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Effects of nano-scale zero-valent iron particles on a mixed culture dechlorinating trichloroethylene

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ABSTRACT

Nano-scale zero-valent iron particles (NZVI) are increasingly being used to treat sites contaminated with chlorinated solvents. This study investigated the effect of NZVI on dechlorinating microorganisms that participate in the anaerobic bioremediation of such sites. NZVI can have a biostimulatory effect associated with water-derived cathodic H_2 production during its anaerobic corrosion (730 ± 30 μmol H_2 was produced in 166 h in abiotic controls with 1 g/L NZVI) or an inhibitory effect upon contact with cell surfaces (assessed by transmission electron microscopy). Methanogens, which are known to compete for H_2 with dechlorinators, were significantly biostimulated by NZVI and methane production increased relative to NZVI-free controls from 58 ± 5 to 275 ± 2 μmol. In contrast, bacteria dechlorinating TCE were inhibited by NZVI, and the first-order degradation rate coefficient decreased from 0.115 ± 0.005 h^{-1} (R^2 = 0.99) for controls to 0.053 ± 0.003 h^{-1} (R^2 = 0.98) for treatments with 1 g/L NZVI. Ethene production from TCE was initially inhibited by NZVI, but after 331 h increased to levels observed for an NZVI-free system (7.6 ± 0.3 μmol ethene produced in 502 h compared to 11.6 ± 0.5 mmol in the NZVI-free system and 3.8 ± 0.3 μmol ethene for NZVI alone). Apparently, cathodic H_2 was utilized as electron donor by dechlorinating bacteria, which recovered following the partial oxidation and presumably passivation of the NZVI. Overall, these results suggest that reductive treatment of chlorinated solvent sites with NZVI might be enhanced by the concurrent or subsequent participation of bacteria that exploit cathodic depolarization and reductive dechlorination as metabolic niches.

1. Introduction

Chlorinated solvents such as trichloroethylene (TCE) have become common contaminants of soil and groundwater and can remain in the subsurface as dense non-aqueous phase liquids (DNAPL), acting as continuous long-term threats to environmental and public health (Christ et al., 2005). The focus of research for the remediation of TCE-contaminated sites has shifted from technologies such as pump and treat, which aim to control expansion of the plume, to treatment of the DNAPL source zones, to reduce the time to reach clean-up standards (Stroo et al., 2003).

A promising technology for DNAPL source-zone remediation is biological degradation by dechlorinating bacteria. Many anaerobic bacteria reductively dechlorinate PCE or TCE to toxic intermediates *cis*-1,2-dichloroethene (*cis*-DCE) (e.g., *Desulfuromonas*, *Sulfurospirillum multivorans*, and *Dehalobacter*) (Holliger et al., 1993; Krumholz, 1997; Luijten et al., 2003; Scholzmuramatsu et al., 1995; Sharma and McCarty, 1996). Of particular interest are members of the genus *Dehalococcoides*, which are the only organisms known to reductively dechlorinate PCE and TCE to vinyl chloride (VC) and ethene. In this pathway, PCE or TCE is sequentially reduced to dichloroethene (DCE) isomers (*cis*-DCE, *trans*-DCE, and 1,1-DCE), vinyl chloride (VC), and finally to the innocuous product ethene (Cupples et al., 2003; Duhamel et al., 2004; Freedman and Gossett, 1989; Gibson and Sewell, 1992; He et al., 2003a,b, 2005; MaymoGatell et al., 1997; Sung et al., 2006). Several field studies have been conducted to evaluate the potential of microbial dechlorination to remediate sites contaminated with chlorinated solvents (Ellis et al., 2000; Hood et al., 2008; Lendvay et al., 2003; Major et al., 2002). However, bioremediation within DNAPL source-zones faces many technical challenges, including (i) low dechlorination rates and long clean-up times, (ii) inefficient supply of suitable electron donors (H_2, acetate) to the dechlorinating bacteria, and (iii) toxicity of high PCE or TCE concentrations to dechlorinating bacteria (Amos et al., 2007).

Nano-scale zero-valent iron (NZVI) has been extensively studied for its potential application in remediation of DNAPL-contaminated sites (Lien and Zhang, 1999, 2001; Liu and Lowry, 2006; Liu

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et al., 2005, 2007; Song and Carraway, 2005; Wang and Zhang, 1997) and has been shown to effectively degrade a large number of chlorinated organic contaminants (Lowry and Johnson, 2004; Zhang, 2003). Highly concentrated (approximately 10 g/L) aqueous slurries of NZVI have already been injected directly into the ground at, or near, the source of contamination sites (Keith and Henn, 2006; Li and Zhang, 2006). NZVI particles offer several advantages for *in situ* applications, such as a large specific surface area, which results in high reaction rates with chlorinated organics. Wang and Zhang (1997) showed that complete dechlorination of 20 mg/L TCE could be accomplished by 2 g NZVI in 100 mL aqueous solution in 1.7 h. Furthermore, given their small size and ability to resist aggregation and remain dispersed in water under the right conditions (Quinn et al., 2005; Saleh et al., 2005, 2008), NZVI can be delivered to contaminated aquifers. Thus, NZVI has been proposed for *in situ* remediation of DNAPL source zones (Gavaskar et al., 2005; Quinn et al., 2005; Saleh et al., 2005). However, the potential influence of NZVI on the community of indigenous microorganisms that participate in the remediation process is unknown.

ZVI can produce cathodic H_2 as it reduces water-derived protons (Daniels et al., 1987; Liu and Lowry, 2006; Liu et al., 2005; Orth and Gillham, 1996; Weathers et al., 1997). Since H_2 is a highly favorable electron donor for microorganisms that biodegrade chlorinated solvents (Aulenta et al., 2006; Daprato et al., 2007; Seshadri et al., 2005), evolution of H_2 during NZVI corrosion suggests the potential enhancement of TCE bioremediation. If reductive treatment of DNAPL with NZVI and microbial dechlorination activity are synergistic, NZVI could (i) abiotically degrade a large fraction of the DNAPL mass in the source-zone directly, while subsequent biological dechlorination might serve as a "polishing" step to remediate residual chloroethenes; (ii) provide electron donors for dechlorinating bacteria to eliminate the need for additional electron donor addition (e.g., lactate); (iii) reduce the time for site closure by quickly dechlorinating the DNAPL mass; and (iv) mitigate the toxicity of DNAPL on dechlorinating bacteria by lowering the aqueous concentration of DNAPL in the source zone. These processes would further increase the attractiveness of NZVI as a DNAPL source-zone treatment alternative. However, these benefits could be offset by the potential toxicity of NZVI to bacteria, which remains to be evaluated.

This study investigated the effects of NZVI on the microbial reduction of TCE in batch microcosms. Specifically, we (i) evaluated the effect of NZVI on a TCE-dechlorinating mixed culture (containing *Dehalococcoides* spp.) to determine whether NZVI has a stimulatory or inhibitory effect on dechlorinating bacteria; and (ii) demonstrated the potential for NZVI to serve as a source of electrons for biological dechlorination via cathodic H_2 evolution. Competition for H_2 between dechlorinating bacteria and methanogens in the consortium is also addressed.

2. Methods

2.1. Chemicals

Trichloroethylene (99%), *cis*-dichloroethylene (*cis*-DCE; 99%), *trans*-1,2-dichloroethylene (*t*-DCE; 98%) and 1,1-dichloroethylene(1,1-DCE; 99%) were purchased from Sigma–Aldrich (St. Louis, MO). A biological buffer 4-(2-hydroxyethyl) piperazine-1-ethansulfonic acid (HEPES; 99.5%) was purchased from Sigma–Aldrich. Methanol (MeOH, HPLC grade) was obtained from Fisher Scientific. NZVI particles were obtained from Toda Kogyo Corporation, Onoda, Japan. The physical and chemical properties of these NZVI can be found in Liu et al. (2005). Gases were obtained from Alltech Associates (Deerfield, IL): methane (1055 ppm_v), ethene (984 ppm_v), ethane (1052 ppm_v), acetylene (1030 ppm_v) and vinyl chloride (VC; 10.8 ppm_v). All gases were balanced with helium and had ±2% variation. Mixed gas (20% CO_2, 80% H_2) and ultra-high purity N_2 were purchased from Matheson Tri-Gas (Houston, TX). All of the other chemicals used were reagent grade or better unless otherwise specified.

2.2. Cultures and medium preparation

A *Dehalococcoides*-containing culture was developed from an anaerobic methanogenic consortium that had shown dechlorination activity for more than a decade in the laboratory (Zheng et al., 2001). This culture was capable of dechlorinating PCE to ethene rapidly and completely (240 μmol/L/d) and was previously used for bioaugmentation of a PCE-contaminated source zone (Adamson et al., 2003; Da Silva et al., 2006). An inoculum of this culture was created by anaerobically transferring 100 mL to a 250-mL serum bottle (Wheaton, Millville, NJ) capped with a Mininert valve. TCE (20 mg/L, dissolved in methanol) was added as a nutrient source. Every 4 days, 10 mL of culture was removed, and 10 mL of fresh nutrient medium was added (hydraulic retention time was 40 days). The microcosm was sparged with pure N_2 for 30 min to purge any traces of chlorinated compounds prior to being inoculated to batch reactors. The pH of the culture was routinely checked with a pH meter and probe (Oakton, IL).

Dehalococcoides spp. concentration was measured by real-time quantitative PCR (Rt-qPCR) (Applied Biosystems 7500, Foster City, CA), using phylogenetic primers and probe as described elsewhere (He et al., 2003). DNA was extracted from 1.8-mL samples of the culture in triplicate using MoBio™ kits (Carlsbad, CA) according to the manufacturer's protocols. A bead-beating device (Mini Beadbeater-8, Biospec, Bartlesville, OK) was utilized for cell lysis. The *Dehalococcoides* spp. cell concentrations were $1.0 \pm 0.2 \times 10^7$ cell/mL after dilution.

Reagent-grade chemicals and Mill-Q water were used to prepare the nutrient medium. The medium was prepared using concentrated stock solutions (10 mL each stock/L medium) of basal salts (40 g/L KCl, 40 g/L $MgCl_2{\cdot}6H_2O$, 40 g/L NH_4Cl, 14 g/L KH_2PO_4, 2.5 g/L $CaCl_2{\cdot}2H_2O$); trace element solution I (50 mg/L $ZnCl_2$, 50 mg/L $MnCl_2{\cdot}4H_2O$, 50 mg/L H_3BO_3, 250 mg/L $CoCl_2{\cdot}6H_2O$, 50 mg/L $NiCl_2{\cdot}6H_2O$, 50 mg/L $Na_2MoO_4{\cdot}2H_2O$) and trace element solution II (1000 mg/L $(NaPO_3)_{16}$, 250 mg/L KI, 50 mg/L NH_4VO_3). Sodium bicarbonate (7–10 g/L) was added as a buffer, and 300 mg/L $Na_2S{\cdot}9H_2O$ and 40 mg/L $FeCl_2{\cdot}4H_2O$ was added to reduce the medium. The medium was kept in an anaerobic chamber at pH 8.1 and ORP of −97 mV.

2.3. Analytical methods

A 100 μL headspace sample was withdrawn from each batch reactor and analyzed for TCE and its daughter-products using a packed column (6 ft. × 1/8 in. o.d. 60/80 carbopack B/1%SP-1000, Supelco, Bellefonte, PA) on a GC (HP5890, Ramsey, MN) equipped with a flame ionized detector (FID). Samples were injected splitless at 250 °C. The oven temperature program (50 °C for 2 min, ramp 40 °C/min to 200 °C, and hold for 4 min) provided adequate separation between TCE and all chlorinated and non-chlorinated reaction products.

TCE, DCE (*cis*-, *trans*-, 1,1-) and gas calibration standard curves were prepared by adding each standard to serum bottles (250 mL) with the same liquid/headspace volume ratio (100/260) as the reactors. Aqueous concentration of TCE and its dechlorinated byproducts were calculated using reported Henry's constants at 22 °C (Gossett, 1987).

Analysis of H_2 was conducted by direct injection of headspace samples (200 μL) into a GC (HP 6890) equipped with a thermal conductivity detector (TCD) and a packed column (H9-Q 60/80 9 ft. 2.0 mm ID 1/8 in. OD, ResTek, Bellefonte, PA). Helium

(20 mL/min) was used as the carrier gas. The inlet temperature was 200 °C, TCD detector was 250 °C. The oven temperature program (35 °C for 1.5 min, ramp 30 °C/min to 200 °C, and hold for 1 min) to ensure TCE and its' intermediates are purged from the column. Calibration standards were prepared as described above.

2.4. Transmission electron microscopy (TEM)

The NZVI particles and NZVI-bacteria system were observed by transmission electron microscopy (TEM) performed with a JEOL 1230 operated at 120 kV (JEOL, Tokyo, Japan). The NZVI samples were prepared by placing drops of NZVI suspension (1 g/L) on 300 mesh copper grids (Ted Pella, Redding, CA), and dried overnight. The NZVI-bacteria sample was prepared by mixing the supernatant of culture and NZVI (1 g/L) together inside anaerobic chamber. This mixture (10 μL) was dried on 400 mesh copper grids (ultrathin carbon type-A, Ted Pella, Redding, CA) and subsequently observed by TEM.

2.5. TCE degradation experiment

Trichloroethylene degradation was studied in 250-mL serum bottles containing 4 mL inoculation culture (25-fold dilution), 100 mg NZVI (1 g/L) and mineral salts medium needed to achieve 100 mL final volume. HEPES buffer (final concentration 60 mmol/L) was used to control a corrosion-induced increase in pH (Liu et al., 2007; Shin and Cha, 2008). These bottles were shaken at 200 rpm at room temperature (22 °C) throughout the experiment. Several batch treatments were examined with NZVI, dechlorinating culture, and their combination in the absence/presence of methanol (electron donor) to treat 20 mg/L TCE (15.2 μmol). The pH was adjusted to 7.2 by adding 1 M NaOH as needed. All treatments were amended with TCE (neat or dissolved in methanol) to provide an initial concentration of 20 mg/L (15.2 μmol). To discern volatilization losses, a sterilized solution (4 mL heat-treated culture) was used as a negative control. Duplicate bottles were established for each treatment. The concentration of TCE and its byproducts were measured over time. A summary of batch experiments is given in Table 1.

2.6. Statistical analyses

Standard deviations were determined for all analytical measurements (which were conducted in triplicate for duplicate treatments) using a Microsoft Excel 2003 spreadsheet, and are depicted as error bars in Figs. 2, 3 and 5. First-order rate constants for TCE degradation (k values) were determined by linear regression of $\ln(C/C_0)$ vs. time data, using Excel. Whether dechlorination was significant (i.e., $k > 0$) was assessed at the 95% confidence level using the Excel regression package.

Table 1
Summary of batch experiments.

Treatment	Components		
	NZVI (mg)	Bacteria ($\times 10^7$ cell/mL)	Methanol (e^- donor) (μL)
Set 1 (control)	0	0	0
Set 2 (NZVI)	100	0	0
Set 3 (mixed culture + methanol)	0	1.0 ± 0.2	100
Set 4 (mixed culture, e^- donor free)	0	1.0 ± 0.2	0
Set 5 (NZVI + mixed culture)	100	1.0 ± 0.2	0
Set 6 (NZVI + mixed culture + methanol)	100	1.0 ± 0.2	100

3. Results and discussion

3.1. Visualization of NZVI-bacterial interactions

The NZVI surface was initially smooth before reacting with TCE (Fig. 1a); however, after reacting with 15.2 μmol TCE in HEPES and mineral salts for 190 h, its shape became irregular and the particle surfaces were altered (Fig. 1b). This was presumably due to partial oxidation and reaction with the mineral salts, which decreased the reactivity of this NZVI (Liu et al., 2007). This change in surface chemistry suggests a decrease in surface reactivity.

These TEM images in the absence (Fig. 1c) and presence (Fig. 1d) of NZVI indicate that NZVI attaches to the bacterial surface. The small size and high surface to volume ratio of the particles result in significant adsorption of NZVI to the bacteria. This has also been observed with other types of NZVI (Lee et al., 2008). There was no evidence of cellular internalization of NZVI.

3.2. Cathodic H_2 analysis

To investigate whether H_2 evolved from NZVI via cathodic corrosion could serve as an electron donor for this mixed dechlorinating culture, three additional abiotic reactors amended with NZVI (1 g/L) were prepared. H_2 accumulation was significantly higher in abiotic reactors, with 730 ± 30 μmol H_2 produced in 166 h (Fig. 2). Reactors amended with the mixed dechlorinating culture accumulated only about 16.5 ± 4.0 μmol after 166 h, indicating that most H_2 produced was consumed by this anaerobic microbial community (e.g., methanogens and dechlorinators).

3.3. Effect of NZVI on methanogens

Methanogens can hinder dechlorination by competing for H_2 with dechlorinating bacteria (Yang and McCarty, 1998). Thus, the effect of NZVI on this competing pathway was studied. Fig. 3 shows that NZVI stimulated methanogenesis dramatically, with methane production during 502 h increasing from 58 ± 5 μmol with methanol alone as electron donor to 275 ± 2 μmol in the presence of NZVI (1 g/L). No methane was detected in the abiotic NZVI reactor.

The probable reason for this biostimulation was the increased amount of H_2 produced by NZVI. It has been previously reported that methanogenesis has a higher H_2 concentration threshold (10.9 ± 3.3 nM) than respiratory dechlorination (2.2 ± 0.9 nM), sulfate reduction (1–1.5 nM), Fe(III) reduction (0.2 nM) and nitrate reduction (0.05 nM) (Yang and McCarty, 1998). Apparently, the relatively high amount of H_2 available led to a preferential biostimulation of methanogens.

3.4. Dechlorination in the presence of NZVI

Trichloroethylene can be degraded both by dechlorinating bacteria as well as directly by NZVI in combined treatment. To compare the reduction rate of different systems, three experiments were conducted simultaneously in duplicate as follows: (i) TCE exposed to 1 g/l NZVI; (ii) TCE with 4 mL (25-fold dilution, 1.0 ± 0.2 × 10^7 cell/mL) dechlorinating culture; and (iii) TCE with both the dechlorinating culture and NZVI. A negative control without NZVI and bacteria was also conducted to discern the volatile and adsorption losses.

The dechlorinating culture (1.0 ± 0.2 × 10^7 cell/mL) without NZVI removed all 15.2 μmol TCE in 46 h ($k_{set\ 3}$ = 0.115 h^{-1}, Fig. 4a). The products were a mixture of *cis*-DCE, VC and ethene. The same culture in the presence of 100 mg of NZVI took 88 h to degrade the same amount of TCE ($k_{set\ 6}$ = 0.053 h^{-1}) and the difference in first-order dechlorination rate coefficients was statistically

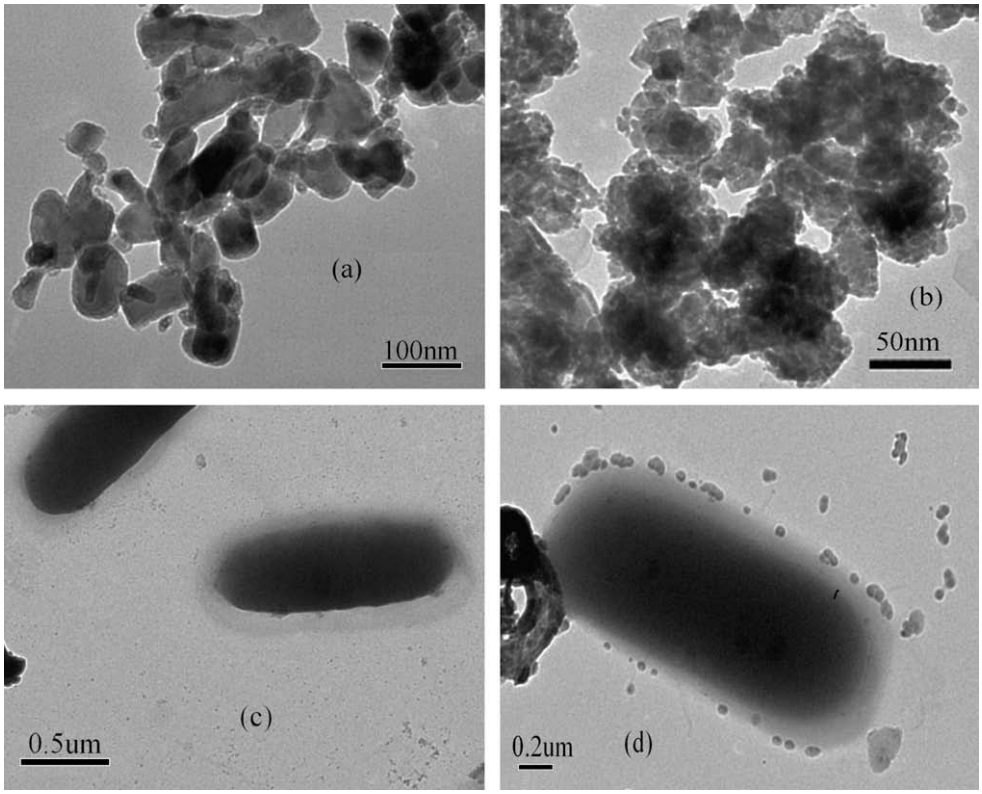

Fig. 1. TEM images of: (a) fresh NZVI; (b) NZVI after reacting with 15.2 μmol TCE in HEPES and mineral salts for 190 h; (c) unidentified bacteria before mixing with NZVI; (d) bacteria after mixing with NZVI (observed after 0.5 h of incubation with 1 g/L NZVI).

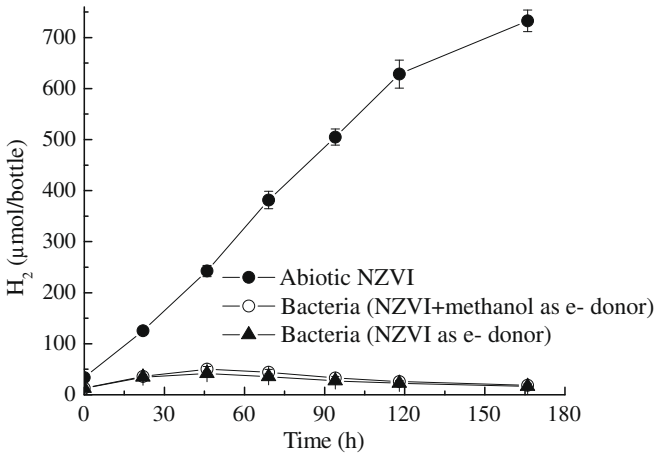

Fig. 2. H_2 evolution and consumption. Compared to the NZVI control (730 ± 30 μmol H_2 produced), only 16.5 ± 4.0 μmol H_2 were left when NZVI (1 g/L) served as the electron donor via cathodic corrosion.

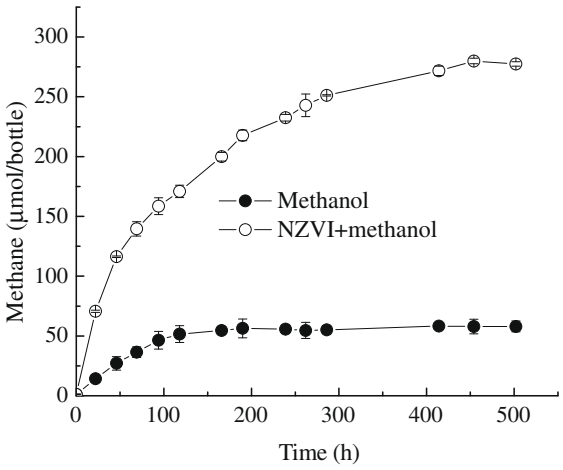

Fig. 3. Effect of NZVI on methanogenesis. Methane production was enhanced from 58 ± 5 μmol (with methanol as electron donor) to 275 ± 2 μmol in the presence of NZVI (1 g/L).

significant ($p = 0.019$). NZVI alone dechlorinated TCE at a much lower rate ($k_{set\ 2} = 0.006\ h^{-1}$, $p = 0.005$). These results suggest that dechlorinating activity was dominated by microbial dechlorination, which was hindered by NZVI. Sterilized cultures (negative controls without NZVI) did not dechlorinate TCE or produce ethene.

The population of dechlorinating bacteria (e.g., *Dehalococcoides* spp.) was expected to be biostimulated by the addition of NZVI. However, in contrast to methanogens, dechlorination activity was inhibited in the presence of NZVI. This result was similar to the effect of surfactants (Tween 80) on this culture. Tween 80 biostimulated methanogens but inhibited *Dehalococcoides* spp. (Amos et al., 2007). Apparently, *Dehalococcoides* spp. are more sensitive to such engineered interventions compared to methanogens.

Even though NZVI can dechlorinate TCE directly, the dechlorination rate of the combined NZVI-bacteria system decreased to less than one-half that of dechlorinating bacteria alone, with k_{obs} decreasing from 0.115 ± 0.005 h^{-1} to 0.053 ± 0.003 h^{-1} ($p = 0.019$). Thus, NZVI inhibited bacterial dechlorination. Although the mode of NZVI inhibition is unclear, it is conceivable that NZVI, which associates with the cell surface (Fig. 1d), affects bacterial membrane functions, which may ultimately result in cell death and lysis.

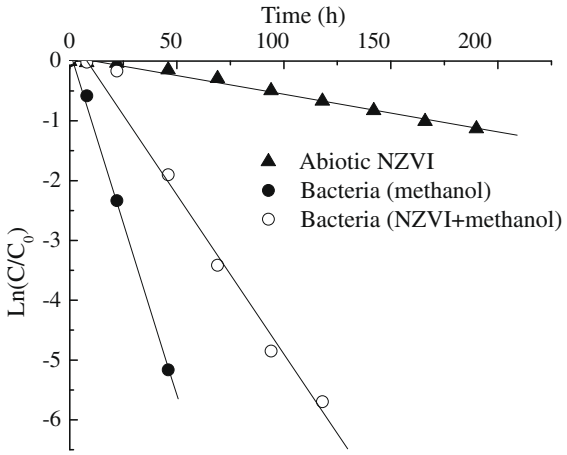

Fig. 4. First-order TCE degradation rate: (▲) $C = C_0e^{-0.006t}$, $R^2 = 0.99$, (●) $C = C_0e^{-0.115t}$, $R^2 = 0.99$, (○) $C = C_0e^{-0.053t}$, $R^2 = 0.98$. The rate constant for TCE biodegradation by dechlorinating culture in the presence of NZVI was $0.053 \pm 0.003\ h^{-1}$, lower than for biodegradation with methanol as sole electron donor ($0.115 \pm 0.005\ h^{-1}$, $p = 0.019$).

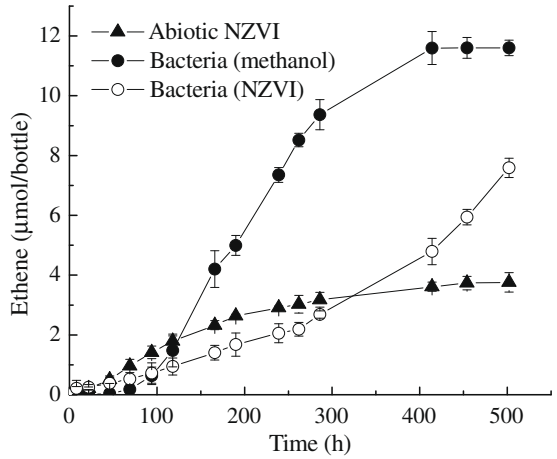

Fig. 5. Ethene production during biological and NZVI-mediated dechlorination. Ethene production was initially inhibited but then increased rapidly to 7.6 ± 0.3 μmol after 337 h.

3.5. Ethene production

It is well known that complete biological dechlorination of TCE follows the sequence of TCE, *cis*-DCE, VC and finally ethene (Freedman and Gossett, 1989; Gibson and Sewell, 1992; MaymoGatell et al., 1997). In contrast, NZVI reduces TCE directly into acetylene, ethene, and ethane, with only trace production of *cis*-DCE and VC (Liu et al., 2005). The production of ethene increased steadily when TCE was abiotically degraded by NZVI, with 3.8 ± 0.3 μmol ethene being produced in 502 h (Fig. 5). In the absence of NZVI, the dechlorinating bacteria produced 11.6 ± 0.5 μmol ethene in 414 h. In the NZVI-amended culture there was a lag time for ethene production in the first 337 h, after which ethene production increased rapidly to 7.6 ± 0.3 μmol. This suggests that the dechlorinating culture recovered from NZVI inhibition after about 300 h. A possible explanation for this observation is that when NZVI was first added to cell suspensions, it was reactive enough to contact and inhibit the dechlorinating bacteria. However, NZVI can be partially oxidized by water and passivated by mineral salts over time (Li and Zhang, 2006; Liu and Lowry, 2006; Liu et al., 2007), which likely decreased its reactivity and toxicity, and allowed the dechlorinating culture to recover.

4. Conclusions

Trichloroethylene dechlorination studies in the presence of NZVI or dechlorinating bacteria or both showed that (i) methanogens were biostimulated in the presence of NZVI; (ii) NZVI initially inhibited dechlorinating organisms, but dechlorination activity and ethane production recovered after a lag period; (iii) H_2 evolved from NZVI via cathodic corrosion can be utilized as electron donor by methanogens and dechlorinating bacteria. Overall, this study suggests that combining microbial reductive dechlorination with NZVI (either concurrently or sequentially) might be a feasible alternative to remediate DNAPL-contaminated sites. Nevertheless, combined NZVI-bioremediation treatment is unlikely to be universally applicable, and pilot studies should be conducted to identify potential critical limitations associated with scale up issues, including the adequate perfusion and distribution of NZVI, the survival of *Dehalococcoides* spp. if injected in bioaugmentation schemes, the need for pH control, and performance in the presence of potentially inhibitory compounds.

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