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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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.

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CHAPTER 1

INTRODUCTION

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.[1,2] Increased and improper handling of TCE has led to spills and extensive contamination of soil and groundwater. TCE is highly toxic and carcinogenic (ATSDR).[3] TCE is often found with one or more organic and inorganic contaminants.[4] Perchlorate (ClO_4^-) is one of the common inorganic contaminants and a chemical oxyanion that often co-occurs with TCE in the groundwater.[5] ClO_4^- is very stable and non-reactive due to its high energy of activation[6] and has adverse health effects on humans because of its interference with the iodide uptake into the thyroid gland.[7]

In situ bioremediation using enrichment cultures is an efficient, sustainable and cost effective treatment method for remediation of TCE and ClO_4^- in groundwater.[8–11] Dechlorinating bacteria transform chlorinated compounds such as TCE, dichloroethene (DCE) and vinyl chloride (VC) to benign product ethene through reductive dechlorination (Figure 1).[12,13]
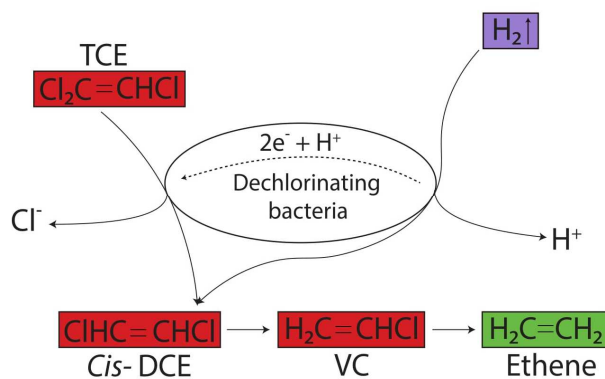
Figure 1. Schematic representing TCE biological reductive dechlorination pathway

Among all dechlorinating bacteria, complete reduction of TCE to innocuous ethene so far has been demonstrated only by *Dehalococcoides mccartyi*.[13,14] *D. mccartyi* reduce TCE to ethene using H_2 as electron donor and acetate as carbon source.[1,15] PRB responsible for ClO_4^- reduction also use H_2 and acetate as electron donors.[7,10] Since dechlorinating bacteria and PRB compete with each other for substrates, achieving simultaneous microbial reduction of TCE and ClO_4^- is challenging. Additionally, dechlorinating bacteria are sensitive to oxygen, which is a by-product of ClO_4^- reduction (Figure 2).
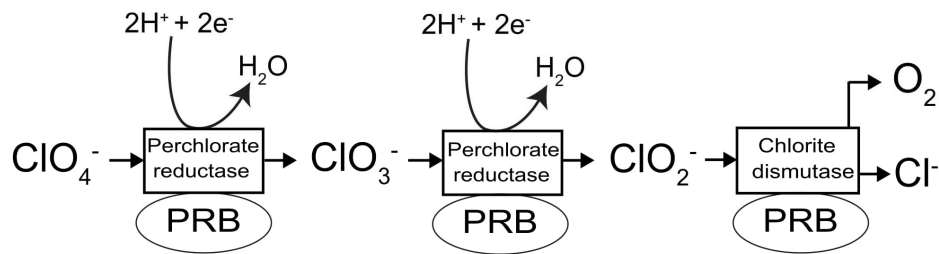
Figure 2. Schematic representing Perchlorate biological reduction pathway.

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_2 and acetate depending on the occurrence and predominance of the respective electron acceptors[16].

H_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^+ during fermentation and reductive dechlorination, respectively. Circumneutral pH is essential for anaerobic reductive dechlorination of TCE as well as microbial perchlorate reduction.[6,13,17] An alternative to organic substrates as a

precursor for H_2 is zero-valent iron (ZVI). ZVI particles (micro (m)-scale and nano (n)-scale) react with water molecules to produce H_2 gas and OH^- ions simultaneously (Eq. 1).

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 (g) + 2OH^- \qquad (1)$$
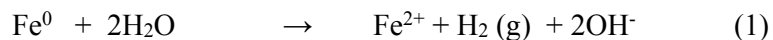
Further, ZVI (both mZVI and nZVI) have shown to effectively reduce TCE to ethene and ethane.[18,19] Although ClO_4^- reduction by ZVI is thermodynamically less favorable because of large activation energy needed for chemical reduction[7,20], steady and fast decrease in ClO_4^- concentration was reported when ZVI was used in combination with PRB.[20,21] Combining bioremediation with ZVI-based chemical reduction could enhance treatment effectiveness by: (i) producing H_2 (Eq. 1), the electron donor for *D. mccartyi* and PRB; (ii) decreasing oxidation-reduction potential (ORP), which leads to an increase in anaerobic microbial activity[22]; (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.[27] 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.
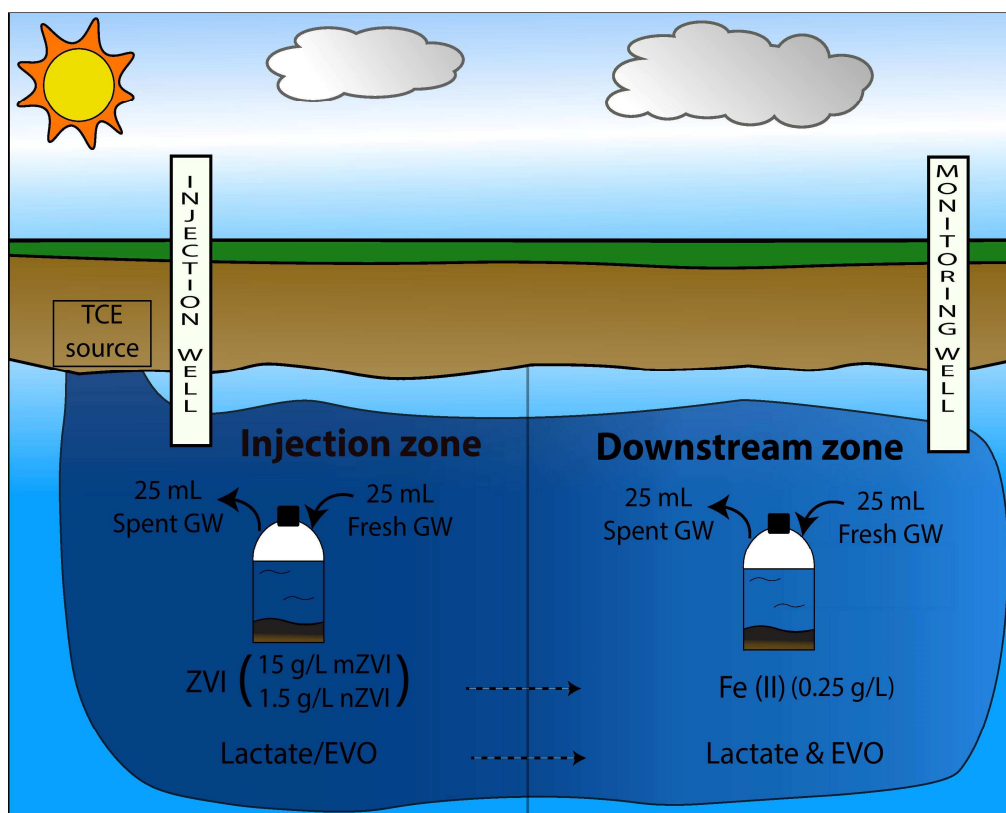
Figure 3. Illustration showing the established microcosm conditions representing the remediation scheme and semi-batch microcosm operation method.

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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_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 $\sim +150$ mV respectively.

2.2 Zero-Valent Irons (ZVIs)

Two mZVIs, Z-Loy™ 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™ (Nano Iron, s.r.o., Czech Republic) with particle diameter

d50 < 50 nm.

2.3 Microbial Inocula

The dechlorinating enrichment cultures used as inocula were ZARA-10,[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).[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

in Table 1. 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 $\sim$ 8 μmol L^{-1} ($\sim$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 $\sim$ 140 μmol L^{-1} ($\sim$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.

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

*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^{TM}1010 PLOT, Supelco). Details on the gas chromatography methods were previously published.[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.[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® 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^- 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^- 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 SO_4^{2-} and NO_3^- were 1.04 μmol L^{-1} and 1.61 μmol L^{-1}, respectively.

CHAPTER 3

RESULTS AND DISCUSSION

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.
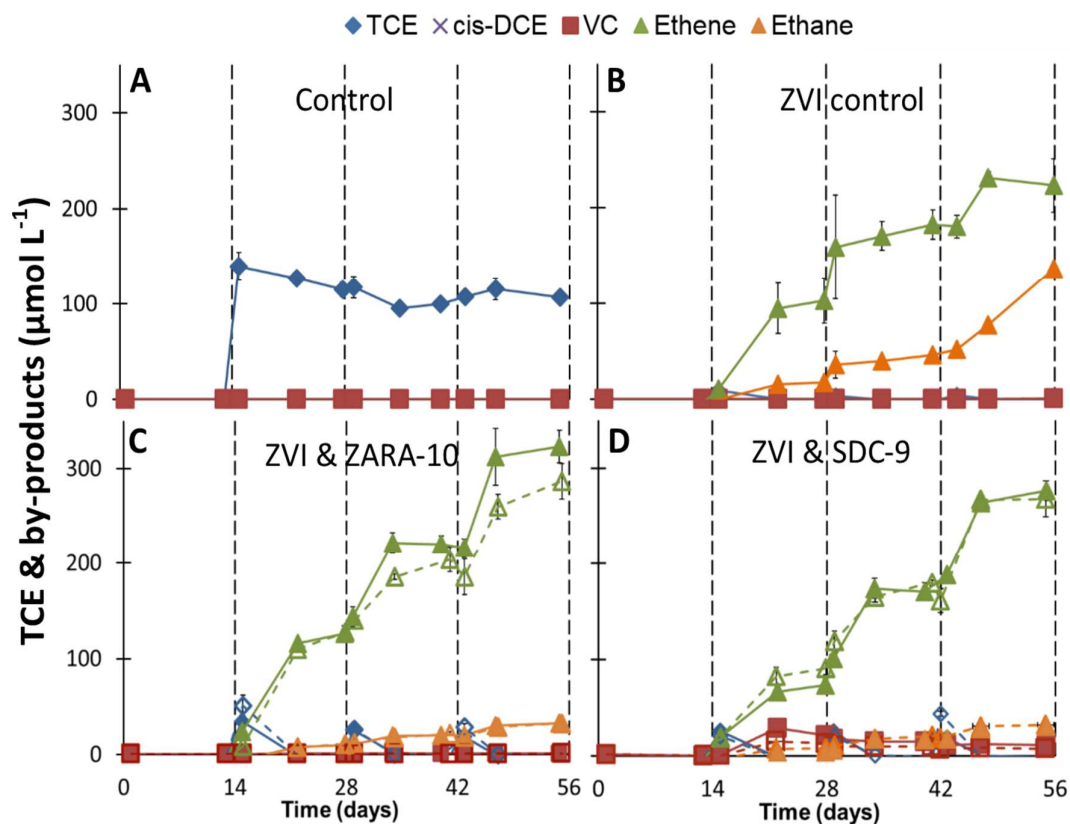
Figure 4. TCE reductive dechlorination in semi-batch microcosms with (A) No amendments (control), (B) ZVI only (ZVI control), (C) ZVI, lactate (filled symbols) or EVO (empty symbols) & ZARA-10 culture and (D) ZVI, lactate (filled symbols) or EVO (empty symbols) & SDC-9 culture. The mZVI and nZVI concentrations 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.
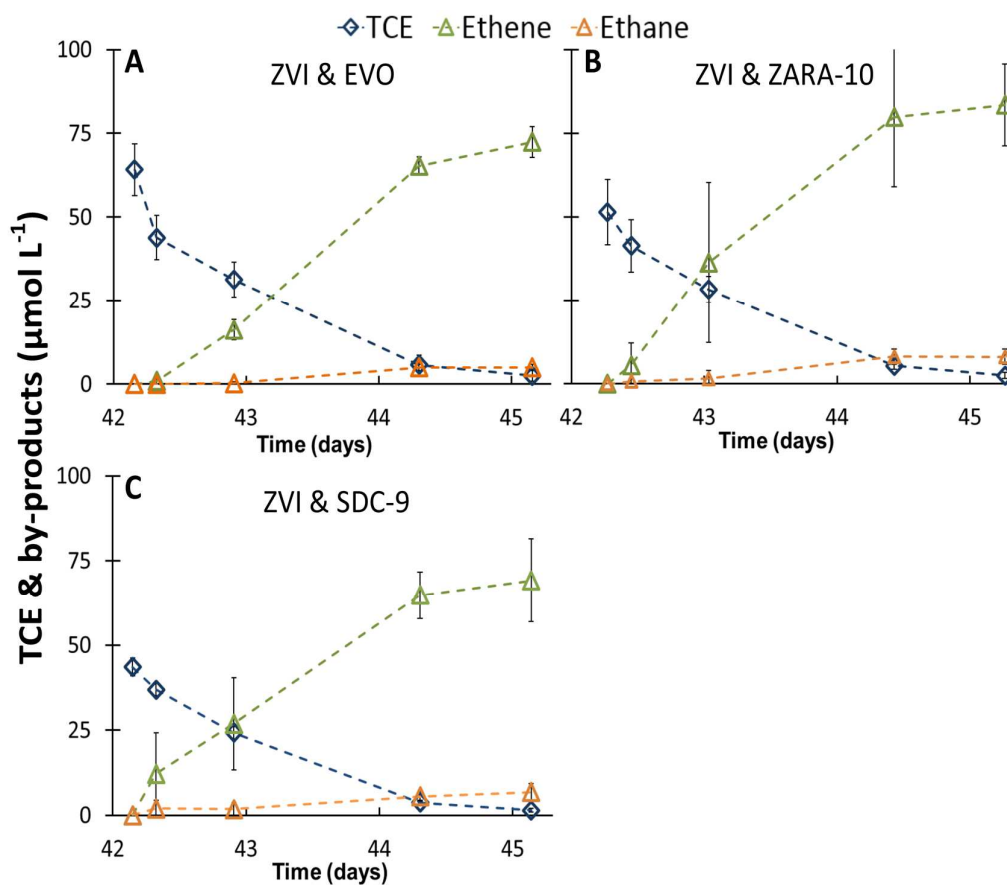
Figure 5. TCE reductive dechlorination in semi-batch microcosms containing ZVI and (A) EVO, (B) ZARA-10 culture with EVO, (C) SDC-9 culture with EVO. Data are average of triplicate microcosms and error bars indicate standard deviation of the mean.

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 *tceA* & *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.

ClO_4^- was present in the site groundwater at a concentration of 1.8 μmol L^{-1} ClO_4^-. As seen in Figure 3A, microcosms without ZVI/amendments showed complete 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 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} ClO_4^- still present at day 56. Results from our study reveal a detrimental effect of ZVI on microbial ClO_4^- reduction. ZVI-mediated abiotic ClO_4^- was likely negligible in our microcosms due to high activation energy required for chemical reduction of ClO_4^-, as demonstrated in previous studies.[20,35]
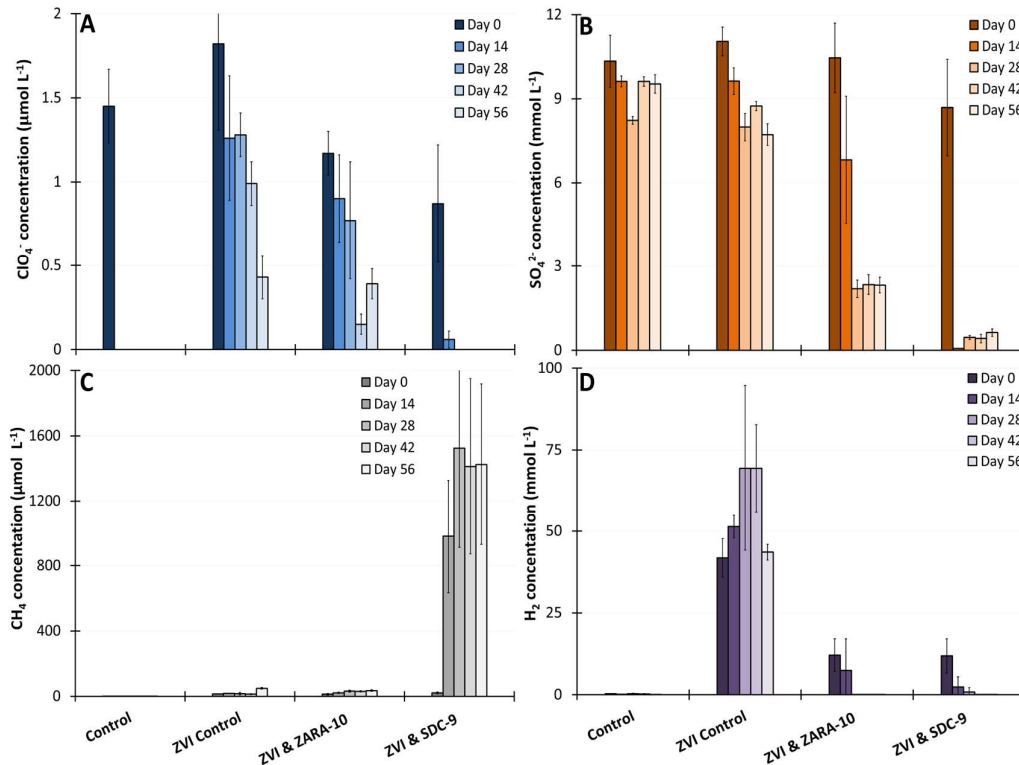
Figure 6. (A) ClO_4^- reduction, (B) SO_4^{2-} reduction, (C) CH_4 production and (D) H_2 production & consumption in semi-batch microcosms containing 15 g L^{-1} mZVI and 1.5 g L^{-1} nZVI. Data are averages of triplicate microcosms and error bars indicate one standard deviation of the mean.

3.2 ZVI enriched undesired H2 utilizing microbes

Anaerobic conditions established and H_2 produced by ZVI oxidation can lead to stimulation of undesired H_2 utilizing microbes such as methanogens and sulfate reducers. These H_2 utilizing microbes compete with desired dechlorinating bacteria and PRB when H_2, acetate and nutrients might be limited. In order to understand potential competing microbial processes in the site, we studied alternative H_2 consuming anaerobic microbial processes enhanced in presence of ZVI in the microcosms. Bicarbonate reduction to methane (methanogenesis) was one microbial

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process enhanced drastically in the SDC-9 enrichment culture. This is reflected by production of ~1450 μmol 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 $\pm$ 30 mV).
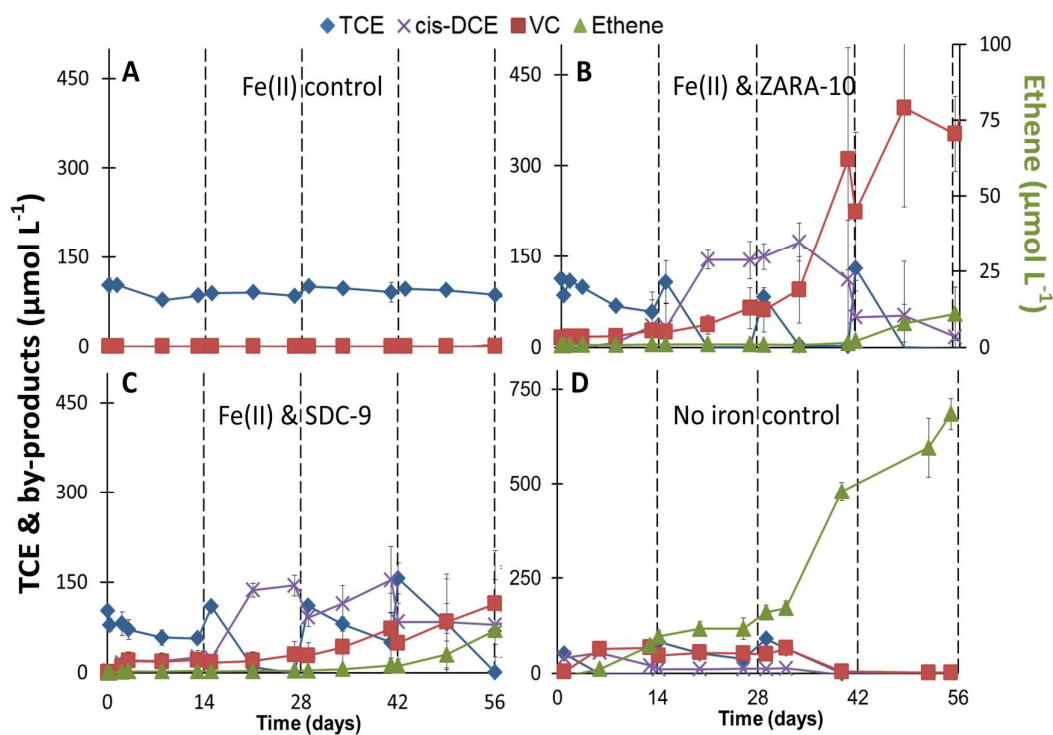
Figure 7. TCE reductive dechlorination in semi-batch microcosms with (A) Fe (II),
lactate & EVO (Fe (II) control), (B) Fe (II), lactate, EVO & ZARA-10, (C) Fe (II),
lactate, EVO & SDC-9 and (C) No ZVI/Fe (II), EVO & SDC-9 (No iron control). The
Fe (II) concentration was 0.25 g L^{-1}. Data are averages 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.
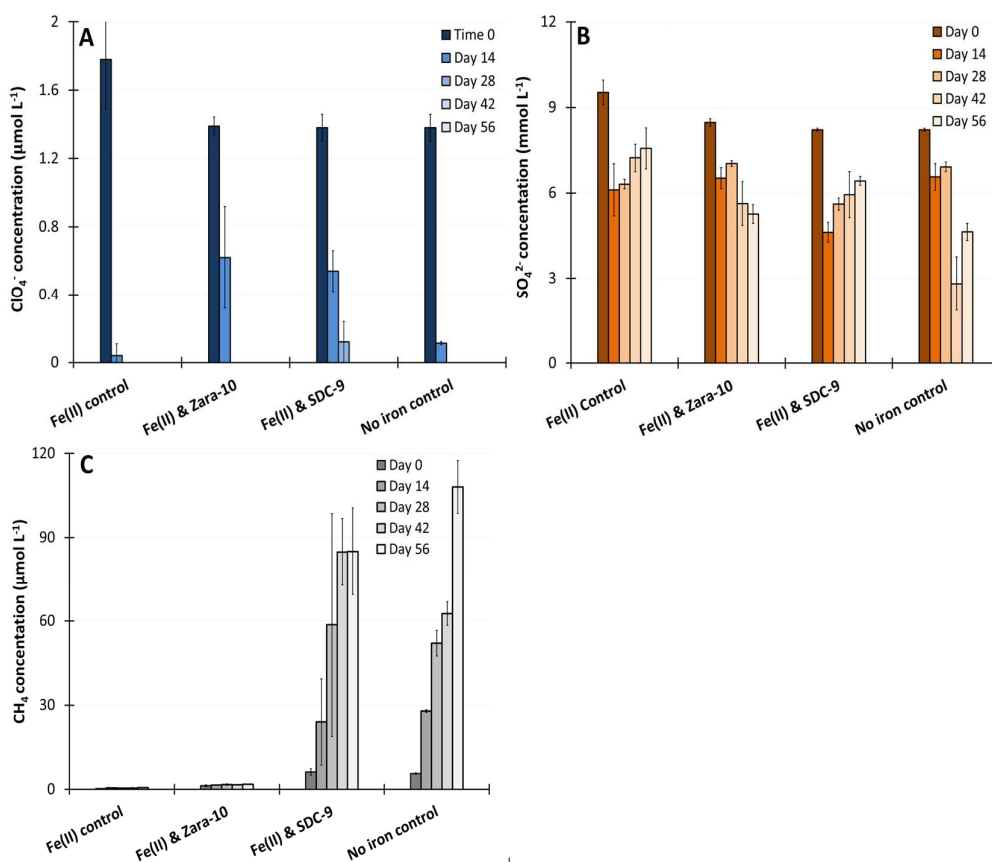
Figure 8. (A) ClO₄⁻ reduction, (B) SO₄²⁻ reduction, (C) CH₄ production and (D) H₂ production & consumption in semi-batch microcosms containing Fe(II). Data are averages of triplicate microcosms and error bars indicate standard deviation of the mean.

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

the Fe (II) microcosms compared to the ZVI microcosms. Microcosms with ZARA-10 produced 1.8 μmol $^{-1}$L methane and microcosms with SDC-9 produced 85 μmol L^{-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_4^{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_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_4^{2-} reduction in the presence of SDC-9 culture were similar (Figs. 8B & 8C). This indicates that neither methanogenesis nor SO_4^{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_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.[38,39] (ii) Fe (II) ions can react with dissolved O_2 and generate reactive oxygen species such as hydroxyl radicals through Fenton's chemistry.[40,41] Reactive oxygen species can potentially induce oxidative stress causing dysfunction of proteins or DNA and microbial death.[42] Also, ferryl ion (Fe (IV)) that could be generated from oxidation of Fe (II) by dissolved O_2 at neutral pH, can produce hydroxyl radicals reacting with water molecules and induce oxidative stress to the bacterial cells.[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 *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 bioremedition and fuel dechlorination of TCE and its by-products to yield the desired, benign end product ethene with simultaneous ClO_4^- reduction.

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