site lacks Dhc	Complete dechlorination unlikely, may observe cis-DCE accumulation Site may require bioaugmentation	
≥1 x 10^7	≥1 x 10^7	≥1 x 10^7	≥1 x 10^7	Dhc and *vcrA/bvcA/tceA* are the same	Entire Dhc population has *tceA*, *vcrA* and *bvcA* gene	Potential for complete dechlorination very high. VC stall unlikely-sites with *vcr*A above 1 x 10^7/L typically have detectable ethene	
≥1 x 10^7	ND	≥1 x 10^7	ND	Total Dhc and *bvcA*/are the same *vcrA/tceA* ND	Dhc at high concentrations entire Dhc population has *bvcA* gene	Potential for complete dechlorination high. VC stall unlikely	
≥1 x 10^7	≥1 x 10^7	ND	ND	Total Dhc and *vcrA*/are the same *bvcA/tceA* ND	Dhc at high concentrations entire Dhc population has *vcrA* gene	Potential for complete dechlorination high. VC stall unlikely-sites with *vcr*A above 1 x 10^7/L often have detectable ethene	
≥1 x 10^7	ND	ND	≥1 x 10^7	Total Dhc high; *vcrA* and *bvcA* non-detect *tceA* same as Dhc	High concentration of Dhc, entire Dhc population has *tceA* but lacks the *vcrA/bvcA* genes	Likelihood for VC accumulation high as *vcrA* and *bvcA* both ND	
1 x 10^7	1 x 10^5	1 x 10^6	1 x 10^7	Total Dhc and *tceA* is significantly higher 10-100 fold) than *vcrA/bvcA*	*Dhc* population consists of different types, some with the *vcrA/* gene (10%) some with *bvcA* gene (1%) all contain *tceA* gene	VC-accumulation possible; Dhc:*vcrA*:*bvcA*:*tceA* ratios may evolve over the course of remediation	
1 x 10^7	1 x 10^7	1 x 10^6	ND	Total Dhc is high *vcrA/bvcA* high *tceA* ND	*tceA* negative population	cDCE to ethene dechlorination likely PCE and TCE dechlorination possible via *pceA* commonly found in other dechlorinators such as *Dehalobacter*	

 = favorable for complete dechlorination,  = some potential for VC stall  = complete dechlorination unlikely

Gene-Trac® *vcrA*/*bvcA*

Gene-Trac® *vcrA and bvcA* tests quantify VC-reductase genes that produce enzymes that convert VC to non-toxic ethene; a critical step in reductive dechlorination. The VC reductase genes (*vcrA, bvcA*) (Müller et al., 2004; Krajmalnik-Brown et al., 2004) produce enzymes found in many (but not all) Dhc. The *vcrA* gene is reported to be the most commonly identified VC reductase gene in the environment, whereas *bvcA* is generally less common but can predominate especially in more oxidizing groundwater (van der Zaan et al., 2010) and possibly where DCE is dominant. The *vcrA* gene can be used for tracking bioaugmentation cultures including KB-1® and is typically present at a 1:1 ratio with total Dhc whereas the *bvcA* gene is not predominant in the KB-1® culture and is present at less than a 1:1 ratio with total Dhc, therefore *bvcA* is not generally used for tracking KB-1® bioaugmentation and may be negative even after bioaugmentation with KB-1®.

Positive Gene-Trac® *vcrA, bvcA* Tests

Positive Gene-Trac® *vcrA* or *bvcA* tests indicate that the Dhc population has the *vcrA* and/or the *bvcA* gene and complete dechlorination to ethene is likely. As a minimal requirement, *vcrA* and/or *bvcA* copies exceeding 10^5/L combined with observed increases over time (i.e., cell growth) are required for robust VC dechlorination (van der Zaan et al., 2010). In one study, more than 90% of samples where *vcrA* enumeration exceeded 1 x 10^7 gene copies/L of groundwater had detectable ethene (Dennis, 2009). The enzyme produced by the *bvcA* genes has also been shown to degrade 1,2-DCA directly to ethene (Grostern and Edwards 2009) and the *bvcA* is used for tracking the KB-1® 1,2-DCA culture.

Non-Detect in Gene-Trac® *vcrA*/*bvcA* Test

A ND in the Gene-Trac® *vcrA* and *bvcA* test indicates that *vcrA/bvcA* gene sequences in the sample were below the detection limit of the assay. In cases where *vcrA/bvcA* are ND the chances of VC accumulation are increased compared to samples with detectable *vcrA/bvcA*. In such cases, *tceA* may promote limited and slow cometabolic degradation of VC to ethene (Lee et al., 2008) that may account for (generally low) detections of ethene where *vcrA* and *bvcA* are ND.

Gene-Trac® *tceA*

Gene-Trac® *tceA* test targets the trichloroethene reductase gene that produces an enzyme that primarily converts TCE to *c*DCE and VC. Studies have shown that this gene is commonly expressed under more oxidized conditions compared to *vcrA* (van der Zaan et al., 2010). Note the *tceA* gene is not predominant in the KB-1® culture and therefore *tceA* is not used for tracking KB-1® bioaugmentation.

Positive *tceA* test

A positive *tceA* test indicates that the Dhc population has the potential to dechlorinate TCE to cDCE and VC and VC to ethene cometabolically at relatively slow rates (Lee et al. 2008). Detection of *tceA* in the absence of *vcrA/bvcA* also indicates an increased likelihood for VC accumulation. The enzyme produced by *tceA* is also reported to dehalogenate 1,2-DCA and 1,2 dibromoethane (Magnussen et al., 2000).

Negative *tceA* test

A ND *tceA* test indicates that the Dhc population may lack the ability to convert TCE to cDCE and VC, nevertheless, conversion of PCE to cDCE is relatively common amongst other dechlorinators that harbor the *pceA* gene (Maillard et al., 2003; Wagner et al., 2012). Therefore *tceA is* not essential for complete dechlorination of TCE provided that *pceA* harboring microorganisms are present. Gene-Trac® Dhb (*Dehalobacter)* and Gene-Trac® Geo (*Geobacter*) can be used to quantify these common *pceA* containing microorganisms.

Sites with mixed Dhc populations

At some sites the Dhc population is homogenous while other sites have Dhc populations that are mixtures of different Dhc types. These scenarios can lead to differing proportions for Gene-Trac® Dhc *vcrA bvcA* and *tceA* test results. If the numerical results of Gene-Trac® *vcrA, bvcA or tceA* tests are identical to those obtained in the Gene-Trac® Dhc test it suggests that the entire Dhc population contains that gene. In other cases, Gene-Trac® *vcrA, bvcA, tceA* results may differ significantly (i.e., more than an order of magnitude) from total Dhc. For example, the *vcrA* gene may be 100-fold lower than the total Dhc. This scenario would suggest that only 1% of the Dhc population harbors the *vcrA* gene and the remaining 99% of the Dhc population does not contain the *vcrA* gene. In such cases the proportions of the functional genes may change over time (e.g., the proportion of *vcrA* may increase as the VC concentration increases favoring Dhc that contain *vcrA*).

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Appendix E: Validation of KB-1 Transport via EK with Column Design 2

In this column setup, KB-1 bacteria were injected directly into the cathode end of one core for EM validation, and at the anode end of the second core for EO validation. Both cores in this validation round were 102 mm long. Direct current of 16 mA, equivalent to a current density of 0.5 mA/cm^2, was applied for five days, which was estimated to be an adequate breakthrough time for KB-1 at the opposite end of the core from the injection site.

The volume of KB-1 solution injected into each well appeared to be limited by the volume of the well. Results suggested that EO could transport KB-1 within the specified time frame (6.0×10^7 *vcrA* gene copies/L porewater detected near the cathode), but EM could not (not detected above quantitation limit near the anode). However, conclusions of the success of EK transport could not be made because:

- Breakthrough time for KB-1 transport was not known;
- The volume of KB-1 injected into the wells may have been negligible relative to the volume of pore spaces within the cores; and
- One replicate for each test may not be representative due to natural heterogeneity within the cores.

Appendix F: Gene-Trac® Functional Gene Assay Reports, Post-EK Treatment

SiREM
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Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: Dave Reynolds, Geosyntec Consultants
Project: EK-TAC
Customer Reference: NCRESDEV.TAC15.05/1195

SiREM Reference: S-4354
Report Date: 20-Jun-17
Data Files: iQ5B-FGA-QPCR-0947
iQ5B-DB-FGA-QPCR-0646

Table 1: Test Results

Sample ID	VC Reductase (*vcrA*)		BAV1 VC Reductase (*bvcA*)		TCE Reductase (*tceA*)	
	Percent *vcrA* (1)	Gene Copies/Gram	Percent *bvcA* (1)	Gene Copies/Gram	Percent *tceA* (1)	Gene Copies/Gram
Proj B-EK-8A-Puck 2	0.003 - 0.008 %	2×10^4	NA	7×10^3 U	NA	7×10^3 U
Proj B-EK-8A-Puck 3 mid	NA	7×10^3 U	NA	7×10^3 U	NA	7×10^3 U
Proj B-EK-8A-Puck 3 top	NA	5×10^3 U	NA	5×10^3 U	NA	5×10^3 U
Proj B-EK-8A-Puck 3 bot	NA	7×10^3 U	NA	7×10^3 U	NA	7×10^3 U
Proj B-EK-8A-Puck 4	0.0007 - 0.002 %	2×10^3 J	NA	7×10^3 U	NA	7×10^3 U

See final page for notes.

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Jennifer Wilkinson
Senior Laboratory Technician

Approved: Ximena Druar
Ximena Druar, B.Sc.
Genetic Testing Coordinator

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Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: David Reynolds, Geosyntec
Project: EK TAC
Customer Reference: NCRESDEV.TAC15.05/1195

SiREM Reference: S-4402
Report Date: 19-Jul-17
Data Files: iQ5A-FGA-QPCR-0954/0955
iQ5A-DB-FGA-QPCR-0653/0654
iQ5A-TBA-QPCR-0278

Table 1a: Test Results

Sample ID	VC Reductase (*vcrA*)		BAV1 VC Reductase (*bvcA*)		TCE Reductase (*tceA*)	
	Percent *vcrA* [1]	Gene Copies/Gram	Percent *bvcA* [1]	Gene Copies/Gram	Percent *tceA* [1]	Gene Copies/Gram
5wk_Puck 2	0.2 - 0.5 %	3×10^4	NA	6×10^3 U	NA	6×10^3 U
5wk_Puck 3Top	0.4 - 1 %	6×10^5	0.001 - 0.004 %	2×10^3 J	NA	6×10^3 U
5wk_Puck 3Mid	0.7 - 2 %	2×10^6	0.002 - 0.005 %	6×10^3	NA	6×10^3 U
5wk_Puck 3Bot	0.8 - 2 %	3×10^6	0.002 - 0.005 %	6×10^3	NA	6×10^3 U
5wk_Puck 4	0.4 - 1 %	1×10^6	0.001 - 0.004 %	4×10^3 J	NA	6×10^3 U
9wkA_Puck 2	0.4 - 1%	1×10^6	0.009 - 0.03 %	3×10^4	NA	6×10^3 U
9wkA_Puck 3Top	0.05 - 0.2 %	1×10^5	0.001 - 0.004 %	3×10^3 J	NA	6×10^3 U
9wkA_Puck 3Mid	0.03 - 0.09%	2×10^5	0.0005 - 0.002 %	3×10^3 J	NA	6×10^3 U
9wkA_Puck 3Bot	0.2 - 0.5 %	1×10^6	0.003 - 0.008 %	2×10^4	NA	6×10^3 U
9wkA_Puck 4	0.4 - 1 %	4×10^5	0.008 - 0.02 %	7×10^3	NA	5×10^3 U
9wkB_Puck 2	0.5 - 1 %	8×10^5	0.007 - 0.02 %	1×10^4	NA	6×10^3 U
9wkB_Puck 3Top	0.3 - 1 %	1×10^6	0.003 - 0.009 %	1×10^4	NA	6×10^3 U
9wkB_Puck 3Mid	0.6 - 2 %	1×10^6	0.01 - 0.04 %	3×10^4	NA	6×10^3 U
9wkB_Puck 3Bot	0.3 - 0.9 %	1×10^6	0.007 - 0.02 %	3×10^4	NA	6×10^3 U
9wkB_Puck 4	0.2 - 0.6 %	6×10^5	0.004 - 0.01 %	1×10^4	NA	6×10^3 U

See final page for notes.

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Appendix G: Analytes Extracted from Cores, Post-EK Treatment

Table K-1 Anions, VFAs, and VOCs extracted from core 1 (baseline) after EK treatment.

Location	Chloride mg/L	Nitrite mg/L	Nitrate-N mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE µg/g	cDCE µg/g	VC µg/g	DHGs µg/g
Puck 2	20	<0.1 U	<0.1 U	2.7	198	1.1	<0.4 U	<0.5 U	<0.3 U	0.5	<0.4 U	<0.7 U	0.14	0.33	<0.06 U	<0.01 U
Puck 3 Top	23	<0.1 U	<0.1 U	5.4	25	1.0	<0.4 U	<0.5 U	<0.3 U	<0.2 U	<0.4 U	<0.7 U	0.74	0.25	<0.06 U	<0.01 U
Puck 3 Middle	20	<0.1 U	<0.1 U	5.0	6.7	0.8	<0.4 U	5.6	<0.3 U	<0.2 U	<0.4 U	<0.7 U	0.22	0.47	<0.07 U	<0.01 U
Puck 3 Bottom	16	8.0	<0.1 U	4.2	26	0.9	<0.4 U	<0.5 U	<0.3 U	0.6	<0.4 U	<0.7 U	0.30	0.53	<0.06 U	<0.01 U
Puck 4	19	8.5	<0.1 U	3.2	10	0.8	<0.4 U	25	0.9	<0.2 U	<0.4 U	<0.7 U	0.30	0.57	<0.05 U	<0.01 U

Notes:

U below reporting limit

Table K-2 Anions, VFAs, and VOCs extracted from core 2 (5-week incubation) after EK treatment.

Location	Chloride mg/L	Nitrite mg/L	Nitrate-N mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE µg/g	cDCE µg/g	VC µg/g	DHGs µg/g
Puck 2	10	<0.1 U	<0.1 U	1.8	54	<0.1 U	<0.04 U	<0.05 U	<0.03 U	<0.02 U	<0.04 U	<0.07 U	<0.03 U	<0.03 U	<0.05 U	<0.01 U
Puck 3 Top	12	<0.1 U	<0.1 U	2.1	41	<0.1 U	<0.04 U	<0.05 U	<0.03 U	<0.02 U	<0.04 U	<0.07 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U
Puck 3 Middle	13	<0.1 U	<0.1 U	3.3	23	<0.1 U	<0.04 U	0.17	<0.03 U	0.24	<0.04 U	<0.07 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U
Puck 3 Bottom	12	<0.1 U	<0.1 U	2.2	22	<0.1 U	<0.04 U	0.38	<0.03 U	0.92	<0.04 U	<0.07 U	<0.03 U	<0.03 U	<0.07 U	<0.01 U
Puck 4	16	6.6	<0.1 U	2.7	18	<0.1 U	<0.04 U	0.48	<0.03 U	1.39	<0.04 U	<0.07 U	<0.03 U	<0.03 U	<0.05 U	<0.01 U

Notes:

U below reporting limit

Table K-3 Anions, VFAs, and VOCs extracted from core 3 (9-week incubation) after EK treatment.

Location	Chloride mg/L	Nitrite mg/L	Nitrate-N mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE µg/g	cDCE µg/g	VC µg/g	DHGs µg/g
Puck 2	13	<0.1 U	<0.1 U	2.2	347	<0.1 U	<0.04 U	2.8	<0.03 U	0.1	<0.04 U	<0.1 U	<0.03 U	<0.03 U	<0.05 U	<0.01 U
Puck 3 Top	13	<0.1 U	<0.1 U	1.7	400	<0.1 U	<0.04 U	0.3	<0.03 U	<0.02 U	<0.04 U	<0.1 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U
Puck 3 Middle	18	<0.1 U	3.7	2.5	416	<0.1 U	<0.04 U	3.9	<0.03 U	0.1	<0.04 U	<0.1 U	<0.03 U	<0.03 U	<0.05 U	<0.01 U
Puck 3 Bottom	14	<0.1 U	<0.1 U	2.3	416	<0.1 U	<0.04 U	0.3	<0.03 U	<0.02 U	<0.04 U	<0.1 U	<0.02 U	<0.02 U	<0.05 U	<0.01 U
Puck 4	18	<0.1 U	1.5	2.5	496	<0.1 U	<0.04 U	4.5	<0.03 U	0.2	<0.04 U	<0.1 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U

Notes:

U below reporting limit

Table K-4 Anions, VFAs, and VOCs extracted from core 4 (9-week incubation) after EK treatment.

Location	Chloride mg/L	Nitrite mg/L	Nitrate-N mg/L	Sulfate mg/L	Phosphate mg/L	Bromide mg/L	Lactate mg/L	Acetate mg/L	Propionate mg/L	Formate mg/L	Butyrate mg/L	Pyruvate mg/L	TCE µg/g	cDCE µg/g	VC µg/g	DHGs µg/g
Puck 2	54	<0.1 U	<0.1 U	5.0	325	6.2	<0.4 U	28	0.6	<0.2 U	<0.4 U	<0.7 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U
Puck 3 Top	59	<0.1 U	<0.1 U	5.4	371	6.6	<0.4 U	102	2.3	0.8	<0.4 U	<0.7 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U
Puck 3 Middle	63	<0.1 U	<0.1 U	6.9	401	7.3	<0.4 U	68	1.9	0.8	<0.4 U	<0.7 U	<0.02 U	<0.02 U	<0.05 U	<0.01 U
Puck 3 Bottom	51	<0.1 U	<0.1 U	4.8	336	5.6	<0.4 U	57	1.6	0.8	<0.4 U	<0.7 U	<0.03 U	<0.03 U	<0.05 U	<0.01 U
Puck 4	58	<0.1 U	<0.1 U	5.0	386	6.4	<0.4 U	139	2.5	<0.2 U	<0.4 U	<0.7 U	<0.03 U	<0.03 U	<0.06 U	<0.01 U

Notes:

U below reporting limit