Trade-offs in Utilizing of Zero-Valent Iron for Synergistic Biotic and Abiotic
Reduction of Trichloroethene and Perchlorate in Soil and Groundwater

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved July 2017 by the
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ARIZONA STATE UNIVERSITY
August 2017
ABSTRACT

The advantages and challenges of combining zero-valent iron (ZVI) and microbial reduction of trichloroethene (TCE) and perchlorate (ClO$_4^-$) in contaminated soil and groundwater are not well understood. The objective of this work was to identify the benefits and limitations of simultaneous application of ZVI and bioaugmentation for detoxification of TCE and ClO$_4^-$ using conditions relevant to a specific contaminated site. We studied conditions representing a ZVI-injection zone and a downstream zone influenced Fe (II) produced, for simultaneous ZVI and microbial reductive dechlorination applications using bench scale semi-batch microcosm experiments. 16.5 g L$^{-1}$ ZVI effectively reduced TCE to ethene and ethane but ClO$_4^-$ was barely reduced. Microbial reductive dechlorination was limited by both ZVI as well as Fe (II) derived from oxidation of ZVI. In the case of TCE, rapid abiotic TCE reduction made the TCE unavailable for the dechlorinating bacteria. In the case of perchlorate, ZVI inhibited the indigenous perchlorate-reducing bacteria present in the soil and groundwater. Further, H$_2$ generated by ZVI reactions stimulated competing microbial processes like sulfate reduction and methanogenesis. In the microcosms representing the ZVI downstream zone (Fe (II) only), we detected accumulation of cis-dichloroethene (cis-DCE) and vinyl chloride (VC) after 56 days. Some ethene also formed under these conditions. In the absence of ZVI or Fe (II), we detected complete TCE dechlorination to ethene and faster rates of ClO$_4^-$ reduction. The results illustrate potential limitations of combining ZVI with microbial reduction of chlorinated compounds and show the potential that each technology has when applied separately.
ACKNOWLEDGMENTS

Special thanks to Dr. Rosa Krajmalnik-Brown, who trusted me and gave me the opportunity to work on this research project with no prior experience in soil and groundwater remediation. As my advisor and mentor, she guided me and supported me constantly in my ups and downs. Thanks for inspiring me and always making sure I felt comfortable and happy working in the lab. I would like to convey my heartfelt thanks to my mentor, guide and well-wisher Dr. Anca Delgado. This would not have been possible without her exceptional support, constant motivation and training right from the day I started working in lab. Sincere thanks to Dr. Greg Lowry and Dr. Laurie Lapat-Polasko for providing timely valuable help and support.

I would like thank all my friends and co-workers in the Swette Center, who helped me professionally as well as personally and made my work place a memorable and happy one. I also sincerely thank the Center for Bio-mediated & Bio-inspired Geotechnics (CBBG) for providing an amazing platform to perform the research work. I have to acknowledge Aatikah Mouti, very dedicated intern for being very helpful in the project. Thanks to Carole Flores for being the sweetest person in the Swette Center and making the work place comfortable.

I would not have been here without the love and support from my family. Thanks for all the motivation and encouragement to pursue this master’s degree. I would like to dedicate this thesis for my family.

This research work is supported by Matrix New World Engineering Inc.
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<td>17</td>
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Trichloroethene (TCE) is a chlorinated solvent that has been extensively used as industrial solvent, degreasing agent for mechanical parts (especially in aircraft engines), and intermediate in the manufacture of several other chemicals. Increased and improper handling of TCE has led to spills and extensive contamination of soil and groundwater. TCE is highly toxic and carcinogenic (ATSDR). TCE is often found with one or more organic and inorganic contaminants. Perchlorate (ClO$_4^-$) is one of the common inorganic contaminants and a chemical oxyanion that often co-occurs with TCE in the groundwater. ClO$_4^-$ is very stable and non-reactive due to its high energy of activation and has adverse health effects on humans because of its interference with the iodide uptake into the thyroid gland.

*In situ* bioremediation using enrichment cultures is an efficient, sustainable and cost effective treatment method for remediation of TCE and ClO$_4^-$ in groundwater. Dechlorinating bacteria transform chlorinated compounds such as TCE, dichloroethene (DCE) and vinyl chloride (VC) to benign product ethene through reductive dechlorination (Figure 1).

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**Figure 1.** Schematic representing TCE biological reductive dechlorination pathway
Among all dechlorinating bacteria, complete reduction of TCE to innocuous ethene so far has been demonstrated only by *Dehalococcoides mccartyi*.\textsuperscript{13,14} *D. mccartyi* reduce TCE to ethene using H\textsubscript{2} as electron donor and acetate as carbon source.\textsuperscript{1,15} PRB responsible for ClO\textsubscript{4}\textsuperscript{-} reduction also use H\textsubscript{2} and acetate as electron donors.\textsuperscript{7,10} Since dechlorinating bacteria and PRB compete with each other for substrates, achieving simultaneous microbial reduction of TCE and ClO\textsubscript{4}\textsuperscript{-} is challenging. Additionally, dechlorinating bacteria are sensitive to oxygen, which is a by-product of ClO\textsubscript{4}\textsuperscript{-} reduction (Figure 2).

![Diagram of Perchlorate Reduction Pathway]

**Figure 2.** Schematic representing Perchlorate biological reduction pathway.

Moreover, other microbial terminal electron accepting processes (e.g., iron reduction, sulfate reduction, bicarbonate reduction to methane and acetate) could potentially compete with dechlorinating bacteria and PRB for H\textsubscript{2} and acetate depending on the occurrence and predominance of the respective electron acceptors.\textsuperscript{16}

H\textsubscript{2} and acetate are commonly supplied through fermentation of organic substrates including lactate, emulsified vegetable oil, methanol, and ethanol. Moreover, both fermenting bacteria and dechlorinating bacteria decrease pH due to production of H\textsuperscript{+} during fermentation and reductive dechlorination, respectively. Circumneutral pH is essential for anaerobic reductive dechlorination of TCE as well as microbial perchlorate reduction.\textsuperscript{6,13,17} An alternative to organic substrates as a

\textsuperscript{2}
precursor for H₂ is zero-valent iron (ZVI). ZVI particles (micro (m)-scale and nano (n)-scale) react with water molecules to produce H₂ gas and OH⁻ ions simultaneously (Eq. 1).

$$\text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2(\text{g}) + 2\text{OH}^-$$ (1)

Further, ZVI (both mZVI and nZVI) have shown to effectively reduce TCE to ethene and ethane.¹⁸,¹⁹ Although ClO₄⁻ reduction by ZVI is thermodynamically less favorable because of large activation energy needed for chemical reduction⁷,²⁰, steady and fast decrease in ClO₄⁻ concentration was reported when ZVI was used in combination with PRB.²⁰,²¹ Combining bioremediation with ZVI-based chemical reduction could enhance treatment effectiveness by: (i) producing H₂ (Eq. 1), the electron donor for D. maccartyi and PRB; (ii) decreasing oxidation-reduction potential (ORP), which leads to an increase in anaerobic microbial activity²²; (iii) generating OH⁻ to counter balance H⁺ produced due to fermentation and reductive dehalogenation; and (iv) co-reducing other chlorinated organic solvents (e.g., chloroform, carbon tetrachloride) which may inhibit microbial activity in chlorinated ethenes enrichment cultures.²³,²⁴

On one hand, dechlorinating bacteria prefer to respire more chlorinated compounds over the lesser chlorinated ones as the former yields more energy.²⁵ On the other hand, for the abiotic dechlorination by ZVI, according to Arnold et al.¹⁸ and stroo et al.²⁶, the order of reactivity is VC > DCEs > TCE > PCE. Hence, accumulation of toxic intermediates like cis-DCE and VC can potentially be negated when combining microbiological and ZVI abiotic reactions. These synergistic benefits strongly encourage a ZVI-enhanced bioremediation scheme for removal of chlorinated solvents and perchlorate in groundwater. In spite of being theoretically synergistic, the limitations and disadvantages in application of the two technologies
simultaneously are not completely understood and field peer-reviewed studies are limited. The objective of this study was to evaluate the limitations of simultaneous application of ZVI and bioremediation for detoxification of TCE and ClO$_4^-$.

In this study, we used soil and groundwater from a Superfund site contaminated with TCE and ClO$_4^-$. We established laboratory conditions representative of a ZVI-enhanced bioremediation scheme (ZVI injection zone and a downstream zone with influent water having a low redox potential and containing dissolved Fe (II) derived from the ZVI. The conceptual design is shown in Figure 3. Using semi-batch microcosm experiments, we evaluated the impact of ZVI and availability of electron and carbon source on TCE and ClO$_4^-$ degradation in conditions representative of each zone.

**Figure 3.** Illustration showing the established microcosm conditions representing the remediation scheme and semi-batch microcosm operation method.
CHAPTER 2
MATERIAL AND METHODS

2.1 Aquifer Materials
TCE-contaminated aquifer material (groundwater and soil) was obtained from a confidential Superfund Site with the consent of U.S. Environmental Protection Agency (US-EPA). Pump-and-treat has been used at the site for over three decades to remove TCE and ClO\textsuperscript{4}. Soil cores from up to 170 m depth were homogenized in an anaerobic glovebox before using in microcosm experiments. pH and ORP of the groundwater are 7.78 ± 0.10 and ~ + 150 mV respectively.

2.2 Zero-Valent Irons (ZVIs)
Two mZVIs, Z-Loy\textsuperscript{TM} MicroMetal (OnMaterials, Escondido, CA) with mean particle diameter 2-3 µm and carbonyl iron powder OM (BASF, Florham Park, NJ) with mean particle diameter < 10 µm were used in this study. The nZVI product utilized was NANOFER STAR W\textsuperscript{TM} (Nano Iron, s.r.o., Czech Republic) with particle diameter d\textsubscript{50} < 50 nm.

2.3 Microbial Inocula
The dechlorinating enrichment cultures used as inocula were ZARA-10,\textsuperscript{28,29} maintained in our laboratory for over 5 years in a continuously stirred tank reactor, and the commercially available SDC-9 culture (CB&I, Woodlands, TX).\textsuperscript{30}

2.4 Semi-Batch Microcosm Experiments
Experiments were carried out in microcosms (120 mL glass serum bottles) with 25 g of soil, 75 mL of groundwater medium. The experimental conditions tested are shown
The medium was site groundwater amended with 200 mg L\(^{-1}\) yeast extract, 560 mg L\(^{-1}\) lactate, and/or 170 mg L\(^{-1}\) EVO, and 10 mM phosphate buffer. The microcosms were sealed with butyl rubber stoppers and aluminum crimps. The fermentable substrates used to deliver electron donor and carbon source were sodium lactate (60% syrup; Sigma-Aldrich, St. Louis, MO) and emulsified vegetable oil (EVO) product, EOS Pro (EOS Remediation. LLC, Raleigh, NC). The experiments were conducted in semi-batch cycles (14 days per cycle) in which, 25 mL of spent liquid from the microcosms were removed and replaced with 25 mL of fresh groundwater with amendments. A schematic representation of semi batch cycles is presented in Figure 3. Four semi-batch cycles (56 days) were conducted and a total of 75 mL liquid from the microcosms were replaced (1 hydraulic retention time). The microcosms were incubated statically at room temperature in the dark.

In the microcosms with ZVI (representing the injection zone), TCE was added initially to attain a concentration of ~ 8 µmol L\(^{-1}\) (~1000 µg L\(^{-1}\); typical concentration in groundwater at the Phoenix-Goodyear Airport Superfund Site). Starting with the second semi-batch cycle (day 14), TCE was added at ~ 140 µmol L\(^{-1}\) (~18.4 mg L\(^{-1}\)).

### Table 1. Experimental conditions established in semi-batch microcosms with 25 g soil and 75 mL of site groundwater. All conditions were tested in triplicate.

<table>
<thead>
<tr>
<th>Zone</th>
<th>mZVI/nZVI (g L(^{-1}))</th>
<th>Fe(II) (g L(^{-1}))</th>
<th>Phosphate (mM)</th>
<th>Yeast extract (mg L(^{-1}))</th>
<th>Lactate (mg L(^{-1}))</th>
<th>EOS Pro (mg L(^{-1}))</th>
<th>ZARA-10 culture (mL)</th>
<th>SDC-9 culture (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZVI control</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>Injection zone</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15/1.5*</td>
<td>0</td>
<td>10</td>
<td>200</td>
<td>560</td>
<td>0</td>
<td>4*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15/1.5*</td>
<td>0</td>
<td>10</td>
<td>200</td>
<td>560</td>
<td>0</td>
<td>0</td>
<td>4*</td>
<td></td>
</tr>
<tr>
<td>15/1.5*</td>
<td>0</td>
<td>10</td>
<td>200</td>
<td>0</td>
<td>170</td>
<td>4*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15/1.5*</td>
<td>0</td>
<td>10</td>
<td>200</td>
<td>0</td>
<td>170</td>
<td>0</td>
<td>4*</td>
<td></td>
</tr>
<tr>
<td>Fe(II) control</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downstream zone</td>
<td>0</td>
<td>0.25*</td>
<td>10</td>
<td>200</td>
<td>560*</td>
<td>170</td>
<td>4*</td>
<td>0</td>
</tr>
<tr>
<td>0.25*</td>
<td></td>
<td></td>
<td>10</td>
<td>200</td>
<td>560*</td>
<td>170</td>
<td>4*</td>
<td>0</td>
</tr>
<tr>
<td>0.25*</td>
<td></td>
<td></td>
<td>10</td>
<td>200</td>
<td>560*</td>
<td>170</td>
<td>0</td>
<td>4*</td>
</tr>
<tr>
<td>No iron control</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>170</td>
<td>0</td>
<td>4*</td>
<td></td>
</tr>
</tbody>
</table>

*Indicates addition only at time 0 only.
2.5 Chemical analyses

Chlorinated ethenes, ethene, ethane and methane were measured by injecting 200 µL gas samples from headspace in gas chromatograph (Shimadzu GC-2010; Columbia, MD) equipped with a flame ionization detector (FID) and an Rt-QS-BOND capillary column (Restek; Bellefonte, PA). The detection limit for TCE was 0.0004 µmol L\(^{-1}\). H\(_2\) was measured using a gas chromatograph (Shimadzu GC-2010; Columbia, MD) equipped with a thermal conductivity detector (TCD) and a fused silica capillary column (Carboxen\textsuperscript{TM}1010 PLOT, Supelco). Details on the gas chromatography methods were previously published.\textsuperscript{28,29} The concentrations of chlorinated ethenes and ethene in the liquid were calculated based on gas-liquid equilibrium by using experimentally determined Henry's constants (\(K_H\)) for each compound at 30 °C.\textsuperscript{29} The concentration reported are nominal concentration in the system (µmol L\(^{-1}\)).

Total gas volume in the headspace of the microcosms was measured using Perfektum\textsuperscript{®} matched numbered hypodermic syringes (Sigma-Aldrich, St. Louis, MO). pH was measured using Sartorius pH bench top meter (Thermo Scientific, Waltham, MA). Oxidation-reduction potential (ORP) was measured using ORP110-GS standard ORP probe (Hach, Loveland, CO).

Perchlorate, sulfate, and nitrate were measured using ion chromatography (IC). Liquid samples for IC analysis were filtered through 0.2 µm membrane filters (PVDF membrane, Pall Life Sciences Acrodisc Syringe Filters, Port Washington, NY). We quantified ClO\(_4^\text{-}\) using Dionex ICS 3000 instrument with a Dionex IonPac AG16 pre-column, Dionex IonPac AS16 column, an eluent concentration of 50 mM potassium hydroxide (KOH), and 1 mL min\(^{-1}\) flow rate. The detection limit for ClO\(_4^\text{-}\) was 0.025 µmol L\(^{-1}\) (2.5 µg L\(^{-1}\)). We analyzed SO\(_4^{2-}\) and NO\(_3^-\) using a Dionex ICS 3000 IC equipped with a Dionex IonPac AG18 pre-column and a Dionex IonPac.
AS18 column, employing an eluent gradient from 15 mM KOH to 40 mM KOH and an eluent flow rate of 1 mL min$^{-1}$. The detection limits for $\text{SO}_4^{2-}$ and $\text{NO}_3^{-}$ were 1.04 µmol L$^{-1}$ and 1.61 µmol L$^{-1}$, respectively.
3.1 ZVI inhibited biological reduction of TCE and ClO$_4^-$

We evaluated simultaneous TCE and ClO$_4^-$ reduction in the presence of ZVI in semi-batch microcosms containing soil and groundwater from a Superfund Site.

**Figure 4.** TCE reductive dechlorination in semi-batch microcosms with (A) No amendments (control), (B) ZVI only (ZVI control), (C) ZVI, lactate (filled symbols) or EVO (empty symbols) & ZARA-10 culture and (D) ZVI, lactate (filled symbols) or EVO (empty symbols) & SDC-9 culture. The mZVI and nZVI concentrations were 15 and 1.5 g L$^{-1}$, respectively. Data are averages of triplicate microcosms and error bars indicate standard deviation of the mean.
As seen in Figure 4A, TCE dechlorination was absent in the microcosms without any amendments, these microcosms were set up as controls. ZVI addition also produced up to ~70 mmol L$^{-1}$ H$_2$ (Fig. 6D; ZVI control) and significantly reduced the ORP from 25.3 ± 50 mV to −320 ± 35 mV. We added two enrichment cultures to the ZVI microcosms in order to understand the interaction of each culture when they encounter high concentration of ZVI during application of the ZVI for enhanced bioremediation. Due to OH$^-$ generation from Eq. 1, the pH reached to ~9 in the ZVI microcosms and was adjusted to ~7.6 using 2M HCl solution on day 42 of the experiment to avoid pH as a limiting factor for biological reductive dechlorination. However, because of the rapid abiotic dechlorination of TCE, dechlorinating bacteria were limited by availability of electron acceptor in both the enrichment cultures. TCE dechlorination rates in the microcosms with ZVI & EVO were similar, regardless of the enrichment culture added. In order to determine TCE dechlorination rates in the ZVI microcosms, we carried out time-intensive measurement of TCE and by-products with frequent sampling. Figure 5 depicts rapid TCE dechlorination and transformation to ethene and ethane.
Figure 5. TCE reductive dechlorination in semi-batch microcosms containing ZVI and (A) EVO, (B) ZARA-10 culture with EVO, (C) SDC-9 culture with EVO. Data are average of triplicate microcosms and error bars indicate standard deviation of the mean.

The average TCE reduction rates in ZVI & EVO microcosms were 24.75 µmol L⁻¹ d⁻¹ with a maximum of 56.63 µmol L⁻¹ d⁻¹ and 19.09 µmol L⁻¹ d⁻¹ with a maximum of 37.22 µmol L⁻¹ d⁻¹ for ZARA-10 and SDC-9 culture, respectively (Fig. 5B & 5C). The TCE added during each semi-batch cycle was completely converted to ethene and ethane within >3 days without accumulation by-products cis-DCE and VC (Figure 5A-C), supporting the fact that microbial reductive dechlorination was either not occurring or had a minimal contribution to degradation in the microcosms.
with high concentration of ZVI. Prior studies have reported that mZVI doses > 15 g L\(^{-1}\) and nZVI doses > 0.05 g L\(^{-1}\) showed inhibiting effects on dechlorinating enrichment cultures\(^{31}\) and nZVI showed decrease in \textit{tceA} & \textit{vcrA} gene expression.\(^{32}\) Overall, rapid abiotic TCE reduction to ethene and ethane in the presence of high concentration of ZVI made TCE unavailable for dechlorinating bacteria in the enrichment cultures.

\[ \text{ClO}_4^- \] was present in the site groundwater at a concentration of 1.8 µmol L\(^{-1}\) \text{ClO}_4^-. As seen in Figure 3A, microcosms without ZVI/amendments showed complete \text{ClO}_4^- reduction within 14 days of incubation. This reduction was sustained for three additional semi-batch cycles (56 days) after addition of fresh groundwater containing \text{ClO}_4^- at the beginning of each cycle. These results suggest that the groundwater from the site contained a robust indigenous population of PRB. PRB are ubiquitous in nature and they have relatively simple nutritional requirements.\(^{7,33,34}\) However, as seen in Fig. 6A, in the microcosm that had ZVI but no other amendments (ZVI control), addition of ZVI inhibited the microbial activity of the indigenous PRB with ~0.4 µmol L\(^{-1}\) \text{ClO}_4^- still present at day 56. Results from our study reveal a detrimental effect of ZVI on microbial \text{ClO}_4^- reduction. ZVI-mediated abiotic \text{ClO}_4^- was likely negligible in our microcosms due to high activation energy required for chemical reduction of \text{ClO}_4^-, as demonstrated in previous studies.\(^{20,35}\)
3.2 ZVI enriched undesired H2 utilizing microbes

Anaerobic conditions established and H2 produced by ZVI oxidation can lead to stimulation of undesired H2 utilizing microbes such as methanogens and sulfate reducers. These H2 utilizing microbes compete with desired dechlorinating bacteria and PRB when H2, acetate and nutrients might be limited. In order to understand potential competing microbial processes in the site, we studied alternative H2 consuming anaerobic microbial processes enhanced in presence of ZVI in the microcosms. Bicarbonate reduction to methane (methanogenesis) was one microbial
process enhanced drastically in the SDC-9 enrichment culture. This is reflected by production of \(~1450 \text{ } \mu\text{mol} \text{ } \text{L}^{-1}\) methane in 56 days (Fig. 6C). Our results are in agreement with previous work\(^{36,37}\), in which nZVI stimulated sulfate reducers and methanogens, inhibiting dechlorinating bacteria in dechlorinating enrichment cultures.

Sulfate (SO\(_{4}^{2-}\)) reduction is also a potential competing microbial electron accepting process for TCE dechlorination and ClO\(_{4}^{-}\) reduction. Groundwater from the site contained a very high SO\(_{4}^{2-}\) concentration (11.05 ± 0.51 mmol L\(^{-1}\)). Abiotic sulfate reduction by ZVI was minimal (Fig. 6B; ZVI control). The extent of sulfate reduction increased considerably by the addition of bioaugmentation cultures (Fig. 6B). The microcosms with ZARA-10 culture showed significant SO\(_{4}^{2-}\) reduction and SDC-9 culture showed almost complete SO\(_{4}^{2-}\) reduction in the ZVI microcosms (Fig. 6B). Therefore, given the limitation of TCE for dechlorinating bacteria and inhibition of indigenous PRB by ZVI, the excess H\(_{2}\) and anoxic condition stimulated undesired methanogens and SRB.

3.3 Fe (II) led to incomplete TCE reduction and Complete ClO\(_{4}^{-}\) reduction

Downstream conditions following the application of ZVI for enhancing bioremediation of TCE and ClO\(_{4}^{-}\) have not been studied yet. Oxidation of ZVI with water molecules yield water-soluble Fe (II) ions that can migrate downgradient with the flow of groundwater. In order to evaluate the effect of Fe (II) on dechlorinating enrichment cultures, we established microcosms containing 0.25 g L\(^{-1}\) Fe (II) ions with ZARA-10 and SDC-9 cultures. Addition of Fe (II) produced anoxic conditions in the microcosms (ORP = - 210 ± 30 mV).
Figure 7. TCE reductive dechlorination in semi-batch microcosms with (A) Fe (II), lactate & EVO (Fe (II) control), (B) Fe (II), lactate, EVO & ZARA-10, (C) Fe (II), lactate, EVO & SDC-9 and (C) No ZVI/Fe (II), EVO & SDC-9 (No iron control). The Fe (II) concentration was 0.25 g L$^{-1}$. Data are averages of triplicate microcosms and error bars indicate standard deviation of the mean. Note that panel (D) has a different scale in y-axis.

As seen in Figures 7B and 7C, both enrichment cultures showed significant biological TCE dechlorination activity in the presence of Fe (II) in contrast to the ZVI microcosms. However, TCE dechlorination by both enrichment cultures were incomplete in the presence of Fe (II), leading to accumulation of cis-DCE and VC. Interestingly, SDC-9 culture showed much faster rate of dechlorination without accumulation of toxic by-products in the microcosms without any iron species to reduce ORP (absence of ZVI/Fe (II); Fig. 7D). Ethene production initiated within 7
days of incubation and ethene was the major product from day 14 onwards (Fig. 7D). This suggests that Fe (II) inhibited biological reductive dechlorination in the microcosms, despite producing anoxic conditions).

The inhibitory effect of 0.25 g L$^{-1}$ Fe (II) on native groundwater PRB’s ability to reduce ClO$_4^-$ was evaluated in presence of both the cultures. Unlike ZVI, Fe (II) ions did not inhibit the indigenous PRB of soil/groundwater. As seen in Figure 8A, Fe (II) microcosms with either of ZARA-10 and SDC-9 culture showed complete ClO$_4^-$ reduction within 42 days of treatment. In agreement with the finding that groundwater contains robust PRB (from control microcosms; Fig. 4A), the rate of ClO$_4^-$ reduction was highest in Fe (II) in the absence of either enrichment culture (Fig. 8A). Also, contrary to what we observed for ZVI, no inhibition was observed in the absence of iron species (Fig. 8A). SDC-9 culture in the absence of Fe (II)/ZVI, either aided the most or posed the least competition to the indigenous PRB present in groundwater and the rate of ClO$_4^-$ reduction was higher than both the enrichment cultures in presence of Fe (II) (Fig. 8A). Overall, Fe (II) ions showed negligible inhibitory effect in ClO$_4^-$ reduction by PRB and both cultures successfully reduced ClO$_4^-$ completely within 42 days of treatment.
3.4 Reactive oxygen species could have inhibited dechlorinating bacteria in the Fe (II) microcosms

In order to understand the inhibition source for complete TCE dechlorination to ethene in the Fe (II) microcosms, pH was evaluated as a factor. pH values were in the range of 6.6 ± 0.3, which is beneficial pH range for dechlorinating bacteria. Following pH, competing microbial processes (methanogenesis and SO$_4^{2-}$ reduction) were evaluated in the presence of Fe (II). Methane production trends were different in

**Figure 8.** (A) ClO$_4^-$ reduction, (B) SO$_4^{2-}$ reduction, (C) CH$_4$ production and (D) H$_2$ production & consumption in semi-batch microcosms containing Fe(II). Data are averages of triplicate microcosms and error bars indicate standard deviation of the mean.
the Fe (II) microcosms compared to the ZVI microcosms. Microcosms with ZARA-10 produced 1.8 µmol \textsuperscript{-1}L methane and microcosms with SDC-9 produced 85 µmol L\textsuperscript{-1} methane by day 56, which is ~ 5% and ~6% of methane produced in presence of ZVI by respective cultures (Figs. 6C & 8C; scales are different). Similarly, SO\textsubscript{4}\textsuperscript{2-} reduction in the Fe (II) microcosms with enrichment cultures was also significantly less than the ZVI microcosms (Figs. 6B & 8B). This suggests Fe (II) did not simulate competing methanogens and SRB as much as ZVI did, due to H\textsubscript{2} production by ZVI and difference in ORP change.

When Fe (II) microcosms were compared with no-iron control microcosms, which showed no inhibition to dechlorination, methane production and SO\textsubscript{4}\textsuperscript{2-} reduction in the presence of SDC-9 culture were similar (Figs. 8B & 8C). This indicates that neither methanogenesis nor SO\textsubscript{4}\textsuperscript{2-} reduction is likely the competing microbial processes that inhibited TCE dechlorination in Fe (II) microcosms. Inhibition of complete TCE dechlorination to ethene in the Fe (II) microcosms may be attributed to two possible scenarios: (i) The competition for H\textsubscript{2} and acetate from iron-reducing bacteria that reduce Fe (III) (derived from oxidation of Fe (II) in the microcosms). Fe (III) has been reported as an electron acceptor strictly competing with TCE.\textsuperscript{38,39} (ii) Fe (II) ions can react with dissolved O\textsubscript{2} and generate reactive oxygen species such as hydroxyl radicals through Fenton’s chemistry.\textsuperscript{40,41} Reactive oxygen species can potentially induce oxidative stress causing dysfunction of proteins or DNA and microbial death.\textsuperscript{42} Also, ferryl ion (Fe (IV)) that could be generated from oxidation of Fe (II) by dissolved O\textsubscript{2} at neutral pH, can produce hydroxyl radicals reacting with water molecules and induce oxidative stress to the bacterial cells.\textsuperscript{41,43}
CHAPTER 4
SUMMARY AND CONCLUSIONS

High concentration of ZVI dechlorinated TCE rapidly and could potentially inhibit biological reductive dechlorination and perchlorate reduction near the injection zone of the remediation site.

Methanogens and SRB proliferate in the presence of ZVI and can compete for electron donor, carbon substrate, and nutrients with dechlorinators at the injection zone or downstream of the injection zone.

Simultaneous injection of ZVI and \textit{D. mccartyi} containing enrichment culture could be detrimental and potentially lead to incomplete TCE reduction and accumulation of toxic TCE daughter products in the subsurface. Further research with simulation of the contaminated site more closely implementing soil-packed columns with continuous groundwater flow can provide valuable information to optimize the strategy of delivering ZVI and bioaugmentation culture. Application of enrichment culture sequentially downstream of ZVI injection could potentially negate the possibility of incomplete dechlorination and aid dechlorinating bacteria by providing anoxic conditions and maintaining circumneutral pH.

Such investigations could help us make optimal use of the potential synergies involved in ZVI enhanced bioremediation and fuel dechlorination of TCE and its by-products to yield the desired, benign end product ethene with simultaneous ClO$_4^-$ reduction.
REFERENCES


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