Passivation of zero-valent iron by denitrifying bacteria and the impact on trichloroethene reduction in groundwater

Liang Chen, Song Jin, Paul H. Fallgren, Fei Liu and Patricia J. S. Colberg

ABSTRACT

Zero-valent iron (ZVI) application in groundwater remediation is limited by its vulnerability to passivation, which significantly decreases its surface reactivity. Both biological and chemical processes can potentially passivate ZVI, although the understanding of biological passivation is limited. This study was conducted in bench-scale reactors packed with fresh ZVI or ZVI pre-exposed to nitrate (NO3) and in the presence or absence of a denitrifying bacterial enrichment (DNBE). The first-order rate coefficients (k) for NO3 reduction by ZVI in the presence and absence of DNBE were 0.20 and 0.09 s−1, respectively, suggesting that both ZVI and microbes contribute to NO3 removal. Abiotic reduction of nitrate was observed in reactors with trichloroethene (TCE) if ZVI was present; however, it resulted in reduced rates of TCE reduction (k = 0.29 s−1) when compared to reactors with fresh ZVI and no nitrate (k = 0.55 s−1). The TCE reduction efficiency decreased by 49% (k = 0.15 s−1) in the presence of DNBE, suggesting that microbial growth on ZVI or catalyzed oxidation of ZVI surface can inhibit TCE reduction by ZVI. Contrary to the presumption that denitrification may decrease ZVI passivation by nitrate, results from this study suggest that denitrifying bacteria actually exacerbate ZVI passivation.

Key words | dechlorination, denitrification, passivation, TCE, trichloroethene, ZVI

INTRODUCTION

Permeable reactive barriers (PRBs) are a technology commonly employed for in situ remediation of groundwater impacted by contaminants such as nitrate (NO3) and chlorinated solvents. Due to their tendency to accept electrons, these contaminants are typically removed by reductive processes. Zero-valent iron (ZVI) is a material commonly used in PRBs to achieve reductive remediation of contaminants in the groundwater (Gu et al. 2002; USEPA 2005; Phillips et al. 2010). For example, as described in Equation (1), reduction of the chlorinated solvent trichloroethene (TCE) by ZVI is an abiotic process that mainly follows a beta-elimination mechanism with acetylene as an intermediate and ethene as the final product (Taeyoon et al. 2004; USEPA 2005; ITRC 2005; Phillips et al. 2010).

\[
C_2HCl_3 + 3Fe^0 + 3H^+ \rightarrow C_2H_4 + 3Fe^{2+} + 3Cl^- \quad (1)
\]

In contrast, microbial mediated reductive dechlorination of TCE is a sequential process whereby toxic intermediates, such as dichloroethenes (DCEs) and vinyl chloride (VC), often accumulate as a result of its incomplete degradation (USEPA 2005; ITRC 2005; Chen et al. 2011).

One of the key issues facing the continued application of ZVI in groundwater remediation is its vulnerability to surface oxidation, deposits and precipitation, which can significantly decrease its reactivity and result in passivation (Daniel & Li 2001; Ritter et al. 2002; USEPA 2005; ITRC 2005). Both chemical and biological processes can potentially passivate ZVI in the groundwater environment.

Nitrate (NO3) is one of the most common groundwater constituents known to oxidize ZVI, forming an oxidized layer that may be composed of hematite (Fe2O3), goethite (FeOOH), and other oxyhydroxide mineral phases (Farrell et al. 2000; Phillips et al. 2000; Ritter et al. 2003; Devlin & Allin 2005; Liu et al. 2007; Luo et al. 2010; Reinsch et al. 2010). These iron oxides may, in turn, inhibit both electron transfer and catalytic hydrogenation by reducing surface contact between the contaminants of concern and the ZVI, which results in decreased rates of contaminant removal (Phillips et al. 2000; Devlin & Allin 2005).
Microorganisms are known to influence the performance of ZVI in PRBs (Gu et al. 1999; Liang et al. 2000; Phillips et al. 2000; Gu et al. 2002; Da Silva et al. 2007; Luo et al. 2010; Phillips et al. 2010; van Nooten et al. 2010). For example, iron-reducing bacteria can restore some reducing ability of ZVI by reducing ferric iron to ferrous iron (Da Silva et al. 2007; van Nooten et al. 2010). However, it has been reported that microbial fouling, mineral precipitation, and gas formation caused by growth and activity of indigenous microorganisms, results in decreasing pollutant reduction rates due to decreasing contact between the pollutant and ZVI (O’Hannesin & Gillham 1998; Liang et al. 2000; Gu et al. 2002).

Denitrifying bacteria (DNB) are common in groundwater and utilize NO₃⁻ as their terminal electron acceptor during organic carbon oxidation, producing CO₂ and N₂ as end products. In addition to the potential for these gases to occupy pores in a PRB, we hypothesized that DNB might also contribute directly to ZVI passivation. The ZVI may serve as an electron source for bacterial growth, resulting in surface oxidation of the ZVI and formation of less conductive metal oxides. In addition, when sufficient carbon (e.g. organic compounds) and electron (e.g. ZVI) sources are available in the groundwater where a ZVI PRB is installed, microbial growth on the ZVI surface is highly likely; the biofilm that forms may also reduce the ZVI as well as physically prevent its contact with target contaminants.

Literature reports on the role that bacteria may play in passivation of ZVI are very few; therefore, we designed a laboratory study to evaluate the impact of a denitrifying bacterial enrichment (DNB) on ZVI passivation. TCE reduction was evaluated in laboratory experiments conducted in bench-scale batch reactors packed with fresh ZVI or with ZVI pre-exposed to NO₃⁻ and in the presence and absence of a consortium of bacteria that contained an active population of DNB.

**MATERIALS AND METHODS**

**Denitrifying bacterial enrichments**

Sludge from an anoxic denitrification reactor was collected from the Dry Creek Water Reclamation Facility in Cheyenne, Wyoming, USA. The sludge sample was used as inoculum to enrich for denitrifying bacteria in large glass bottles with septa closures. Artificial groundwater was amended with NO₃⁻ for a final concentration of 20 mg L⁻¹ (NaNO₃, Sigma-Aldrich, St Louis, MO, USA). Glucose was provided as electron donor and carbon source (10 mg L⁻¹). The artificial groundwater contained (per liter): 58.8 mg NaHCO₃; 6.9 mg K₂CO₃; 100 mg CaCl₂; 65 mg MgCl₂; 0.25 mL of a trace mineral supplement (ATCC). The pH of the artificial groundwater was 7.5. Nitrate was re-amended to the enrichment cultures over a period of six weeks (about 10 times) at room temperature (22 ± 1 °C), whenever concentrations were depleted to non-detectable levels (<0.50 mg L⁻¹) as determined by ion chromatographic analysis (Dionex, Sunnyvale, CA, USA).

**Batch reactors**

A batch type experiment was conducted at room temperature (22 ± 1 °C). The reactors consisted of 250-mL glass bottles sealed with polytetrafluoroethylene (PTFE) septa and aluminum crimp seals. In one set of treatments (no TCE added), the following reactors were established in triplicate (Table 1): (1) sand + NO₃⁻; (2) sand + NO₃⁻ + DNBE; (3) sand + NO₃⁻ + ZVI; and (4) sand + NO₃⁻ + DNBE + ZVI. Each reactor was amended with glucose as a carbon source for the bacterial enrichment for a final concentration of 10 mg/L. The reactors with ZVI contained 200 g ZVI

<table>
<thead>
<tr>
<th>Material Designations</th>
<th>Treatment design for the batch-type experiment</th>
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<tbody>
<tr>
<td>Sand + NO₃⁻</td>
<td>1 2 0 600 10 10 1,000</td>
</tr>
<tr>
<td>Sand + NO₃⁻ + DNBE</td>
<td>1 2 0 600 10 10 1,000</td>
</tr>
<tr>
<td>ZVI + sand + NO₃⁻</td>
<td>1 2 200 400 10 10 1,000</td>
</tr>
<tr>
<td>ZVI + sand + NO₃⁻ + DNBE</td>
<td>1 2 200 400 10 10 1,000</td>
</tr>
<tr>
<td>Fresh ZVI + sand + NO₃⁻</td>
<td>2 200 400 10³ 100</td>
</tr>
<tr>
<td>Fresh ZVI + sand</td>
<td>2 200 400 – – 1,000</td>
</tr>
</tbody>
</table>

DNBE: denitrifying bacterial enrichment.
Fresh ZVI: not pre-exposed to nitrate.
TCE only added in set 2.
(HCA 150, 100 mesh; Hepure Technologies Inc., Wilmington, DE, USA) and 400 g of Ottawa quartz sand (EMD Chemicals, Gibbstown, NJ, USA). The reactors without ZVI contained 600 g of sand. The reactors containing bacteria (DNBE) were inoculated with 50 mL of an active denitrifying enriching culture. The DNBE was a mixed culture that exhibited high levels of denitrifying activity. Although numbers of organisms were not monitored in the reactors, the inoculum addition process was standardized so that replicate reactors should have contained an equal number of bacteria at the start of each experiment. All reactors were purged with nitrogen gas for 30 min to reduce dissolved oxygen concentrations. In the reactors with TCE, this step preceded TCE addition.

Nitrate in the form of NaNO₃ was added to all reactors for a final concentration of 10 mg NO₃ L⁻¹. As nitrate decreased to non-detectable levels, it was replenished to 10 mg L⁻¹. This was repeated nine times before reactors were amended one final time for an initial concentration of 10 mg L⁻¹ as NO₃.

A second set of batch reactors (Table 1) was established to evaluate the impact of nitrate amendments on ZVI reactivity and the presence of nitrate-reducing bacteria on TCE reduction. Nitrate was added to the reactors for an initial concentration of 10 mg L⁻¹. With one exception, repeated nitrate amendments were made as described for the first set of reactors. TCE was added to all reactors for a final concentration of approximately 1.000 µg L⁻¹.

This second set of reactors had two additional reactors. One set contained ZVI + sand + NO₃, but was not repeatedly amended with nitrate before addition of TCE. These reactors were meant to simulate in situ conditions, one would expect before passivation of ZVI. A second reactor type contained ZVI + sand only to simulate in situ conditions in the absence of any possibility of ZVI passivation. No glucose was added to these two types of reactor, but otherwise was prepared like all others in the study. Both of these reactor types will be referred to as having ‘fresh’ ZVI in the discussion that follows.

Analytical methods

Trichloroethene in the batch reactors was extracted in dichloromethane and analyzed on a HP 5,890 gas chromatograph with a 5972 mass spectrometer (Santa Clara, CA, USA). The column used was an Agilent DB-VRX Column (30 m × 0.25 mm × 1.4 µm). Nitrate was measured with a Dionex-100 ion chromatograph and AS14 column (Sunnyvale, CA, USA). Solid samples from the reactors were collected at the end of the experiment and dried under N₂ at ~20 °C. The solid samples were analyzed by field emission scanning electron microscopy (FESEM) to observe any qualitative changes on the ZVI surface.

RESULTS AND DISCUSSION

The effects of PRB on microbial communities and on autotrophic microbial denitrification are well documented in the literature. However, little information exists in the literature on the effects the microorganisms have on ZVI passivation. The following results present evidence of direct passivation by microbial activity, likely from the denitrifying bacteria in the DNBE.

In the first set of batch reactors, which contained no TCE, the reduction of NO₃ was monitored over 24 h. As presented in Figure 1, there was no NO₃ reduction in reactors containing sand only, while reactors containing sand and the DNBE exhibited clear evidence of microbial NO₃ reduction. As expected, all reactors containing ZVI exhibited much higher rates of NO₃ reduction than reactors without ZVI. The differences in NO₃ removal between ZVI reactors with and without the DNBE are indistinguishable at 24 h ([NO₃] / [NO₃]₀ of 0.07 and 0.03, respectively); however, based on the fraction of NO₃ remaining in the ZVI-free reactors with the DNBE after 48 h (0.21), it is reasonable to assume that at least some of the NO₃ removal in the ZVI + DNBE reactors (as much as 20%) may be due to reduction by the zero-valent iron.

Figure 1 | Reduction of nitrate (NO₃⁻) in batch reactors over time in the absence of TCE. Concentrations of nitrate measured at 0, 2, 4, 8, 12, 24, and 48 h are plotted relative to the initial NO₃⁻ concentration. Symbols used: ▼ sand + nitrate (no ZVI); △ sand + nitrate + denitrifying bacterial enrichment, DNBE (no ZVI); ◯ ZVI + sand + nitrate + DNBE; ▲ ZVI + sand + nitrate. Error bars represent the standard deviation of triplicate reactors.
Assuming first-order kinetics for NO₃⁻ reduction by ZVI, the first-order rate coefficients (k, s⁻¹) were determined by Equation (2):

$$k = \frac{t}{\ln\left(\frac{[NO_3^-]_i}{[NO_3^-]_t}\right)}$$

(2)

where, t is the reaction time (s) and $[NO_3^-]_i/[NO_3^-]_t$ is the inverse of the fraction of NO₃⁻ that was reduced at t.

A higher k value for NO₃⁻ reduction was realized for reactors amended with ZVI, sand, and the DNBE (0.20 s⁻¹) than in reactors with only ZVI and sand (0.09 s⁻¹). These values suggest that DNBE enhanced the NO₃⁻ reduction in the presence of ZVI (see Table 2); however, as stated previously, almost 20% of the observed reduction was likely due to the ZVI. During sampling for NO₃ analyses, a mean pH value of 10 was measured in the ZVI reactors; this apparently was not inhibitory to the DNBE. Elevated pH in ZVI media is consistent with observations described by the USEPA (2005) and ITRC (2005).

Figure 2 summarizes NO₃⁻ and TCE removal rates in batch reactors that were amended with TCE. As may be seen in Figure 2(a), there was no NO₃⁻ reduction in reactors containing sand only, while the DNBE in reactors containing sand only were apparently inhibited in the presence of TCE, as indicated by the high fraction of NO₃⁻ remaining (0.87) after 24 h. Based on these results, TCE appears to be toxic to DNB, which is consistent with other studies where TCE has been shown to inhibit NO₃⁻ reducing bacteria (Arcangeli & Arvin 1997; Halsey et al. 2005; Kocamemi & Cecen 2007; Singh & Olson 2010). These results also suggest that in the presence of TCE, the reduction of NO₃⁻ in all reactors containing ZVI was due to direct reduction by the ZVI with little or no contribution from the bacteria.

A much higher k value for NO₃⁻ reduction was realized for the ‘fresh’ ZVI + sand + NO₃⁻ amended reactors (0.43 s⁻¹) compared to reactors that were repeatedly exposed to NO₃⁻, which had k values ranging from 0.09 to 0.20 s⁻¹. These calculated differences in k values (see Table 2) suggest that the repeated additions of NO₃⁻ to the ZVI matrix before commencement of the experiment caused some oxidation (i.e. passivation) of the ZVI that, in turn, resulted in a diminished capacity of the zero-valent iron to reduce NO₃⁻.

As presented in Figure 2(b), TCE concentrations in reactors containing no ZVI exhibited little or no change over 24 h, while all reactors with ZVI removed TCE to non-detectable levels within 12-24 h. In reactors containing ‘fresh’ ZVI + sand or ‘fresh’ ZVI + sand + NO₃⁻, TCE was reduced completely within 12 h with k values of 0.55 and 0.27 s⁻¹ respectively.

Table 2 | First-order rate coefficients for reduction of nitrate in the presence and absence of TCE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No TCE k, s⁻¹</th>
<th>With TCE k, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand + NO₃⁻</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Sand + NO₃⁻ + DNBE</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>ZVI + sand + NO₃⁻</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>ZVI + sand + NO₃⁻ + DNBE</td>
<td>0.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Fresh ZVI + sand + NO₃⁻</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

DNBE: denitrifying bacterial enrichment.
Fresh ZVI: not pre-exposed to nitrate.
The fractions of TCE remaining in reactors amended with ZVI pre-exposed to NO₃ (ZVI + sand + NO₃ and ZVI + sand + NO₃ + DNBE) were 0.21 and 0.16, respectively, after 12 h. The k value calculated for TCE reduction in the presence of the DNBE was ~49% lower (0.15 s⁻¹ vs. 0.29 s⁻¹; see Table 3) than that in the absence of DNBE. These results indicate that DNBE can inhibit TCE reduction in a ZVI PRB, possibly by directly accepting electrons from ZVI, or by competing for reactive sites on the ZVI surface, resulting in decreasing TCE reduction by ZVI. The k values for TCE reduction were determined by Equation (3),

\[ k = \frac{t}{\ln([\text{TCE}]_i/[\text{TCE}]_f)} \]

where, [TCE]ᵢ/[TCE]ₖ is the inverse of the concentration fraction of TCE remaining in the reactors.

Analysis of the reactor solids by FESEM (Figure 3) suggest evidence of both oxidation and mineral precipitation on the ZVI surfaces, particularly in the ZVI reactors that had been repeatedly exposed to DNBE and NO₃ before the experiment. The formation of mineral precipitate layers (e.g. Fe₂O₃ and FeOOH) on the surface of ZVI decreases available sites for TCE reduction. These observations are consistent with other studies reporting decreasing TCE reduction efficiency during oxidization of ZVI by DNBE and nitrate (Phillips et al. 2010) and prevention of contact between TCE and the ZVI reactive surface (O’Hannesin & Gillham 1998; Liang et al. 2000; Gu et al. 2002).

In summary, the presence of DNBE substantially decreased the ZVI reactivity, as shown in its decreased rate of TCE reduction. This was likely attributed to microbial growth on the ZVI surface, nitrate-induced ZVI oxidation, microbially (NDBE) catalyzed ZVI oxidation, or the combination of all these pathways. Overall, these results suggest that NO₃-reducing bacteria may exacerbate ZVI passivation beyond that attributed to ZVI oxidation by NO₃ alone. This study offers some interesting new insights into the role that bacteria may play in ZVI passivation, an issue that clearly warrants additional research and attention when ZVI is used in remediation of contaminants existing in subsurface matrices. Knowledge on inhibiting microbial populations including the NDBE may be applied to alleviate microbially catalyzed passivation of ZVI, therefore extending the life-span of ZVI in various remediation practices.

Table 3 | First-order rate coefficients for reduction of TCE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>k, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand + NO₃</td>
<td>0.004</td>
</tr>
<tr>
<td>Sand + NO₃ + DNBE</td>
<td>0.006</td>
</tr>
<tr>
<td>ZVI + sand + NO₃</td>
<td>0.29</td>
</tr>
<tr>
<td>ZVI + sand + NO₃ + DNBE</td>
<td>0.15</td>
</tr>
<tr>
<td>Fresh ZVI + sand + NO₃</td>
<td>0.55</td>
</tr>
<tr>
<td>Fresh ZVI + sand</td>
<td>0.54</td>
</tr>
</tbody>
</table>

DNBE: denitrifying bacterial enrichment.
Fresh ZVI: not pre-exposed to nitrate.
ACKNOWLEDGEMENTS

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