ARSENIC RELEASE FROM DECHLORINATION REMEDIATION PROCESSES OF BIOSTIMULATION AND BIOAUGMENTATION

by

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

MASTER OF SCIENCE

in

Civil and Environmental Engineering
(Environmental Engineering)

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Logan, Utah

2015
ABSTRACT

Arsenic Release From Dechlorination Remediation Processes of Biostimulation and Bioaugmentation

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Utah State University, 2015

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Biostimulation and bioaugmentation are remediation processes commonly used for dechlorination of trichloroethylene (TCE) in contaminated aquifers, but these processes can also enhance arsenic (As) mobilization, causing As concentrations that exceed the drinking water limit. Column laboratory studies were designed to evaluate the effectiveness of different carbon and energy sources, with and without bioaugmentation, for TCE dechlorination over depth and time. Factors affecting As mobilization and solubilization were observed to determine if As would be an unfortunate byproduct of TCE removal. The TCE-contaminated aquifer solids used in this study were collected from near Hill Air Force Base (UT). This basin-fill aquifer contains As minerals associated with volcanic deposits.

Large (15.2 cm diameter, 183 cm long) columns were packed with TCE-contaminated aquifer solids and fed with groundwater, TCE, and a carbon and energy
source (whey, two formulations of emulsified oil, or no carbon control) over 7.5 years. This column study showed a snap shot of changes in As biogeochemistry at the end of 7.5 years. Subsequently, small (7.62 cm diameter, length) columns were fed with groundwater, TCE, and a carbon source (whey, lactate, or no carbon control) with columns being sacrificed over 160 days. This study was designed to observe changes with time.

Each carbon source produced reducing conditions (Fe(II) production, NO₃-N depletion, SO₄ reduction, and sulfide production), which was the driving force for TCE dechlorination, but also As reduction and therefore As mobilization. Arsenic was leached out of the sediments in all carbon-treated columns. The addition of whey resulted in 50% of the sediment As being leached from the large columns. With the addition of emulsified oils, As was attenuated towards the bottom of the columns through association with carbonate minerals. In the small columns, the addition of lactate or whey resulted in increased association of As(III) with carbonate minerals as well as with other soluble minerals over time, but over 30% of the As in the sediments was leached out of these columns. Conditions developed with carbon addition are conducive for full dechlorination of TCE, as well as As solubilization, resulting in remediation of TCE but pollution of groundwater by As.
PUBLIC ABSTRACT

Arsenic Release From Dechlorination Remediation Processes of Biostimulation and Bioaugmentation

Suzy Smith

Arsenic (As), a known carcinogen, is a groundwater contaminant in many parts of the world. Arsenic contamination is enhanced through carbon addition, such as biostimulation, a remediation process, which has been used to remove trichloroethylene (TCE) from sediment and groundwater. Two studies were designed to evaluate the effect of different carbon sources on the removal of TCE through dechlorination and on As solubilization and mobilization in response to carbon addition.

The first set of columns (15.2 cm diameter, 183 cm long) used whey, Newman Zone® standard surfactant emulsified oil, Newman Zone® nonionic surfactant emulsified oil, and no carbon controls as carbon and energy sources and were fed for 7.5 years. The second set (7.62 cm diameter and length) used whey, lactate, and no carbon control as carbon sources with columns being dismantled and analyzed over a 5-month time period.

These studies showed that reducing conditions, caused by the carbon sources, was the driving force for As mobilization as As(V) was reduced to the more mobile As(III). Total As mass in the sediment was lost with all carbon treatments within the first study with whey having a greater loss; however, within the second study, both whey and lactate treatments had the same extent of As mass loss over time. The results also indicated that some As could be attenuating with carbonates or other highly soluble minerals.
ACKNOWLEDGMENTS

I would first like to thank Joan McLean, my advisor and employer for the past six years, for the knowledge, experience, and training she has shared with me. I would also like to thank my committee members and professors, Ryan Dupont and Laurie McNeill, for the instructions they have given me over the years.

Special thanks to the Utah Water Research Laboratory and all those that helped with this project, especially Sarah Kissell for being my associate and friend as we designed and implemented this project.

I would like to thank my family, especially Mom and Dad, for their support and encouragement over my entire life and the countless prayers that went up in my behalf from all my family members. Also a thank you to my friends that were close by to pick me up when I was feeling down and there to celebrate when things were working out well.

It is hard to express all the appreciation that I feel for all of you. Thank you from the bottom of my heart.

Suzy Smith
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Arsenic (As) is a known carcinogen. Millions of people have been exposed to As at concentrations above the drinking water limit (10 µg/L) (USEPA 2001) due to contamination of groundwater from the local geology. The concentration of As released in groundwater has been related to the microbial reductive dissolution of iron (Fe) oxides (Cummings et al. 1999) and the direct reduction of As by dissimilatory arsenic reducing bacteria (Zobrist et al. 2000). Organic carbon (as the source of carbon and energy) and microbes aid in the reductive dissolution process and cause conditions conducive for As solubilization. Organic carbon and the population of Fe and As reducing microbes may be natural or added through human activity (He et al. 2009; Fendorf et al. 2010), such as biostimulation and bioaugmentation.

Biostimulation (addition of an organic carbon source) and bioaugmentation (addition of microbes with a desired function) are commonly used technologies for the remediation of groundwater contaminated with chlorinated solvents, such as trichloroethylene (TCE) (Macbeth et al. 2004; Rahm et al. 2006). These imposed reducing conditions, conducive for microbial dechlorination processes leading to production of innocuous ethene, may also lead to the reductive dissolution of Fe oxides and increased concentrations of As in groundwater (Lee et al. 2005a; McLean et al. 2006). Arsenic release potential is not often considered when biostimulation and bioaugmentation are applied. However, it is critical to evaluate the release of As in aquifer systems where As is bioavailable in the geology.
Throughout the western and southwestern United States, basin-fill aquifers are associated with As in groundwater due to geologic material connected with volcanic activity (Anning et al. 2012). The Basin and Range province, covering Nevada and Utah, show elevated concentrations of As in the geologic material. Hill Air Force Base (HAFB) is located on the eastern edge of the Basin and Range province and has a long history of TCE disposal and contamination of groundwater. It was placed on the Superfund National Priorities List in 1987 and has 12 operable units (OU). Biostimulation and bioaugmentation have been utilized at Hill as remediation processes, however, they may cause problems with As solubilization and mobilization due to the reduction processes that occur from the carbon and energy sources applied to the groundwater. It is critical to evaluate the geochemistry and microbial community that develop under biostimulation and bioaugmentation that affect the release of As from aquifer material at HAFB, and other basin-fill aquifers throughout the Western US.

**Hypotheses**

1. Addition of a carbon and energy source will stimulate microbial reductive dissolution of Fe and As minerals leading to mobilization of As.

2. The extent of As mobilization will be dependent on the type of the carbon and energy source added to the system.

3. Bioaugmentation with a culture containing dechlorinating bacteria, as well as known dissimilatory iron reducing bacteria (DIRB), will promote more extensive mobilization of As than biostimulation alone.
Objectives

1. Determine the extent of As solubilization, as estimated using pore water chemistries and sequential extractions of the mineral phases, and mobilization based on mass balance calculations, and their relationship to Fe reductive dissolution, using data from a long-term, large column study designed to evaluate the effect of carbon and energy source addition on TCE dechlorination. Columns (183 cm in length and 15.2 cm in diameter) were packed with aquifer solids from OU5 HAFB and leached with TCE containing groundwater with the addition of either whey, two formulations of emulsified oil, or no carbon as a control with and without bioaugmentation. After 7.5 years the columns were sectioned. The pore water and solid phase were analyzed for general water quality and sediment properties, such as pH, EC, elemental analysis and specific parameters relevant to As solubilization. This objective is described in Chapter III.

2. Monitor small columns (7.6 cm in length and 7.6 cm in diameter packed with aquifer solids from OU5 HAFB) over time to evaluate the extent of dechlorination of TCE contaminated groundwater and As solubilization and mobilization with the addition of either whey or lactate as the carbon and energy sources, with bioaugmentation. Effluent from the columns was analyzed throughout the duration of the study for general water quality parameters as well as redox conditions such as Fe(II), nitrate, sulfate and Eh. Solid phase biogeochemical processes that control As solubilization and mobilization were monitored and analyzed at select sampling times to see progression over time. This objective is described in Chapter IV.
CHAPTER II
LITERATURE REVIEW

Arsenic Speciation

Arsenic has several oxidation states with arsenate (As(V)) and arsenite (As(III)) being the most common in groundwater. The speciation of As is important to know since As(III) is generally more toxic than As(V) (Sharma and Sohn 2009). The speciation of As is controlled by the Eh and pH of the system. Under oxidized conditions, As(V) dominates with three $pK_a$ values ($pK_1 = 2.2$, $pK_2 = 6.97$, $pK_3 = 11.53$) and As(III) dominates in reducing conditions also with three $pK_a$ values ($pK_1 = 9.22$, $pK_2 = 12.13$, $pK_3 = 13.4$) (Su and Puls 2008). Under typical environmental pH (5-9), $\text{HAsO}_4^{2-}$ and $\text{H}_3\text{AsO}_4^-$ are the dominant As(V) species and $\text{H}_3\text{AsO}_3$ is the dominant As(III) species.

Arsenic in the Environment

Arsenic minerals are classified as either primary or secondary. Primary minerals, such as arsenopyrite (FeAsS) and realgar (AsS), naturally occur in the environment whereas secondary minerals (e.g., arsenolite (As$_2$O$_3$) and pitticite (Fe$_x$(AsO$_4$)$_y$(SO$_4$)$_z$*nH$_2$O)) are formed when primary minerals are altered due to exposure to the atmosphere and surface water or groundwater (Drahota and Filippi 2009). Arsenic can be associated with other mineral phases such as Mn oxides (Amirbahman et al. 2006). However, the association with Fe oxides has been the major focus as the mechanism of As release to groundwater (Dixit and Hering 2003; Lee et al. 2005b; Tufano et al. 2008; Fendorf et al. 2010).
Reduction processes that cause
As release and mobilization

Arsenic within minerals is immobile; however, As can be mobilized due to several processes including the reductive dissolution of Fe oxides and the direct microbial reduction of As(V). Horneman et al. (2004) and van Geen et al. (2004) examined As mobilization and the dissolution of Fe oxyhydroxides from sediment profiles and sediment incubations, respectively, in groundwater sediments from Bangladesh. They suggested that the release of As was linked to and only occurred after the reduction of Fe(III). Niedhardt et al. (2014) attributed an increase in As to the dissolution of Fe oxyhydroxides in wells in the Bengal Delta. Cummings et al. (1999) demonstrated in contaminated lake sediments that dissimilatory Fe reducing bacteria (DIRB) can mobilize As(V) and Corsini et al. (2010) demonstrated that in flooded soils, microbial Fe reduction was the major process contributing to As mobilization. Lee (2013) found that *Shewanella putrefaciens* (Fe(III) reducing bacteria) increased As mobilization.

Studies have contrasted with the results above and shown evidence that As release is decoupled from Fe reduction. Using a single bacterial strain *Sulfurospirillum barnesii*, capable of growth using Fe(III) or As(V) as the terminal electron acceptor (TEA), Zobrist et al. (2000) demonstrated that the reductive dissolution of Fe oxides was not necessary for As reduction and solubilization. Masscheleyn et al. (1991) found that some As was released before Fe dissolution but the amount of As increased with increasing amounts of Fe oxide dissolution.

Tufano et al. (2008) compared Fe(III) and As(V) reduction. They found that As(V) can be directly reduced by microbes on mineral surfaces or can be released into
solution with the reductive dissolution of Fe minerals. If As(V) is reduced to As(III), more As will be mobilized because As(III) does not sorb as strongly as As(V) to Fe minerals. The study concluded that As sorption is controlled by both As(V) reduction and the reductive dissolution of hydrous ferric oxides (HFOs) and therefore both As(V) and Fe(III) reduction will contribute to As mobilization.

Smedley and Kinniburgh (2002) reviewed studies that further described As release, including studies that showed evidence of As release after flooding and development of anaerobic conditions. When conditions become anaerobic, a sequence of reduction reactions occurs: O$_2$, NO$_3^-$, Mn(IV), Fe(III), and SO$_4^{2-}$ (Stumm and Morgan 1996). As(V) reduction is expected to occur between Fe(III) and SO$_4^{2-}$ reduction (Smedley and Kinniburgh 2002). Islam et al. (2004) used microcosms of sediments from the Bengal Delta to show that As release occurs after Fe reduction and can be explained with the sequence of terminal electron acceptors where Fe has a higher redox potential.

Both Fe and As reduction processes require microbes that can reduce Fe and/or As when there is a bioavailable carbon and energy source. The required microbes are DIRB and dissimilatory arsenate reducing bacteria (DARB). Neidhardt et al. (2014) reported the addition of sucrose to test wells in the Bengal Delta caused an increase in Fe and As; however As solubilization was attenuated by the repartitioning of As onto the remaining or newly formed Fe(II) minerals. The type of added organic carbon also influences the rate and extent of As solubilization as it contributes to the establishment of the microbial community and biogeochemical processes that influence the extent of Fe and As reactivity (McLean et al. 2006). A link was made between inputs of organic
carbon with an increased prevalence of organisms which reduce As(V) via the arsenate respiratory reductase (arrA) gene (Lear et al. 2007). It has also been shown that native organic carbon, even in low concentrations, drives Fe, As, and sulfate reduction reactions causing As solubilization in microcosms (Mirza et al. 2014).

**Mobilization of As during biostimulation of TCE**

He et al. (2009) reported mobilization of As with the addition of molasses at a field site at Devens Reserve Forces Training Area (Devens, MA). Hering et al. (2009) discussed further the behavior of As as it became mobilized at this site and showed that the mobilization of As was limited by natural attenuation processes, with As being sequestered within 60 meters of the injection site. The dominant processes to attenuate As are precipitation, coprecipitation, and adsorption (Hering et al. 2009). Precipitation and adsorption reactions can occur at a plume boundary with the oxidation of Fe(II) and As(III) with precipitation of Fe(III) oxyhydroxides (Cozzarelli et al. 2001). The As(V) adsorbs to the newly formed Fe(III) oxides as well as to minerals containing manganese (Mn(III,IV)) (Amirbahman et al. 2006). Adsorption is dependent on the oxidation state of As and the mineralogy at specific sites. Tufano et al. (2008) noted that As adsorbed on ferrihydrite to a greater extent than on goethite and hematite. Another factor to be considered is that the release of other ligands, such as phosphate (PO$_4^{3-}$), can compete with As for re-adsorption to remaining mineral surfaces (Fendorf et al. 2010).

Although natural attenuation may remove As from groundwater released to solution with biostimulation, He et al. (2009) suggested that continual inputs of an organic carbon source would overwhelm the natural attenuation capacity of the system.
and therefore mobilize As to a greater extent. Limiting the duration of carbon addition to stay within the natural attenuation capacity of the downstream aquifer must be considered.

**Microbial TCE Dechlorination**

TCE dechlorination requires both an organic carbon source and bacteria capable of dechlorinating TCE. Both the organic carbon source and the type of microorganisms will have an effect on the rate and extent of TCE dechlorination (Bhowmik et al. 2009; Lee et al. 2011). TCE is a terminal electron acceptor (TEA) and can be partially dechlorinated to trans- or cis-dichlorethylene (DCE) and vinyl chloride (VC) and fully dechlorinated to ethene. Various microbes partially dechlorinate TCE (Löffler et al. 2000); however *Dehalococcoides mccartyi* strains (DHC) have been shown to be the only microbes that can fully dechlorinate TCE (Maymo-Gatell et al. 1997; Rahm et al. 2006).

**Studies from Hill Air Force Base**

Field and laboratory studies have been performed on sediments and groundwater at HAFB to determine steps for remediation of TCE-contaminated groundwater. Laboratory microcosm studies using various carbon sources to stimulate dechlorination of TCE contaminated aquifer solids from HAFB showed minimal TCE dechlorination with dissolution of As (Dupont et al. 2003). Addition of soluble or low solubility carbon sources led to release of As in concentrations that exceeded the drinking water limit (>10 µg/L), with over 50% of it released as As(III) (McLean et al. 2006). If HAFB desires to use carbon sources as a remediation option, they must consider the possibilities of As release. TCE dechlorination would be expected to take place after utilization of other
bioavailable TEAs, including Fe(III) and As(V) as well as sulfate (McLean et al. 2006; Wei and Finneran 2013).
CHAPTER III
ARSENIC RELEASE INTO GROUNDWATER RESULTS FROM
DECHLORINATION REMEDIATION PROCESSES OF BIOSTIMULATION AND
BIOAUGMENTATION

Abstract
Arsenic (As) contamination of groundwater has been reported world-wide. Most reported occurrences of As in groundwater in excess of the drinking water limit are from natural sources; the release of As however can be amplified by anthropogenic inputs of carbon, including additions associated with the remediation of chlorinated solvents such as trichloroethylene (TCE). Large laboratory columns (15.2 cm diameter, 183 cm long) packed with aquifer solids from a TCE contaminated site near Hill Air Force Base (UT) were fed with groundwater containing TCE and were biostimulated, bioaugmented, and monitored over 7.5 years for TCE dechlorination. This aquifer is located within the Great Basin, a basin-fill aquifer with As bearing geologic features. The objective of this study was to evaluate the biogeochemical changes that occurred over the 7.5 years of feeding the columns with either whey, Newman Zone® standard surfactant emulsified oil, or Newman Zone® nonionic surfactant emulsified oil compared with a no carbon control that affect As solubilization and reduction. The sediments and pore water were analyzed in 10.16 cm sections down each column for water and sediment quality parameters, and parameters descriptive of redox conditions and the geochemistry of As. The whey treatment showed approximately 50% of the total As in the solid phase had been leached out of the columns. The oil treatments showed a loss of 10-30% of total As. Arsenic
solubilization and reduction were associated with strongly reducing conditions developed with addition of whey leading to dissolution of even crystalline Fe oxides that released As from these minerals. Arsenic was attenuated within the oil treated columns as seen by an increase in As associated with carbonates in the lower layers of the columns. The original objective of the study was to determine the effect different carbon sources had upon dechlorination of TCE, but these analyses show that the consequence of full dechlorination of TCE is the mobilization of As in the groundwater.

Introduction

Arsenic is a known carcinogen and poses a health threat to people in regions of the world with As in their drinking water supply (Smith et al. 1992). It is becoming a growing problem as populations become increasingly more dependent on groundwater for their potable water needs (Nordstrom 2002). The concentration of As in groundwater has been related to the microbial reductive dissolution of Fe oxides (Cummings et al. 1999), host minerals for As (Drahota and Filippi 2009), and the direct reduction of As by dissimilatory arsenic reducing bacteria (Zobrist et al. 2000). Organic carbon, as a carbon source and electron acceptor, is necessary for the reductive dissolution process as its use by microbes causes the development of conditions conducive for As solubilization. These conditions may be imposed through natural processes or through human activity (He et al. 2009; Fendorf et al. 2010). Biostimulation, the addition of a carbon and energy source, and bioaugmentation, the addition of microbial inoculum with a desired metabolic capability, are commonly used technologies for the remediation of groundwater contaminated with chlorinated solvents, such as trichloroethylene (TCE) (Macbeth et al.
These imposed reducing conditions, conducive for microbial dechlorination processes leading to production of innocuous ethene, may also lead to the reductive dissolution of Fe oxides and increased concentrations of As in groundwater (Lee et al. 2005a; McLean et al. 2006; Neidhardt et al. 2014).

He et al. (2009) reported mobilization of As with the addition of molasses at a field site at Devens Reserve Forces Training Area (Devens, MA). Hering et al. (2009) discuss further the behavior of As as it became mobilized at this site. The extent of mobilization of As was limited by natural attenuation processes, with As being retained through sorption within 60 meters of the injection site. The dominant processes to attenuate As are precipitation, coprecipitation, and adsorption (Hering et al. 2009). Precipitation and adsorption reactions can be expected to occur at the plume boundary with the oxidation of Fe(II) and Mn(II) resulting in the precipitation of Fe(III) oxyhydroxides (Cozzarelli et al. 2001) and minerals containing manganese (Mn(III,IV)) (Amirbahman et al. 2006).

He et al. (2009) suggested however, that continual inputs of an organic carbon source would overwhelm the natural attenuation capacity of the system and therefore mobilize As to a greater extent. Limiting the duration of carbon addition, to stay within the natural attenuation capacity of the downstream aquifer, must be considered. Choosing the source of organic carbon and how much to add are critical decisions when attempting to control As mobilization, but may be counter to efficient dechlorination.

Hill Air Force Base (HAFB), Utah, has a long history of TCE disposal and contamination of groundwater. Laboratory microcosm studies using various carbon
sources to stimulate dechlorination of TCE contaminated aquifer solids from HAFB, showed limited production of dichloroethene, with dissolution of As (Dupont et al. 2003). Addition of soluble or low solubility carbon sources led to the release of As in concentrations that exceeded the drinking water limit (>10 µg/L), with over 50% of the released As as As(III) (McLean et al. 2006). HAFB is located on the eastern edge of the Basin and Range Province, covering Nevada and Utah. Elevated concentration of As in the geologic material is associated with volcanic activity. Similar basin-fill aquifers are found throughout the western and southwestern United States (Anning et al. 2012).

To further study the effects of carbon source on TCE dechlorination in HAFB aquifer solids, TCE contaminated aquifer solids were packed into large columns (183 cm in length and 15.2 cm diameter) and treated with various carbon sources that included whey and two formulations of a commercial emulsified oil remediation substrate, a no carbon added control, and treatments with and without bioaugmentation using a culture of known dechlorinating bacteria that were incorporated into the study design (McLean et al. 2015). Complete dechlorination was observed with the addition of whey to the columns and partial dechlorination occurred with the addition of emulsified oils; bioaugmentation accelerated the process of TCE dechlorination, but the same endpoints as observed with bioaugmentation were eventually achieved with only biostimulation for all treatments. After 7.5 years of operation these columns were dismantled and analyzed for geochemical parameters describing redox conditions, changes in Fe mineralogy, reductive dechlorination, and As chemistry and mineralogy.
The objective of this study was to determine the extent of As solubilization over the 7.5-year study, based on mass balance calculations, and observe changes in As mineral association at the end of the study. In particular, the relationship with Fe, as estimated using sequential extractions, was explored as a function of the carbon and energy source added during treatment.

**Materials and Methods**

Column construction and operations are described in McLean et al. (2015). Briefly, aquifer solids were collected by trenching into the vadose zone and saturated zone across a TCE plume from Hill AFB Operable Unit 5 (OU5) property located in Sunset, Utah. General characteristics of the aquifer solids are summarized in Table 3-1. Large, 15.2 cm diameter and 183 cm long, downward-flow-through soil columns were constructed from glass process pipe. The eight columns were packed with solids to a bulk density of 1.6 g/cm³. Site groundwater was collected as needed (every 4 to 5 weeks) during the study from the Hill AFB OU5 groundwater recovery site. The groundwater was stored at 4°C prior to use in the columns. The groundwater was slightly alkaline (pH = 7.63, total alkalinity = 276 mg CaCO₃/L, electrical conductivity, EC = 0.092 Sm⁻¹) and low in dissolved organic carbon (DOC) (DOC = 6 mg/L) and As (< 2 µg/L).
Table 3-1 Aquifer solids characterization from HAFB, OU5.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (standard units)</td>
<td>8.2</td>
</tr>
<tr>
<td>EC (dS/m)</td>
<td>0.4</td>
</tr>
<tr>
<td>Organic Carbon (%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>73</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>20</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>7</td>
</tr>
<tr>
<td>CEC (cmol/kg)</td>
<td>6.5</td>
</tr>
<tr>
<td>Total As (mg/kg)</td>
<td>2.55</td>
</tr>
<tr>
<td>Total Fe (mg/kg)</td>
<td>5420</td>
</tr>
<tr>
<td>Total Mn (mg/kg)</td>
<td>120</td>
</tr>
<tr>
<td>TCE (mg TCE/kg aquifer solid)</td>
<td>&lt;1.63</td>
</tr>
<tr>
<td>Ratio of HCl extractable Fe(II)/Fe(III) (mg/kg)</td>
<td>8/230</td>
</tr>
<tr>
<td>Microbially reducible Fe (III) (mg/kg)</td>
<td>700</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sequential Extraction</th>
<th>% of total Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe associated with exchange sites</td>
<td>0.00</td>
</tr>
<tr>
<td>Fe associated with carbonate</td>
<td>1.5</td>
</tr>
<tr>
<td>Fe associated with organic</td>
<td>1.7</td>
</tr>
<tr>
<td>Fe associated with Mn oxide</td>
<td>1.0</td>
</tr>
<tr>
<td>Amorphous Fe oxides</td>
<td>9</td>
</tr>
<tr>
<td>Crystalline Fe oxides</td>
<td>56</td>
</tr>
</tbody>
</table>

Groundwater was sparged with nitrogen before application to the columns to decrease the amount of TCE and oxygen in the water. The water was pumped into the top of the columns at approximately 9 cm/day to match field groundwater flow rates. TCE was fed continuously into each column at a concentration of approximately 10 mg/L via a split stream of TCE saturated deionized water that was mixed with site groundwater at a ratio of approximately 1:200. Whey (Gossner Cheese, Logan UT) was added to two columns (Column W and W-B) at 1,000 mg whey/L (502 mg C/L). Newman Zone® standard surfactant emulsified oil (product #1906722) was added to two columns (Column EO and EO-B) and Newman Zone® nonionic surfactant emulsified oil (product #1906730) was added to two other columns (Column EON and EON-B). Two columns
were used as No Carbon controls (Column C and C-B). Whey was added continuously via a peristaltic pump to Column W and W-B. The Newman Zone products contained 4% lactate and 46% soybean oil plus surfactants. This provided a fast (lactate) and a slow (soybean oil) release carbon source. Emulsified oils with ionic and nonionic surfactant were applied to columns EO, EO-B, EON, and EON-B as a single addition of 35 mL (13.3 g C/dose equivalent to 24,000 mg C/L pore volume) at the top of the column, and reapplied to all emulsified oil columns after 2 years of operation. After 7.4 years, Newman Zone® emulsified oil plus nonionic surfactant was reapplied to column EON-B. The columns were incubated at 16 ± 2° C, the average temperature of the OU5 aquifer.

After 2.4 years of operating the columns with biostimulation, 0.13 L (approximately 1% of the pore volume of the soil columns) of an anaerobic mixed dechlorinating culture derived from the Bachman Road culture (DBR culture) (Löffler et al. 2000), were added to one of the two columns amended with each carbon donor: Column W-B (whey), Column EO-B (emulsified oil with ionic and nonionic surfactant), Column EON-B (emulsified oil plus nonionic surfactant), and Column C-B (no carbon control).

After 7.5 years of operation, the columns were dismantled. From the top of the column, 15 cores to a depth of 10.16 cm were removed using a 2.54 cm diameter sampling tube with internal plastic sleeves. The remaining soil (approximately 58% of the total volume) from that depth was removed. The plastic sleeves were immediately capped and placed into an anaerobic glove bag under nitrogen to be processed. Triplicate cores were randomly selected from the 15 cores for analyses of As, Fe, and other
geochemical parameters. The next 10.16 cm layer was then cored with the sampling tube. The top seven layers were continuous, whereas there was a 5.08 cm gap between Layers 7, 8, 9, and 10 and a 20.3 cm gap between Layers 11 and 12.

The solids from each core were placed in a Ziploc bag and mixed to obtain a homogenous mixture. The solids were placed in two 50 mL 0.2 µm UltraClean® Maxi Plasmid Spin Filters (Mo Bio Laboratories, California) and centrifuged at 10,000 g for 15 minutes to separate out the pore water. The centrifuge tubes were returned to the glove bag where the solids from each of the two centrifuge tubes were re-combined and mixed. The pore water was also re-combined and immediately analyzed for Fe(II) using a ferrozine method (Lovley and Phillips 1986) with a Genesys 10UV-Vis spectrophotometer (Thermo Scientific) at 562 nm with a 1 cm cell. The pore water was then analyzed for pH and EC using standard procedures. Pore water was preserved with nitric acid for elemental analysis using an inductively coupled plasma mass spectrometer (ICPMS) (Agilent 7500c). Arsenic speciation was preserved with 0.25M EDTA, 5% addition (McCleskey et al. 2004). As(V) and As(III) were separated on a C-18 column with an isocratic elution with 5 mM tetrabutylammonium hydroxide, 3 mM malonic acid in 5% methanol at pH 5.8 with a liquid chromatograph (Agilent 1200 Series) and detection with ICPMS with a helium collision cell to minimize polyatomic interferences from ArCl.

Total elemental analysis in the solids was determined by ICPMS after microwave assisted acid digestion (USEPA Method 3052). The method detection limit (MDL) for As and Fe are 0.02 µg/L and 0.05 µg/L, respectively. This analysis was performed on all
layers including the “gapped” layers to determine total mass of Fe and As throughout the column at the end of the 7.5-year study. Fe and As mineralogy for the aquifer solids were characterized by chemical extraction using a modification of the sequential extraction procedure of Amacher (1998). Aquifer solids (1 g) were placed into a 50-mL centrifuge tube and the minerals extracted were operationally defined by separate extract solutions as 1) carbonates (1 M ammonium acetate for 24 hours); 2) amorphous Fe oxides (25 mL of 0.25 M hydroxylamine hydrochloride in 0.25 M hydrochloric acid for 2 hours at 50 °C); and 3) crystalline Fe oxides (10 mL of 0.3 M ammonium oxalate plus 0.3 M oxalic acid and 5 mL of 0.3 M ascorbic acid for 15 minutes in a boiling water bath and finally washed with 5 mL of 0.3 M ammonium oxalate plus 0.3 M oxalic acid and diluted to a final volume of 100 mL). The supernatant was filtered (0.45 µm) after centrifuging. To determine the mass of extracting solution left on the solids, the centrifuge tubes were weighed between each extraction step. The mass of As and Fe extracted was corrected for the mass of each element associated with this residual solution from the previous extractant. The extracts were analyzed for trace elements by ICPMS. The oxidation state of Fe was determined by extracting a 1-g portion of solids with 0.5 M HCl in the anaerobic glove bag and analyzing for Fe(II) using ferrozine (Lovley and Phillips 1986). The extract was also analyzed for total As and total Fe by ICPMS.

Acid volatile sulfides were determined with a 1 M HCl extraction (van Griethuysen et al. 2002). Aquifer solids (2 g) were weighed into a 125-mL glass jar with a teflon-lined lid and a stir bar. Ten mL of sulfide anti-oxidizing buffer (SAOB) were added to a 25-mL glass vial secured in the top of the larger glass jar. Degassed 1 M HCl
(20 mL) was injected through the septum to the soil and the jars were placed on stir plates for 3–4 hours. The SAOB solution was then analyzed for dissolved sulfide using an ion selective electrode. The soil-HCl solution in the glass jar was filtered and analyzed for Fe(II) using the ferrozine method (Lovley and Phillips 1986).

**Statistical Analysis**

An analysis of variance (ANOVA) was carried out using the JMP statistical package (SAS Institute, Inc. 5.01) to determine differences among the columns and among layers within a column for each parameter measured. Triplicate samples were collected at each layer within each column, providing sample replication not treatment replication. A Tukey’s honestly significant difference (HSD) multiple comparison analysis was performed to further explore the differences with a significant (p ≤ 0.05) ANOVA result. All HSD values were based on all columns at all depths.

Differences in As concentration in each solid’s chemical sequential extraction step were found by subtracting values measured in each treatment from those in the control column. A t-test was used to determine if the differences were statistically significant. Only statistically significant values are presented and discussed in this paper for both As and Fe for each treatment.

Correlations were performed among different pore water and solid phase parameters to determine what conditions control As chemistry. Correlations were done for each column separately and for all columns combined. As(III) and As(V) in the pore water were used as the dependent variable in the correlations. These values were log transformed before analysis. Cluster and discriminant analysis were performed to
determine how the columns would cluster with different parameters and which parameters were controlling As chemistry during the course of the experiment.

Results and Discussion

As was leached from the columns with biostimulation and bioaugmentation

The total mass of As remaining in each column after 7.5 years of operation was determined by summing the mass of As from the acid digestion of the solids in each layer (Figure 3-1). With the continuous feeding of whey, with and without augmentation (W-B, W), 54±2% of the total As was leached out of the columns. The amount of As leached was found by subtracting the mass of As in the carbon treated columns from the appropriate control column. Each carbon treated column showed leaching of As compared to the control column. There was no significant difference in leaching of As with pulse feeding of either emulsified oil, 9 ± 6% (EO) to 16 ± 2% (EON). The addition of the DBR culture enhanced As leaching for the oil treatments: 27±2% (EO-B), and 30 ± 2% (EON-B). It is interesting to note that whey was the only carbon source that led to full dechlorination during the study (McLean et al. 2015). The emulsified oils produced only partial dechlorination, with less As leaching compared with the whey treatment.
Figure 3-1. Total mass of As within the sediment of each column after 7.5 years of treatment. Error bars represent the honestly significant difference in a one-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant. ‘B’ represents bioaugmented.

Various microcosm studies with aquifer solids from Bangladesh, West Bengal, and South East Asia have shown that the addition of carbon, usually as acetate or lactate, enhances microbial reduction/dissolution of As, often associated with reductive-dissolution of the host Fe oxide minerals (Lee et al. 2005a; Rowland et al. 2007). The type of added organic carbon contributes to the establishment of the microbial community and biogeochemical processes that influence the extent of Fe and As reactivity (McLean et al. 2006). Addition of whey to microcosms with HAFB solids released twice as much As (70 µg/L) than when lactate was used as the carbon source (McLean et al. 2006), as seen in this present column study with whey versus the emulsified oils.

The added DBR culture contained *Anaeromyxobacter dehalogenans*, *Desulfobulbus sp.*, *Desulfitobacterium sp.*, *Geobacter sp.* and bacteria related to *Chlorobium sp.* as well as the dechlorinators, *Dehalococcoides mccartyi* strains (Zhou
Addition of the culture also provides known As and Fe reducing bacteria, as the arsenic reductase gene (arrA) has been identified in Geobacter sp. (Ohtsuka et al. 2013) and in Desulfitobacterium sp. (Saltikov and Newman 2003). The addition of the culture had no effect on the extent of As reactivity with whey as the carbon source as the amount of As leached is statistically the same, but the As reactivity increased with the two oil treatments (Figure 3-1) where there is a greater loss with bioaugmentation treatments.

Although the total amount of As leached from the oil columns was affected by biostimulation, other measured parameters descriptive of the biogeochemistry and As mineralogy/solution chemistry of these columns were not statistically different with stimulation. Therefore, the remaining results and discussion will be based only on differences among organic carbon treatments without bioaugmentation. The results and graphs of bioaugmented are contained in Appendix A.

**Organic carbon addition influenced geochemical conditions within the columns**

*Reducing Conditions.* Analysis of the effluent over the 7.5 years of the study demonstrated that the whey treated columns developed conditions conducive to sulfate reduction and methane production (McLean et al. 2015). The control columns remained under oxidized conditions whereas the oil treated columns were under Fe reducing conditions.

Analysis of the sediments at the end of the study confirmed reduced redox conditions as defined by the concentration of sulfide and the percent of Fe(II) in the sediments. Sulfide was detected in the top layers of all carbon-amended solids (Figure
3-2a), with the reaction extending deeper in the whey treated column. The control columns contained less than 22% of the total Fe extracted with 0.5 M HCl as Fe(II), whereas in the whey amended columns, after 7.5 years of treatment, 100% of the total Fe extracted with 0.5 M HCl throughout the profile was Fe(II) (Figure 3-2b). The two oil treatments resulted in Fe reduction throughout the profile, but not to the extent observed with whey addition. EON was considered to be “enhanced” with the nonionic surfactant that can increase the solubilization which in turn results in increased Fe(II).

The microbial reductive dissolution of the host Fe oxide is a major mechanism for As release to solution (Cummings et al. 1999; Corsini et al. 2010; Lee 2013) and conditions within all carbon treated columns were conducive to As solubilization. As observed in this study with each treatment, an increasing reducing conditions (Figure 3-2) results in an increase in As release (Figure 3-1).

![Figure 3-2. (a) Sulfide in 1 M HCl extraction and (b) Percent of total Fe in 0.5 M HCl extraction with depth. Error bars represent the honestly significant difference in a two-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant.](image)
pH. The sediment pH for all columns and depths, after 7.5 years of operation, was constant with an average value of 7.85 ± 0.11, which was a decrease from the original pH of 8.2 (Table 3-1). One of the oil treated columns, EON, showed higher variability in pH measurements (7.51 ± 0.71) than the other columns and was excluded in the pH range referenced above. The pH values were in the typical range for groundwater (pH 6.5-8.5), where As is sensitive to mobilization (Smedley and Kinniburgh 2002). The exact relationship between pH and As sorption is dependent on the type of minerals or sediment type (Dixit and Hering 2003). Because the pH values in each column are consistent in this study, any increase in As leaching cannot be exclusively attributed to a change in sediment pH.

*What happened to the As within the columns?*

Arsenic concentrations with depth. Arsenic was leached from all treated columns (Figure 3-1), but it is important to consider the fate of As with depth within each column. With the addition of any carbon source, the concentration of total As in the top 50 cm was statistically the same and 51 ± 9% less than the controls (Figure 3-3). All treated columns were sulfate reducing in the upper layers (Figure 3-2a). The columns amended with whey remained at approximately half the concentration of the controls throughout the full profile while the As concentration in the columns amended with oil increased with depth until 127 cm, where the concentration of As was statistically the same as the controls. The reactivity of As with the addition of the oil may be restricted to the upper layers of these columns, or As leached from the upper layers may have been attenuated in the deeper sediments by redistribution of the solubilized As onto mineral phases.
Figure 3-3. Total As in each column with depth. Error bar represents the honestly significant difference in a two-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant. The upper layers of the oil columns were statistically the same as the whey. At 127 cm the oil columns were statistically the same as the control columns.

As reduction, dissolution, and redistribution within the columns

Biostimulation and bioaugmentation may be effective remediation processes for chlorinated solvents if As is attenuated naturally. Natural attenuation may be observed if As is immobile under ambient conditions (Hering et al. 2009). The total concentration of As in the pore water of the control column was consistently less than 2 µg/L, with the majority of As as As(V) (Figure 3-4a). All carbon treatments led to solubilization of As (Figure 3-4b,c,d). For the two oil treated columns, the concentration of As increased with depth, whereas the maximum concentration of As in the pore water with the whey treatment was in the top, second, and third layers. The majority of As in the pore water for all carbon treatments was As(III), with the exception of As(V) dominating the upper layers of the whey treated column. This same profile was observed with the bioaugmented whey treatment (Appendix A). The increase in the proportion of As(V) in
the pore water of the second and third layers with whey treatments may indicate Fe
reduction had occurred but the solubilized As(V) concentration was not lowered in
solution in this otherwise highly reducing environment. Arsenic was reduced and
transported to the lower layers with the oil treatments and out of the column with whey
treatment.

Figure 3-4. Arsenic speciation concentration in solution phase with depth for
columns (a) Control, (b) Whey, (c) Emulsified Oil, and (d) Emulsified oil with non-
ionic surfactant.

The sequential extraction data demonstrate how the As has been re-distributed
among mineral phases with depth. It is essential to remember that the whey column has
approximately half of the total As compared with the control column (Figure 3-1) and the sequential extraction data support this with the statistically significant losses of As from crystalline and amorphous Fe oxides (Figure 3-5a). There was no evidence of As being gained in any defined mineral phase down the profile with the whey treatment. Below the first two layers, As associated with carbonates was not different than in the control. It is unlikely that the As originally associated with the carbonate minerals with depth was unaltered in this highly reactive column, but without analyses of the oxidation state of As associated with this mineral phase we do not know if the As is as As(III). The upper layers in the oil columns were similar to the whey treatment, with losses from crystalline and amorphous Fe oxides; however the losses decreased with depth and As associated with carbonate minerals increased in the lower layers (Figure 3-5b,c). This, along with the increase of As(III) in the lower layers (Figure 3-4c,d), showed that the native As was reduced throughout the entire column and natural attenuation may be occurring through the association of As with newly formed carbonate minerals in the oil treatments. Others (Cozzarelli et al. 2001; Dixit and Hering 2003; Amirbahman et al. 2006) have observed natural attenuation of As with the formation of Fe and Mn oxides as the groundwater moves into oxic sediments away from the biostimulated treatment zone. In this column study, the conditions within the 1.8 m columns remained under Fe reducing conditions. Under these conditions, and in a calcareous environment, carbonate minerals provided surfaces for natural attenuation of As, as As(III) (Smedley and Kinniburgh 2002).
Figure 3-5. Statistically significant As sequential extraction differences between columns Control and (A) Whey, (B) Emulsified Oil, and (C) Emulsified oil with non-ionic surfactant.

Change in Fe minerals

Because As is so closely associated with Fe minerals, it is also beneficial to look at the total mass of Fe remaining in the column and changes of Fe mineral association among treatments and column depth. The total mass of Fe within each column (Figure 3-6) shows that Fe was not leached out of the system to the same extent as As (Figure 3-1). For the whey treated column, a decrease in amorphous Fe oxide in the lower part of the column and crystalline Fe oxides through the column showed that reducing conditions caused dissolution of Fe minerals. There was an increase in Fe associated with the
carbonate fraction and all HCl extractable Fe was as Fe(II) (Figure 3-2b) from the potential formation of siderite (Figure 3-7), which would explain in part why the Fe was not leached out of the column like As was. The oil treated columns all showed an increase of Fe associated with carbonate minerals, with a decrease in crystalline Fe oxides in only the top layers, whereas with whey, crystalline Fe oxides were reactive throughout the column (Figure 3-7).

Figure 3-6. Total mass of Fe within the soil of each column. The error bars represent the honestly significant difference in a one-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified Oil, and EON: Emulsified Oil with non-ionic surfactant.

Bacteria are able to reduce Fe oxides through electron shuttles and do not have to come into direct contact with the Fe mineral surface to enhance the Fe mineral solubility (Nevin and Lovley 2000; McLean et al. 2006). The addition of whey, an easily fermentable carbon source producing a variety of metabolic products, some of which act as electron shuttles and chelating agents (McLean et al. 2006), would enhance Fe reduction (Figure 3-2b), including dissolution of crystalline Fe oxides and As release
(Figure 3-1). With the oil addition, the crystalline Fe oxides appear to remain non
bioavailable below 25 cm.

![Graphs showing Fe sequential extraction differences between columns.](image)

**Figure 3-7. Statistically significant Fe sequential extraction differences between
columns Control and (A) Whey, (B) Emulsified Oil, and (C) Emulsified Oil with
non-ionic surfactant.**

Statistics describing As changes among columns

Correlations were performed between soluble As(III) or As(V) and all measured
pore water and solid phase parameters to determine which parameters control As
solubilization and mobilization. Soluble As was not correlated with any descriptor of Fe
chemistry, pH, or EC. The only significant correlations were between As(III) and As(V)
in the pore water and As extracted from carbonate minerals, amorphous, and crystalline Fe oxides for the oil treated columns (Table 3-2). All of the defined solid phases are sources of solution phase As. A positive correlation was also seen in the whey column for As associated with the amorphous Fe phase for both As(III) and As(V); however solution phase As(III) and As(V) was either negatively or not correlated with carbonates and crystalline oxides (Table 3-2). The non-significant and negative correlations in the whey column indicate that these solid phases are not influencing the As concentration in the pore water.

Table 3-2 As(III) and As(V) p-values when correlated with sequential extractions. Highlighted p-values are negatively correlated.

<table>
<thead>
<tr>
<th>Correlated variables</th>
<th>Treatment</th>
<th>As(III) p-value</th>
<th>Treatment</th>
<th>As(V) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>As associated with carbonates</td>
<td>W</td>
<td>0.9721</td>
<td>W</td>
<td>0.0127</td>
</tr>
<tr>
<td></td>
<td>EO</td>
<td>&lt;.0001</td>
<td>EO</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>EON</td>
<td>&lt;.0001</td>
<td>EON</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.4649</td>
<td>C</td>
<td>0.1271</td>
</tr>
<tr>
<td>As associated with amorphous</td>
<td>W</td>
<td>0.0046</td>
<td>W</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>EO</td>
<td>&lt;.0001</td>
<td>EO</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>EON</td>
<td>&lt;.0001</td>
<td>EON</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.4813</td>
<td>C</td>
<td>0.5054</td>
</tr>
<tr>
<td>As associated with crystalline</td>
<td>W</td>
<td>0.0493</td>
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<td>&lt;.0001</td>
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<tr>
<td></td>
<td>EO</td>
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<td>EO</td>
<td>0.0003</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>C</td>
<td>0.0303</td>
<td>C</td>
<td>0.1754</td>
</tr>
</tbody>
</table>

Cluster and discriminant analysis were performed in JMP 8 for all columns, with and without bioaugmentation, to determine how the As results from the different columns cluster and what is causing the observed clustering. It was shown that the whey treated columns cluster, the control columns cluster, and the oil columns cluster separately. The
most discriminating variables were ammonium, 0.5 M HCl Fe(II), acetate, and sulfate all of which are indicators of reducing conditions. All carbon treatments led to reducing conditions conducive to As solubilization and reduction. The whey treatment led to highly reducing conditions with extensive solubilization of As, with As associated with all mineral pools, even crystalline Fe oxides.

Conclusions

The extent of TCE dechlorination and As mobilization depends on the organic carbon source. Whey treatment, with and without bioaugmentation, fully dechlorinated the applied TCE (McLean et al. 2015), and had the most As mobilized based on the mass of As leached out of the 1.83 m columns (approximately 50%), followed by the partially TCE dechlorinating bioaugmented oils (approximately 30% As mass mobilization) and the partially dechlorinating, non-bioaugmented oils (approximately 15% As mobilization).

The columns amended with oil, with and without bioaugmentation, still seem to be active throughout the entire column based on the increase in As in the lower layers of the pore water and the As associated with carbonates in the sequential extraction procedure. Attenuation is occurring only with the oil amended columns and the As is associated with carbonates instead of Fe oxides under these still reducing conditions. The role of carbonates in these semi-arid aquifer materials as sources and sinks of As has not been reported but in this study carbonates are the minerals providing sites for natural attenuation with the oil treatments. It appears that the whey treated columns have leached
all the available As as shown by no change in mass with depth and negatively correlated As in the pore water with As in the solid phases.

Adding an organic carbon source to aquifer solids can stimulate microorganisms that dechlorinate TCE; however, it may result in unintended consequences as it can cause an environmentally significant problem with the mobilization of As. With remediation processes, mitigating one pollutant in groundwater cannot lead to degrading water quality by another. Addition of carbon to these studied sediments led to As concentrations in the pore water that exceed the drinking water standard.
ARSENIC SOLUBILIZATION AND MOBILIZATION INTO GROUNDWATER
FROM DECHLORINATION REMEDIATION PROCESS OF BIOSTIMULATION

Abstract

Biostimulation and bioaugmentation have been used to promote the reductive dechlorination of trichloroethylene (TCE), but the addition of a carbon and energy source can cause arsenic (As) solubilization and mobilization to the groundwater. Small laboratory columns (7.62 cm diameter, 7.62 cm length) packed with aquifer solids from a TCE contaminated aquifer near Hill Air Force Base (UT) were treated with groundwater, a carbon source (whey, lactate, or no carbon control), and TCE. This aquifer contains As bearing minerals. The objective of this study was to evaluate the biogeochemistry development within the aquifer solids at selected sacrificing points defined by Fe reduction and progressive dechlorination determined by monitoring the column effluent over time. The sediments and pore water were analyzed at each sacrificing point for water and sediment quality parameters and parameters descriptive of redox conditions and geochemistry of As. The results showed As(V) in the column effluent for all treatments including the control (10-15 µg/L); however, with a carbon and energy source, concentrations of 20-70 µg/L As(III) greatly exceeded the drinking water limit of 10 µg/L in the column effluent. At the final sacrifice point (complete TCE dechlorination), the sediments for both the whey and lactate treated columns were highly reducing which resulted in 30% of the As in the sediment being leached out of the columns by that point in the study. These highly reducing conditions led to the dissolution of even crystalline
mineral structures releasing As from these minerals, with a greater extent of dissolution occurring within the whey treatment. Most (99%) of the produced As(III) was associated with newly formed carbonate and Fe oxide minerals, providing attenuation even within these highly reducing solids. The extent of attenuation however was not enough to lower As in the leachate to concentrations below the MCL. The addition of whey or lactate led to full dechlorination of TCE but also developed conditions for leaching of As(III) into groundwater.

Introduction

Arsenic (As) is a known carcinogen. Millions of people worldwide have been exposed to As at concentrations above the drinking water limit (10 µg/L) (USEPA 2001) due to contamination of groundwater from the local geology. Arsenic release into groundwater has been connected to the microbial reduction of iron (Fe) oxides (Cummings et al. 1999; Tufano et al. 2008) and the direct microbial reduction of As (Zobrist et al. 2000).

Fendorf et al. (2010) reviewed studies that showed these microbial reductive processes require a supply of organic carbon as a carbon and energy source. Microbes, i.e., dissimilatory iron reducing bacteria (DIRB) and dissimilatory arsenate reducing bacteria (DARB), are also required. Neidhardt et al. (2014) reported the addition of sucrose to test wells in the Bengal Delta caused an increase in Fe and As; however As solubilization was attenuated by the repartitioning of As onto the remaining or newly formed Fe minerals. The type of added organic carbon also influences the rate and extent of As solubilization as it contributes to the establishment of the microbial community and
biogeochemical processes that influence the extent of Fe and As reactivity (McLean et al. 2006). A link was made between inputs of organic carbon with an increased prevalence of organisms which reduce As(V) via the arsenate respiratory reductase (arrA) gene (Lear et al. 2007). It has also been shown that native organic carbon, even in low concentrations, drives Fe, As, and sulfate reduction reactions causing As solubilization in microcosms (Mirza et al. 2014).

The remediation processes of biostimulation (addition of an organic carbon source) and bioaugmentation (addition of microbes) are used for dechlorination of chlorinated solvents, such as for the biotransformation of trichloroethylene (TCE) to innocuous ethane (Macbeth et al. 2004; Rahm et al. 2006). However, these processes of adding organic carbon and microbes may lead to an increase in As concentration (Lee et al. 2005a; Neidhardt et al. 2014). The addition of molasses was reported to mobilize As by He et al. (2009) at a field site at Devens Reserve Forces Training Area (Devens, MA). Hering et al. (2009) showed that the mobilization of As was limited by natural attenuation processes (precipitation, coprecipitation, and adsorption) at this site.

Hill Air Force Base (HAFB), Northern Utah, has a history of TCE contamination and therefore field and laboratory studies have been performed on sediments and groundwater at HAFB to determine steps for remediation of TCE-contaminated groundwater, including biostimulation and bioaugmentation. These studies have shown increases in As concentrations (Dupont et al. 2003; McLean et al. 2006). Dupont et al. (2003) saw dissolution of As in laboratory microcosms while observing the effect of TCE dechlorination using various carbon sources on HAFB OU5 sediment. McLean et al.
(2006) showed that As concentrations exceeded the drinking water standard irrespective of the type of carbon and energy source used. McLean et al. (2015) carried out a study with large, flow through columns packed with OU5 aquifer solids which operated for 7.5 years with TCE and various carbon and energy sources (whey, Newman Zone® standard surfactant emulsified oil, and Newman Zone® nonionic surfactant emulsified oil). This study showed that over the course of this long-term study, whey leached out 50% of the mass of As in the column whereas emulsified oil only leached approximately 25%. It appeared some of the As was being attenuated with carbonates in the lower layers of the oil columns (Chapter III). This was a one-time analysis of As in the aquifer solid at the end of 7.5 years; therefore, it remains necessary to study the progression of this As loss with time based on the dechlorination of TCE.

To further analyze findings from the large column study, small bioaugmentation columns (7.62 cm length and 7.62 cm diameter) amended with TCE containing groundwater and whey or lactate as the carbon and energy source, were sacrificed over time. The objective of this study was to evaluate biogeochemistry development within the aquifer solids at selected reaction end points defined by Fe reduction and progressive dechlorination determined by monitoring the column effluent over time. Bacteria community composition and the abundance of functional genes capable of As reduction and TCE dechlorination were analyzed over time as part of the overall project, but are not discussed here.
Materials and Methods

Aquifer Solid Collection

TCE contaminated aquifer solids were collected from a property owned by HAFB in Clinton, Utah using a Geoprobe driller by direct push technology. The cores were collected between 3.05 m and 4.57 m below ground surface in 1.52 m long plastic sleeves. This was the same site where samples were collected for the large column study (Chapter III). The goal was to have the sediment approximately 0.76 m above and 0.76 m below the water table. From field observations, the sediments in the first two 1.52 m cores were completely dry and were discarded. A third core drilled another 1.52 m deeper was determined by visual inspection to be approximately half in the water table. Groundwater depth, determined using a water level meter, was 3.66 m confirming that the samples were collected from the water table zone. To have enough material for column construction, 11 cores were collected from the water table zone by moving the drill rig 0.3 m in between each core drilled. The 1.52 m cores were cut in half to fit into a cooler, capped, put on ice, and taken back to the Utah Water Research Laboratory in Logan, Utah. The sediment cores were combined, air-dried, sieved through a 2 mm sieve, and then mixed.

Aquifer Solid Properties

Aquifer solids characterization was performed using standard analytical methods by the Utah State University Analytical Lab (Table 4-1). Groundwater from HAFB is characterized in Table 4-2.
Table 4-1 Aquifer Solids Characterization

<table>
<thead>
<tr>
<th></th>
<th>OU5</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (standard units)</td>
<td>8.3</td>
</tr>
<tr>
<td>EC (dS/m)</td>
<td>1.46</td>
</tr>
<tr>
<td>Organic Carbon (%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>61</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>26</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>13</td>
</tr>
<tr>
<td>CEC (cmol/kg)</td>
<td>19.1</td>
</tr>
<tr>
<td>CaCO$_3$ (%)</td>
<td>8.7</td>
</tr>
<tr>
<td>Total Arsenic (mg/kg)</td>
<td>10.4</td>
</tr>
<tr>
<td>Total Iron (mg/kg)</td>
<td>9800</td>
</tr>
<tr>
<td>HCl extractable Fe(II)/Fe(III) (mg/kg)</td>
<td>9/400</td>
</tr>
</tbody>
</table>

Table 4-2 Groundwater Characterization

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.64</td>
</tr>
<tr>
<td>EC (ms/cm)</td>
<td>1.4</td>
</tr>
<tr>
<td>Eh (mv)</td>
<td>197</td>
</tr>
<tr>
<td>Chloride (mg/L)</td>
<td>178</td>
</tr>
<tr>
<td>Nitrate-N (mg/L)</td>
<td>7.7</td>
</tr>
<tr>
<td>Sulfate (mg/L)</td>
<td>75.0</td>
</tr>
<tr>
<td>Alkalinity (mgCaCO$_3$/L)</td>
<td>987</td>
</tr>
<tr>
<td>Dissolved Organic Carbon (mg C/L)</td>
<td>6.8</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>54.8</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>83.2</td>
</tr>
<tr>
<td>Sodium (mg/L)</td>
<td>76.4</td>
</tr>
<tr>
<td>Arsenic (µg/L)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Experimental Design**

Aquifer solids were packed into 36 glass pipe serving as the flow through columns (Prism Glass, PRG-8000-71) (7.62 cm length and 7.62 cm diameter) to a bulk density of 1.7 g/cm$^3$. The columns were treated with carbon source addition as whey (650
± 60 mg C/L), lactate (650 ± 60 mg C/L), and no carbon controls in a randomized block design. The number of columns (36) allowed for sacrificing triplicate columns per treatment at four time intervals during the study. Influent and effluent were monitored bi-weekly in triplicate. Initially, site groundwater was pumped through all the columns using multichannel peristaltic pumps at a flow rate of 0.086 mL/min to mimic groundwater flow rates at the site. The flow was upward to minimize gas entrapment and maintain flow rates. These conditions were maintained for 12 weeks. During this time a bromide trace study was conducted on each column to determine the retention time and to verify that the mass of bromide could be recovered. The average retention time measured across all 36 columns was 26.6 ± 3.9 hours, with the average mass recovery of 94 ± 16%.

Initial experimental conditions consisted of pumping site groundwater, 10 mg TCE/L plus whey or lactate through 12 columns for each treatment. A set of control columns was pumped with groundwater and TCE, but no added carbon. The feed solutions were prepared in influent bags (3L, Flex Foil®, SKC, Fullerton, California) each day. Liquid and gas effluent samples were collected at the top of each column using 250 mL glass bottles with 58 mm septa caps (Fisher Scientific, Santa Clara, California) and gas sampling bags (1L, Tedlar, SKC, Fullerton, California), respectively. The peristaltic pumps used Cole Parmer size 13 Viton tubing with Masterflex multichannel pump heads. Other 0.16 cm inner diameter tubing (Cole Parmer, Vernon Hills, Illinois) connected the pump to the bottom of each column, the top of the column to the effluent collection bottle, and the influent to the pumps. Because
TCE is volatile, the system was gas tight and all components were non-plastic (Figure 4-1).

**Influent:**
- Site Groundwater, TCE, Carbon

**Effluent Collection**
- Liquid
- Gas

**Pump to 12 columns**

![Diagram](image)

**Figure 4-1. Schematic diagram for one column within one treatment.**

**Effluent monitoring**

Effluent was weighed and discarded every day from all columns throughout the study. The effluent was analyzed twice a week from each treatment in triplicate for analytes listed in Table 4-3. These measurements provided data to evaluate progressive changes in redox conditions and geochemistry within the columns and were used to determine when to sacrifice and dismantle a set of columns. Chlorinated solvents (TCE, cis-DCE, and VC), dissolved gases (ethene, ethane, methane, carbon dioxide, carbon monoxide, and acetylene), and hydrogen analyses were all done on the effluent samples, but are not addressed here.
Table 4-3 Summary of Effluent Analyses

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Methods/Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Accumet® XL25, Method 4500-H⁺ pH value (APHA et al. 2014)</td>
</tr>
<tr>
<td>DO</td>
<td>Orion 5 Start, Thermo Scientific, Method 4500-O Oxygen (Dissolved) (APHA et al. 2014)</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>Ferrozine (Lovley and Phillips 1986)</td>
</tr>
<tr>
<td>As(III)</td>
<td>Resin separation (Wilkie and Hering 1998) with Inductively Coupled Plasma Mass Spectrometer (ICPMS)</td>
</tr>
<tr>
<td>EC</td>
<td>Accumet® model 30 conductivity meter, Method 2510 Conductivity (APHA et al. 2014)</td>
</tr>
<tr>
<td>Eh</td>
<td>Accumet® XL25, (APHA et al. 2014)</td>
</tr>
<tr>
<td>Inorganic anions/volatile organic acids (VOAs)</td>
<td>Ion chromatography (Dionex Application note 123), Dionex ICS-3000 with AS40 Autosampler</td>
</tr>
<tr>
<td>Metals/Trace Elements</td>
<td>ICPMS Agilent 7500c (EPA Method 6030)</td>
</tr>
</tbody>
</table>

Arsenic speciation was determined by separation of As(III) using a Dowex 1 x 8 anion exchange resin with analysis by ICPMS (Agilent 7500c). An aliquot of effluent (20 mL) was adjusted to pH 4 then passed (first 5 mL discarded) through the Dowex resin in the chloride form (50-100 mesh Bio-Rad) followed by analysis by ICPMS. This resin traps the As(V) allowing only As(III) to pass through it (Wilkie and Hering 1998). The resin was prepared by mixing 200 g of resin with 200 mL of 1 M NaOH for 1 hour. The resin was then filtered through Whatman No. 114 filter paper and remixed with NaOH two more times followed by rinsing with two 200 mL aliquots of DI water. The process was repeated with 200 mL of 1N acetic acid to change the resin to the acetate form with a 5 minute stirring time and rinsing with DI water. Econo-Pac® chromatography columns (Bio-Rad, Hercules, California) were packed with 2-g of resin.
Dissolved Fe(II) was determined using the ferrozine method (Lovley and Phillips 1986) with a Genesys 10 UV-Vis spectrophotometer (Thermo Scientific) at 562 nm with a 1 cm cell. Dissolved Fe (Fe(II)+Fe(III)) and As (As(III)+As(V)) were analyzed on ICPMS and Fe(III) and As(V) were determined by subtraction of Fe(II) and As(III) from the appropriate total. All sample handling was performed in an anaerobic glove bag with a 100% nitrogen atmosphere.

**Trigger Points**

Once Fe reduction was seen in several readings over one week (two total measurements of three columns per treatment), a set of nine columns was sacrificed (described below). This trigger point was selected since conditions within the columns for Fe reduction should be the point when As was being released due to microbial reductive dissolution of Fe minerals, or the direct microbial reduction of As, stimulated with carbon addition.

Monitoring of column effluent continued for the remaining columns until sulfate depletion occurred under both carbon addition treatments, at which point, 10 mL of an anaerobic mixed dechlorinating culture derived from the Bachman Road culture (DBR culture) (Löffler et al. 2000) culture were added to the columns (Day 57) using a syringe through a septum at the connection at the bottom of the column (Appendix B). This ensured that the culture was put directly into column and no oxygen was introduced. The depletion of sulfate verified that redox potential was low enough to provide optimum conditions for the anaerobes in the DBR culture to survive and continue the reduction process for dechlorination. Analysis of the effluent continued as previously discussed;
however, hydrogen analysis in the effluent gas was begun twice a week at this point in the study.

The overall project objective was to determine biogeochemical conditions conducive to TCE dechlorination whereas this subset of the project described in this paper was to observe what happened to As during microbial reduction of TCE. The remaining triggers were based on TCE dechlorination processes. Triggers were based on the Chlorine Number ($N_{Cl}$) that is defined by $N_{Cl} = \sum w_i \text{Cl}_i / \sum \text{Cl}_i$, where $w_i$ is the number of chlorine atoms in molecule $i$, and $\text{Cl}_i$ is the molar concentration of each chlorinated species. This is based on a scale of 0 to 3 where 0 represents no chlorinated species and 3 represents TCE as the only species present (McLean et al. 2015). Three triggers were used: a chlorine number of 2.5-2, 1.5-1, and 0.5-0. Once a trigger was reached from any one treatment, a set of columns was sacrificed and analyzed. Columns were dismantled nine at a time: control, whey, and lactate all in triplicate.

*Column sampling and analyses*

Before the columns were dismantled, effluent was collected into headspace vials for the analyses of dissolved gases, chlorinated solvents, and hydrogen, and into 40 mL amber glass bottles for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Sacrifice columns were placed into an anaerobic glove bag, the glass caps were removed and the solids in the columns were cored using stainless steel borers (0.95 cm in diameter). Core borers were autoclaved prior to use to avoid introducing nonnative microbes into the DNA/RNA extraction and analyses (4 cores~20 g) procedure. One additional borer was used for chlorinated solvents sampling (1 core~5 g). The five cores
were approximately 4.7% of the total column volume. TCE and microbial analyses will be reported in other papers.

The remaining aquifer solids were placed in a Ziploc bag and mixed to obtain a homogenous sample. Approximately 1 g was put onto a pre-dried and weighed aluminum boat for moisture content determination. Approximately 40 g of the mixed solids were placed in 3 50 mL capacity 0.2µm Ultra Clean® Maxi Plasmid Spin Filters (Mo Bio Laboratories, California) and centrifuged. The generated pore water from the three subsamples were recombined and analyzed for pH, Fe(II), EC, Volatile Organic Acids (VOAs: lactate, acetate, butyrate, isobutyrate, valerate, and isovalerate), macro cations and anions, trace elements, and Eh. The collected column effluent was analyzed for DOC/DIC using methods described in Table 4-3. The only change in procedure was the analysis of As(V)/As(III). Pore water was preserved for elemental analysis using nitric acid and for As speciation with 0.25M EDTA, 5% addition (McCleskey et al. 2004). As(V) and As(III) were separated on a C-18 column with an isocratic elution with 5 mM tetrabutylammonium hydroxide, 3 mM malonic acid in 5% methanol at pH 5.8 with a liquid chromatograph (Agilent 1200 Series) and detection with an inductively coupled plasma mass spectrometer (ICPMS) (Agilent 7500c). Pore water Fe (Fe(II) + Fe(III)) and As (As(III)+As(V)) were determined by ICPMS.

Acid volatile sulfide was determined with a 1 M HCl extraction (van Griethuysen et al. 2002). Aquifer solids (2-5 g) were weighed out into a 125 mL glass jar with a teflon-lined lid and a stir bar. Ten mL of sulfide anti-oxidizing buffer (SAOB) was added to a 25 mL glass vial secured in the top of the larger glass jar. Degassed 1 M HCl (20
mL) was injected through the septum to the sediment and the jars placed on stir plates for 3-4 hours. The SAOB solution was analyzed for sulfide using an ion selective electrode (Orion 9616B). Solid procedures are found in Table 4-4 whereas aqueous procedures are found in Table 4-3 with the exception of As(III)/As(V), which is described in the paragraph above.

**Table 4-4 Solid Procedures and Methods**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Digestion with trace element analysis</td>
<td>Modified from USEPA Methods 3052/3050B, ICPMS</td>
</tr>
<tr>
<td>Sequential Extractions with trace element analysis and Fe and As speciation</td>
<td>Modified from Huang and Kretzschmar (2010), ICPMS, Ferrozine analysis for Fe(II) and LC ICPMS of As speciation</td>
</tr>
<tr>
<td>Acid Volatile Sulfide</td>
<td>1 M HCl extraction (van Griethuysen et al. 2002), sulfide ISE</td>
</tr>
<tr>
<td>Oxidations state of Fe and As</td>
<td>0.5 M HCl extraction (Lovley and Phillips 1986), ferrozine and LC-ICPMS</td>
</tr>
</tbody>
</table>

Moisture content was determined for the centrifuged solids along with solid phase pH and EC. Fe and As mineralogy for the aquifer solids was characterized by sequential chemical extraction. Aquifer solids (1 g) were placed into a 50 mL centrifuge tube and the operationally defined As and Fe phases were extracted using the six-step procedure modified from Huang and Kretzschmar (2010). This procedure was developed to preserve the oxidation state of As during the extraction and analysis steps. The extractions are defined, using terminology from Huang and Kretzschmar (2010), as ligand exchangeable As (1), As associated with carbonate minerals (2), Mn oxide
minerals, acid volatile sulfides (AVS), and very amorphous Fe oxides (3), amorphous iron oxides (4), crystalline sulfides (5) and crystalline iron oxides (6) (Table 4-5). The residual phase was found using microwave assisted acid digestion with nitric acid and hydrogen peroxide (Modified USEPA Method 3052/3050B).

Table 4-5 Sequential extractions

<table>
<thead>
<tr>
<th>Target Fraction</th>
<th>Solution</th>
<th>Extract Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ligand Exchangeable</td>
<td>20 mL of 5 mM sodium phosphate monobasic + 0.2% sodium diethyldithiocarbamate trihydrate (w/v), pH 7</td>
<td>12 hours at 15 °C in anaerobic glovebag</td>
</tr>
<tr>
<td>2 Carbonates</td>
<td>20 mL of 1 M ammonium acetate, pH 5</td>
<td>24 hours at 15 °C in anaerobic glovebag</td>
</tr>
<tr>
<td>3 Mn Oxide Minerals, Acid volatile sulfides, Very Amorphous Fe (hydr)oxides</td>
<td>10 mL of 1 M hydrochloric acid + 10% acetic acid (v/v) + 50 mM mercuric chloride</td>
<td>1 hour at 15 °C in anaerobic glovebag, repeat once</td>
</tr>
<tr>
<td>4 Amorphous Fe Oxides</td>
<td>10 mL of 0.2 M ammonium-oxalate buffer (pH 3.25) + 1 mM mercuric chloride</td>
<td>2 hours in the dark at 15 °C in anaerobic glovebag, repeat once</td>
</tr>
<tr>
<td>5 Crystalline Sulfides</td>
<td>10 mL of 4 M nitric acid + 0.5% 1- Pyrrolidinecarbodiithioic acid ammonium salt (w/v)</td>
<td>1 hour in water bath (65 °C), repeat once</td>
</tr>
<tr>
<td>6 Crystalline Fe Oxides</td>
<td>10 mL hydrochloric acid + 10% acetic acid (v/v)</td>
<td>1 hour in water bath (95 °C)</td>
</tr>
</tbody>
</table>

Statistical Analyses

An analysis of variance (ANOVA) was carried out using the JMP statistical package (SAS Institute, Inc. 5.01) to determine differences among the treatments for each
parameter measured over time. Experimental replicates were measured to perform Tukey’s Honestly Significant Difference (HSD) multiple comparisons analysis to further explore the differences with a significant (p ≤ 0.05) ANOVA result.

Differences in As and Fe concentration in each solids’ chemical sequential extraction step were found by subtracting values measured in each treatment from those in the control column. A t-test was used to determine if the differences were statistically significant. Only statistically significant values are presented and discussed below for both As and Fe for each treatment over time.

**Results**

*Indicators of arsenic reactivity in effluent changes over time*

Redox conditions, as indicated by DO, nitrate-N, sulfate, Fe(II), and Eh, within the columns were significant by treatment x time using a two-way ANOVA. The DO values were at or below 1 mg/L within the first week and remained low for the remainder of the study (Figure 4-2a). Nitrate-N was depleted (MDL = 0.5 mg/L) in both carbon treatments within 2 weeks (Figure 4-2b) and dissolved Fe(II) (MDL = 12 µg/L) was quantifiable by Day 20 and Day 23 for whey and lactate treatments, respectively (Figure 4-2c). Sulfate was reduced by 75% by Day 31 for lactate and Day 38 for whey (Figure 4-2d). These redox sensitive parameters remained relatively unchanged from the initial conditions in the control columns. The Eh values for lactate and whey treated columns decreased to below 0 mV by Day 23 and remained between 0 and -150 mV through the duration of the study. The control column Eh values fluctuated between +80 and +220 mV (Figure 4-2e). The addition of a carbon and energy source was necessary to drive the
system reducing. The carbon treated columns were under sulfate reducing conditions by the first sacrifice time (Day 29).

The dramatic decrease and later increase of DO, NO₃-N, and Eh in the control treatment at Day 58 corresponds to the day after the DBR culture was added to each column. This shows that no DO was added when this was performed, but that some lactic acid or residual organic carbon from the biomass in the culture was added with culture addition and was quickly utilized before effluent conditions returned to pre-culture amendment conditions.

The pH of the effluent from the control columns (7.7 ± 0.1) was statistically higher than in the lactate treatment (6.7 ± 0.1), which was higher than in the whey treated columns (6.2 ± 0.2) over the duration of the study. Although the pH decreased by at least one pH unit with the addition of carbon compared with the control, this pH change would not cause the extensive dissolution of mineral associated As that was observed in the carbon amended treatment. However, the sorption of As is controlled by the pH of the system with an increase in As sorption at lower pH (Dixit and Hering 2003).

The type of organic carbon added influences the extent of TCE dechlorination (Bhowmik et al. 2009; Lee et al. 2011; McLean et al. 2015) and As solubilization and mobilization (Chapter III). The control treatment had minimal organic carbon in the effluent (<40 mg/L) throughout the study except at Day 58 when it peaked at 106 mg/L, which was the day after DBR was added (Figure 4-3a). Within the lactate treated columns, lactate concentrations in the effluent were equivalent to the influent concentrations at Day 1 and Day 6 at 650 mg/L, decreased to 240 mg/L at Day 10, and by
Day 15 the added lactate was completely depleted and remained below the MDL (0.4 mg/L) for the remainder of the study. There were high concentrations of VOAs within the whey treatment for the first two sampling points; however, the peaks were too large to quantify and dilutions were not performed so the data are not reported here. At Day 15 and throughout the duration of the study, acetate, propionate, and butyrate had the highest concentrations of all the measured VOAs for the lactate and whey treated columns in the effluent (Figure 4-3b, c, d). Some butyrate was produced within the lactate treatment, but remained below 10 mg/L, a low concentration in comparison to the concentrations of acetate and propionate. Within the whey treatment, there was a downward trend for acetate and butyrate over time. The utilization of the carbon sources is occurring for both whey and lactate treatments as the lactate is being converted to acetate and propionate for the lactate treatment and to acetate and butyrate for the whey treatment. Bacteria that use Fe and/or As as a terminal electron acceptor couple this reaction with utilization of a number of organic substrates as the electron donor, with acetate being commonly reported (Löffler et al. 2000; He et al. 2007). Dissimilatory iron reducing bacteria (DIRB) are commonly observed to utilize acetate as an electron donor, but butyrate and propionate have also been shown to support iron reduction for some DIRB (Lovley 2006).
Figure 4-2. Reducing conditions of columns based on (a) DO, (b) NO3-N, (c) Fe(II), (d) SO4, and (e) Eh. Error bars represent the 95% Confidence Interval. C: no carbon addition, W: Whey (650 mg C/L), and L: Lactate (650 mg C/L). Black vertical lines represent sacrifice periods when triplicate columns were dismantled for each treatment (Day 29, 79, 121, and 161). Derived Bachman Road culture added at Day 57.
Figure 4-3. Effluent concentrations of (a) Dissolved Organic Carbon (DOC), (b) Acetate, (c) Propionate, and (d) Butyrate. C: no carbon addition, W: Whey (650 mg C/L), L: Lactate (650 mg C/L). Concentrations of VOAs for the control were all <0.1 mg/L. Error bars represent the 95% confidence Interval. Black vertical lines represent sacrifice periods when triplicate columns were dismantled for each treatment (day 29, 79, 121, and 161).

As(III) is more mobile and toxic than As(V) (Smedley and Kinniburgh 2002). Both As(III) and As(V) in the effluent were statistically significant with treatment x time in a two-way ANOVA. Arsenic was detected in the effluent at the first sampling point as As(V) (Figure 4-4a) for all treatments. A mere 5 days later, an increase in As(III) was seen in the whey and lactate treated columns whereas As in the effluent of the control
column remained as As(V) (Figure 4-4b). Arsenic, as As(V), was solubilized in all
columns, but reduction to As(III) only occurred with the addition of a carbon and energy
source. The As(V) concentration over time was affected by treatment with the control at
13 ± 1 µg/L, whey at 11 ± 2 µg/L, and lactate at 5 ± 1 µg/L. The As(III) in the effluent
was statistically higher on average over time in the whey treatment (31 ± 4 µg/L) than the
lactate treatment (18 ± 2 µg/L). The increase of As(III) concentration in the control at
Day 58 again corresponds to the day after the DBR culture was added where some
organic acid was added and utilized by the microbes as seen also with a decrease in DO
and NO$_3$-N (Figure 4-1). Large error bars are due to the high variance among the sampled
columns. At the end of the study, the effluent from the same three columns treated with
whey were analyzed several times. One of these columns consistently had lower
concentrations of As(III) than the other two columns.

![Figure 4-4](image)

**Figure 4-4.** Effluent concentrations for (a) As(V) and (b) As(III). Error bars
represent the 95% confidence interval. C: no carbon addition, W: Whey (650 mg
C/L), and L: Lactate (650 mg C/L). Black vertical lines represent sacrifice periods
when triplicate columns were dismantled for each treatment (day 29, 79, 121, and
161).
Changes within the solid over time

Columns were sacrificed at Day 29 based on reduction of Fe in the effluent and at Days 79, 121, and 161 based on the chlorine number (2.5-2, 1.5-1 and 0.5-0, respectively).

pH. The pH of the solids at Sacrifice 1 was 8.6 ± 0.1 for all treatments. At the end of the study, the mean pH of the control treatment was slightly higher with a pH of 8.4 ± 0.02, than the lactate and whey treatments with a pH of 8.1 ± 0.02. The pH was lower with the carbon treatments as carbon dioxide was being produced.

Total As Mass and Changes in Mineral Phases. The total mass of As leached from the columns was estimated from the concentration of As in the effluent and the total volume collected between sacrifice times. Unfortunately, this estimate is low since only dissolved As was analyzed in the effluent. The main interest was the analysis of As(III) and As(V) in the effluent to observe changes in redox with treatments, so total digestions were not routinely performed. It was determined however at one sampling interval that three times the As was associated with the visible Fe precipitated in the collection bottles than was determined for dissolved As. There was also potential precipitation with FeS in the sand layer based on the black colors in the sand at the end of the study. A mass balance therefore cannot be reported with the amount of As in the effluent.

The total mass of As in the solid was significant with treatment x time. No change in total As was seen in the control columns over time (Figure 4-5). Thirty percent of the total As was leached from the whey column by Sacrifice 3, and from the lactate column by Sacrifice 4. Greater removal of As was seen in the large column study (Chapter III.
Figure 3-3) with over 50% loss in the whey and oil treated columns in the top layer, but this increase As leaching occurred over a total of 7.5 years.

![Graph showing As concentrations over time for different treatments.](image)

**Figure 4-5. Total As for each treatment over time.** C: no carbon control, W: Whey (650 mg C/L), L: Lactate (650 mg C/L). Levels not connected by the same letter are significantly different by Tukey Honestly Significant Difference two-way ANOVA (n=3).

Arsenic mobilization and solubilization are affected by Fe reduction (Cummings et al. 1999; Amirbahman et al. 2006). Biogenic Fe(II) is defined as the amount of reduced Fe observed in the pore water and extracted with 0.5M HCl. The initial soil contained 9 mg/kg 0.5M HCl extractable Fe(II). The control columns showed no formation of biogenic Fe(II) since the concentration of HCl extractable Fe(II) remained the same as the initial condition. There was no statistical difference with treatment for biogenic Fe(II) and therefore only the difference in sacrifice number is shown. Extensive reduction of Fe was promoted with the whey and lactate treatments with the biogenic Fe(II) increasing with time (Figure 4-6a). The percent of Fe(II) associated with the aqueous phase was less
than 0.1% for lactate and whey treatments throughout the study. The contribution of aqueous phase Fe(II) at any sacrifice time to the biogenic Fe(II) was therefore insignificant. Use of aqueous phase Fe(II) as the only indicator of Fe reduction can be misleading as the amount of Fe(II) sorbed/precipitated is not quantified (Meng 2015). Monitoring the effluent for Fe(II) was only an estimate of the extent of Fe reduction observed in the columns over time.

The pore water concentrations of As(III) in the lactate and whey treatments were detectable but were not statistically different from the control at Sacrifice 1. However, by Sacrifice 2, the whey treatment resulted in 117 µg/L As(III) and by Sacrifice 4, significantly higher concentrations of As(III) were observed in both the whey (190 µg/L) and lactate (180 µg/L) treatments (Figure 4-6b). There was no statistical difference with treatment for As(V) in the solution phase and therefore only the difference in sacrifice number is shown, with an increase in As(V) in pore water by Sacrifice 2 (Figure 4-6c).

Time and treatment had an effect on the concentration of As(III) in the HCl extraction. The lactate and whey treatment developed conditions with the sediment columns that was conducive for As(III) sorption or co-precipitation with HCl soluble minerals relative to the control treatment (Figure 4-6d). An increase in As(III) in the HCl extraction was seen by Sacrifice 2 but the concentration remained steady through the rest of the study (Figure 4-6e). As with biogenic Fe(II), 99% of the As(III) generated was associated with the solid phase. The aqueous and HCl extractable phases concentrations for As and Fe (Figure 4-6) were statistically the same for Sacrifice 3 and 4.
Figure 4-6. Fe reduction and As solubilization. (a) Biogenic Fe(II) (HCl extractable Fe(II)+aqueous phase Fe(II)) (sacrifice number significant), (b) As(III) in solution phase, (c) As(V) in solution phase (sacrifice number significant), (d) As(III) in the 0.5 M HCl extractable phase (treatment significant), and (e) As(III) in the 0.5 M HCl extractable phase (sacrifice number significant) C: no carbon control, W: Whey (650 mg C/L), L: Lactate (650 mg/L). Levels not connected by the same letter are significantly different by Tukey Honestly Significant Difference in a two-way ANOVA.

Addition of lactate to the columns increased the concentration of As(III) associated with surfaces through ligand exchange (1), and associated with carbonates (2) and other highly soluble minerals (Mn oxides, AVS, and very amorphous Fe oxides, 3) (Figure 4-7a) by Sacrifice 1. As(III) associated with these phases increased with time. For
the first three sacrifice times, the As(III) was associated with minerals extracted in 3,
which included very amorphous Fe oxides, Mn oxides, and AVS. There was no evidence
of the formation of AVS, as determined by a separate 1 M HCl extraction with analysis
of S²⁻ until Sacrifice 4, with a concentration of 220 mg sulfide/kg. Arsenic was associated
with newly formed sulfide minerals as evident by the increasing black coloration of the
sediments with time. For the first three extraction steps, the As(III) was therefore
associated with very amorphous Fe oxides and Mn oxides. There was a corresponding
decrease in As(V) to As(III) associated with soluble minerals (3) at all sampling times.
As(V) was solubilized from crystalline Fe oxides (6) by Sacrifice 3 and crystalline
sulfides by Sacrifice 4. There was 1400 mg/kg Fe released from the extractant for
amorphous Fe oxide (4) at Sacrifice 4. The produced Fe(II) was associated with
carbonates, as siderite, and as newly formed Fe(II) oxides (Figure 4-7b). These newly
formed surfaces provided additional retention sites for As(III) (Figure 4-7a).

The whey treatment enhanced As reduction with the sorption of As(III) to ligand
exchange sites (1) and association with carbonate minerals (2) by Sacrifice 1 (Figure
4-8a). Contrasting to the lactate treatment, carbonate associated As(III) dominated at the
second and third sampling intervals, but As(III) associated with very amorphous Fe
oxides and Mn oxides (3) at Sacrifice 4. Acid volatile sulfides had formed, as determined
by the independent extraction and analysis of S²⁻, by Sacrifice 3 and 4. As(III) may be
associated with these newly formed sulfide minerals. With the addition of whey, there
was 2,400 mg/kg Fe released from the extractant for crystalline sulfides (5) at Sacrifice 3
and 1,400 mg/kg and 3,300 mg/kg from the amorphous Fe oxides (4) and crystalline
sulfides (5), respectively (Figure 4-8b). As(V) was also released from associations with amorphous and crystalline Fe oxides and crystalline sulfides. After 161 days of column operation, the distribution of As(III) and As(V) is still dynamic.

Figure 4-7. Statistically significant (student t-test) sequential extraction differences between the Control and Lactate for (a) As and (b) Fe. Extraction Number 1: Ligand exchangeable As, 2: As associated with carbonates, 3: As associated with Mn oxides, AVS, very amorphous Fe oxides, 4: As associated with Amorphous Fe oxides, 5: As associated with Crystalline sulfides, 6: As associated with Crystalline Fe oxides.
Figure 4-8. Statistically significant (student t-test) sequential extraction differences between the Control and Whey for (a) As and (b) Fe. Extraction Number 1: Ligand exchangeable As, 2: As associated with carbonates, 3: As associated with Mn oxides, AVS, very amorphous Fe oxides, 4: As associated with Amorphous Fe oxides, 5: As associated with Crystalline sulfides, 6: As associated with Crystalline Fe oxides.

Discussion

The addition of organic carbon causes reducing conditions which have been shown to have an effect on As solubilization and mobilization (Chapter III, Fendorf et al. 2010). The addition of both whey and lactate caused reducing conditions within the columns based on the effluent and solid extractions including: the DO being below 1 mg/L, nitrate depletion, sulfate reduction, Eh values below 0 mV, and increases in
biogenic Fe(II). The dosing of carbon in this study was at higher concentrations than used in the large column study (Chapter III). Also, the two carbon sources used here are water-soluble and were continuously fed. In the large column study, the emulsified oils, with low water solubility were used in multiple batch dosings. With the large columns, whey produced highly reducing conditions for complete TCE dechlorination and extensive solubilization and reduction of As, as observed in these small columns with the addition of whey or lactate. The oils in the large column study provided high concentrations of carbon at the column surface, dosing that was equivalent for use in a reactive barrier. The periodic dosing of the oils was not sufficient to support the biogeochemistry necessary for full dechlorination. This lower bioreactivity however, resulted in lower release of As from the columns compared with the continuous addition of whey. We chose to use lactate with the small columns. Lactate is a component of the emulsified oils, providing a fast release carbon source. High dosing and continuous feeding of lactate, as used in this present study, led to similarly reducing conditions as with whey with similar As reactivity. Carbon was not limited with the whey or lactate treatments. Both carbon sources produced highly reducing conditions with 90% of the pore water As reduced to the more mobile and more toxic As(III) which exceeded the MCL. Most of the reduced As, however, was retained on existing or newly formed mineral phases.

The influent concentration of As was 2.4 µg/L and throughout this study, a continuous presence of As(V) was seen in the effluent for the control treatment at concentrations within the range of 10-15 µg/L, values above the MCL. Similar amounts were seen with the whey and lactate treatments over time. Without a carbon source, only
the solubilization of As(V) occurs and only to a limited extent. An added carbon source was needed for reduction of As and increased As solubilization to occur.

Sacrifice 1 was selected to evaluate the geochemistry of As when Fe was in the beginning stages of reduction. Arsenic reduction was evident from the concentration of As(III) in the effluent and by the increase in As(III) associated with ligand exchangeable and carbonates for both lactate and whey treatments, and highly soluble minerals for the lactate treatment. Therefore, As reduction was beginning to occur, along with Fe reduction. The As(V) being reduced was supplied from surface exchange sites and associated with carbonate and very amorphous Fe minerals.

Sacrifice 2 was selected to evaluate the biogeochemical characteristics when TCE had been dechlorinated to dichloroethylene for both whey and lactate treatments. Arsenic continued to be reduced with an increase in As(III) concentrations associated with the ligand exchange, carbonates, and highly soluble minerals for both the whey and lactate treated columns, producing increased pore water concentrations in both carbon treatments. These data illustrated that As reduction continued from the first sacrifice.

Sacrifice 3 was used to evaluate the biogeochemical characteristics when TCE had been dechlorinated to vinyl chloride. As(III) concentrations increased with the first three sequential extraction steps for both the lactate and whey treated columns, with now discernable loss of As(V) from amorphous Fe oxides, crystalline sulfides, and crystalline Fe oxides for the whey treated columns and from crystalline sulfide and crystalline Fe oxides for the lactate treated columns. The Sacrifice 3 data show that As reduction continued and As was being mobilized. Although some of the reduced As was mobilized
into the pore water by Sacrifice 3, 99% remained associated with newly formed solid phases. There was 30% loss of As from the columns, but the association of As with minerals minimized the full impact of As leaching from the columns.

Sacrifice 4 evaluated the sediment once TCE dechlorination was nearly complete (ethene production) for both lactate and whey treatments. For both whey and lactate treatments, 30% of the As originally in the sediment had leached out of the columns. The dissolution/desorption of As(V) continued to be associated with soluble minerals, carbonates, and amorphous Fe oxides as with other sacrifice periods, and also from minerals that are considered insoluble, crystalline Fe sulfides and crystalline Fe oxides. With the addition of carbon and energy sources such as the emulsified oils used in Chapter III or acetate (McLean et al. 2006), crystalline Fe minerals remain insoluble since microbes cannot access the Fe(III) in the mineral structure, but these mineral phases are accessible with the addition of whey or lactate as the carbon and energy sources.

The addition of whey or lactate led to highly reducing conditions and extensive biological activity generating a biochemically rich environment and supporting a diverse microbial community that utilized chelating agents and electron shuttles resulting in dissolution of crystalline Fe(III) minerals. Researchers have suggested competition among microbes for the utilization of different terminal electron acceptors (McLean et al. 2006; Shani et al. 2013). In these columns, such competition was eliminated by the high dosing of soluble carbon and energy sources and the addition of the DBR culture. This culture was added to provide known dechlorinating bacteria, but the culture also contains DIRB and DARB (Löffler et al. 2000; McLean et al. 2015). The use of a less readily
bioavailable carbon source and no added bacteria would result in less Fe reduction and As solubilization and limited rate of TCE degradation, as seen with the emulsified oil addition in the large column study (Chapter III). Addition of glucose to microcosm studies using sediment from another site in northern Utah again caused extensive dissolution of Fe(III) crystalline minerals with the released and reduction of As. Without added carbon, the native microbes utilized the native organic carbon resulting in dissolution of As from carbonate minerals not Fe oxides, a reaction that was masked by the aggressive dissolution of Fe(III) minerals with the added carbon (Meng 2015).

**Conclusions**

This study was conducted to determine how As is solubilized and mobilized when organic carbon is added as a remediation process for TCE. The comparison between whey and lactate showed different VOAs in the effluent, although both produced acetate. Microbial community response to the addition of whey versus lactate and the development of DIRB and DARB are being investigated as another part of this overall project. Regardless, the Fe reduction and As biogeochemistry were similar with the addition of these bioavailable carbon sources.

The continuous effluent concentrations of As(V) (10-15 µg/L) in all treatments, including the control, indicates that the dissolution/desorption of As(V) was occurring even without an exogenous carbon source via abiotic processes. With a carbon source, the more mobile and toxic As(III) is in concentrations that exceed the MCL by as much as six times.
Slight differences were seen with the whey and lactate treated columns in the extent of As(III) associated with mineral phases, but ligand exchangeable As(III) and As(III) associated with carbonates and highly soluble minerals accounted for all of the retention of As(III) in both treatments. The addition of whey led to dissolution and reduction of As(V) from more insoluble mineral phases than was observed in the lactate treatment. The high amount of carbon added to the columns resulted in the biogenic dissolution of even crystalline structures in both treatments resulting in some sorption and precipitation of As(III) to existing or newly formed soluble minerals, but there were not enough sorption sites to attenuate all of the reduced As.

Arsenic was transported out of the columns over time. With the whey treatment, a net loss of As was seen by Sacrifice 3 whereas the loss of As in the lactate treatment did not occur until Sacrifice 4. However, the same extent of As loss (30%) was seen for both the whey and lactate treatments by the end of the study suggesting that the type of carbon source in this case is not significant in regards to the end result of As mobilization and solubilization. This is most likely due to the large amount of continuous carbon overwhelming the system. If biostimulation as a remediation technique for TCE is desired, care should be taken to avoid solubilizing and mobilizing As regardless of the type of organic carbon being utilized to drive the biogeochemistry.
CHAPTER V
SUMMARY AND CONCLUSIONS

Two different column studies were performed that showed As mobilization and solubilization can occur as a result of the remediation processes of biostimulation and bioaugmentation for the intended dechlorination of TCE. The first study used large (15.2 cm diameter, 183 cm long) flow through columns, with the evaluation of As and Fe biogeochemistry in the solids at the end of 7.5 years. The second study monitored the effluent of small (7.62 cm diameter, 7.62 cm length) flow through columns over a 161 day period, with columns being sacrificed in triplicate to evaluate changes in As and Fe geochemistry within the solid phase at four time intervals (29, 79, 121, and 161 days) throughout the study.

The large column study was designed to observe how different carbon sources (whey, Newman Zone® standard surfactant emulsified oil, and Newman Zone® nonionic surfactant emulsified oil) affected the dechlorination of TCE. Columns were also bioaugmented with a culture containing known dechlorinating bacteria to assist in the dechlorination process. When the columns were dismantled, after 7.5 years of operation, the As and Fe were analyzed within different mineral phases using sequential extractions. Over 50\% of the As in the original sediment leached out of the columns treated with whey, regardless of whether the column was bioaugmented or not. Treatment with the emulsified oils resulted in a 10-30\% loss of As from the columns. A greater loss of As was seen with the bioaugmented columns compared to the non-augmented columns with the oil treatment. Arsenic associated with the solid phase was depleted throughout the
length of the whey treated columns, whereas depletion of As was observed only over the
top 60 cm of the oil treated columns, with As accumulating in the lower layers that was
associated with carbonate minerals. Addition of whey led to reducing conditions through
the column for reduction and mobilization of As out of the columns, whereas reduction
only occurred in the upper layers of the oil treated columns, with attenuation of the
produced As by carbonate minerals with depth.

The small column study was designed and implemented after the large column
study and was designed to determine the geochemical conditions that occurred at
progressive reducing and dechlorinating steps. Lactate and whey were used as the carbon
sources. Column effluent was measured for redox conditions, TCE, and TCE byproducts
to determine when columns should be sacrificed over time. Arsenic was seen in the
effluent in the control column between 10 and 15 µg/L as As(V) and in higher
concentrations (10-70 µg/L) in the carbon treated columns, mainly as As(III), which
corresponds with the Fe reduction. As(III) precipitated or sorbed onto existing or newly
formed minerals and the formation of AsS minerals occurred as the system went more
reducing, providing retention of As(III) within the columns. Even with some attenuation,
30% of the As in the sediment was leached out of the columns in the whey treatment by
Sacrifice 3 and 4 and with the lactate treatment by Sacrifice 4. This is less than the large
column study, which had a 50% loss of As with whey and oil columns in the top layer.
This greater loss occurred over a greater amount of time (2740 days) than the 161 day
small column study.
The use of oil in the large column study showed only partial dechlorination and limited bioavailability of Fe and As. With the small columns, both carbon sources led to the same end point. Whey seems to provide an environment where more dissolution of the crystalline minerals occurs, but there was no difference between whey and lactate overall in terms of the extent of As dissolution and mobilization over this 161 day column study.

Both of these studies showed that the reducing conditions caused by the carbon source additions triggered As reduction even to the extent of leaching out of columns, but there is a possibility that As can be retained onto various mineral phases, including carbonate minerals as it moves away from these highly reduced zones. The attenuation of As was demonstrated in the large column with the oil treatments. The active dechlorinating zone was at a depth of 10.16 cm. Arsenic was also solubilized within this zone but accumulated with depth, associating with carbonate minerals. These lower layers of the oil treated columns were under Fe reducing conditions. Research on natural attenuation of As at field biostimulated and bioaugmented sites for TCE remediation have reported the natural attenuation of As at the oxidized edge of the plumes, with the oxidation of Fe and Mn forming oxides that provide surfaces for As retention in these oxidized plume margins. This study also shows retention of As within a reduction zone by carbonate minerals and newly precipitated Fe(II) carbonates.

The selection of the type of carbon and energy source is important to both TCE dechlorination and As mobility as the full dechlorination of TCE also resulted in the maximum amount of As reduction and mobility. With less readily bioavailable carbon
addition (less fermentable) or lower carbon dosing, however, less As is solubilized and has potential retention at some distance away from the source. Under these conditions, however, TCE dechlorination is expected to be limited.

There are a number of military installations in the western and southwestern US that are in need of TCE groundwater remediation. These regions, however, are also associated with basin-fill aquifers that contain naturally high levels of As in the geologic deposits due to ancient volcanic activity (Anning et al. 2012). Remediation strategies for dechlorination of TCE that involve biostimulation and bioaugmentation could have an impact on water quality with the release of As into groundwater under these imposed reducing conditions. The best way to have full TCE dechlorination and not have As contamination would be to determine ways to attenuate the As under these reducing conditions.
CHAPTER VI
ENGINEERING SIGNIFICANCE

The outcome of these overall projects was the identification of a set of conditions, including both the microbial community and the geochemistry, that are necessary for full dechlorination of TCE. This knowledge provides engineers the opportunity to target remediation of TCE from HAFB and other locations based on microbial and geochemical conditions at a site. However, the imposed anaerobic conditions necessary for dechlorination of TCE may also cause As release. Results from this study contribute to the understanding of the biogeochemical conditions that lead to As solubilization, mobilization, and potential As attenuation under biostimulation and bioaugmentation conditions.

Arsenic solubilization, mobilization, and attenuation in aquifer solids is a complex problem dependent on many factors including oxidation-reduction processes, Fe mineralogy, pH, sulfate, phosphate, and the microbial community. Knowing the initial conditions of a site will allow engineers to determine if As release will occur, and potentially how to mitigate this enhanced mobility through subsequent mineral attenuation.

Arsenic oxidation state and mineral association changes occurring under the imposed reducing conditions will help determine the processes under which As is solubilized and mobilized. This study also observed how the As and Fe solid phases changed with depth and over time which provides insight for engineers into the factors
that should be monitored and the potential for natural attenuation to occur with existing or newly formed mineral phases with an emphasis on carbonate minerals.

Overall, engineers will use data from these studies to properly identify if As release will occur with biostimulation and bioaugmentation to remediate TCE, which variables are most important to monitor, and what can be done to help with the attenuation of As. It will also have implications where organic carbon is added, intentionally (biostimulation) or inadvertently, to soils or sediments such as through irrigation or from release of landfill leachate to adjacent, vulnerable aquifers.
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APPENDIX A Bioaugmentation Graphs
Figure A-1. (a) Sulfide in 1 M HCl extraction and (b) Percent of total Fe in 0.5 M HCl extraction with depth. Error bars represent the honestly significant difference in a two-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant. ‘B’ represents bioaugmentation.

Figure A-2. Total As in each column with depth. Error bar represents the honestly significant difference in a two-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant. ‘B’ represents bioaugmentation.
Figure A-3. Arsenic speciation concentration in solution phase with depth for bioaugmented columns (a) Control, (b) Whey, (c) Emulsified Oil, and (d) Enhanced Emulsified Oil.
Figure A-4. Statistically significant As sequential extraction differences between bioaugmented columns Control and (A) Whey, (B) Emulsified Oil, and (C) Enhanced Emulsified Oil
Figure A-5. Total mass of Fe within the soil of each column. Error bars represent the honestly significant difference in a two-way ANOVA with n=3. C: Control, W: Whey, EO: Emulsified oil, EON: Emulsified oil with nonionic surfactant. ‘B’ represents bioaugmentation.
Figure A-6. Statistically significant Fe sequential extraction differences between bioaugmented columns Control and (A) Whey, (B) Emulsified Oil, and (C) Enhanced Emulsified Oil.
Table A-1 As(III) and As(V) p-values when correlated with sequential extractions. Highlighted p-values are negatively correlated.

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<th>Treatment</th>
<th>As(V) p-value</th>
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APPENDIX B Bioaugmentation Addition Photos for Small Columns
Figure B-1. Photos of the process of the addition of the Derived Bachman Road culture using syringe and adding through the septum below column.