Regeneration of iron for trichloroethylene reduction by *Shewanella alga* BrY

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Abstract

Zero valent iron (ZVI), the primary reactive material in several permeable reactive barriers, is often oxidized to ferrous or ferric iron, resulting in decreased reactivity with time. Iron reducing bacteria can recover the ferric iron to its ferrous form, prolonging the reduction of chlorinated organic contaminants. In this study, the reduction of Fe(II,III) oxide and Fe(III) oxide by a strain of iron reducing bacteria of the group *Shewanella alga* BrY (*S. alga* BrY) was observed in both aqueous and solid phases. *S. alga* BrY preferentially reduced dissolved ferric iron over the solid ferric iron. In the presence of iron oxide the Fe(II) ions produced by *S. alga* BrY efficiently reduced trichloroethylene (TCE). On the other hand, Fe(II) produced by *S. alga* BrY covered the reactive surfaces of ZVI iron filings and inhibited the reduction of TCE by ZVI. The formation of precipitates on the iron oxide or Fe 0 surface was confirmed by scanning electron microscopy. The results suggest that iron-reducing bacteria in the oxidized Fe 0 barriers can enhance the removal rate of chlorinated organic compounds and influence on the long-term performance of Fe 0 reactive barriers.

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Keywords: Trichloroethylene; Dechlorination; Iron reducing bacteria; *Shewanella alga* BrY; Permeable reactive barrier

1. Introduction

In recent years, Fe 0 -based permeable reactive barriers (PRBs) have been promoted as alternatives to conventional pump and treat systems (Blowes et al., 1997). Fe 0, also referred to as zero valent iron (ZVI), is readily available and has shown a potential to remediate contaminated subsurface. The use of ZVI can therefore lead to significant cost savings since it requires less energy and materials to operate than other alternative remediation methods. The degradation process involves the corrosion of ZVI, which provides the electrons necessary for reduction of compounds such as heavy metals, chlorinated organics, nitroaromatics, and nitrate (Agrawal and Tratnyek, 1996; Blowes et al., 1997; Gu et al., 1998; Furukawa et al., 2002).

The accumulation of corrosion products on Fe 0 surface can affect reduction either by inhibiting contaminant access to the metal surface or by forming new sites for contaminant adsorption, reaction, and catalysis to occur (Johnson and Tratnyek, 1994; Scherer et al., 1999). Several laboratory and field studies involving the long-term performance of Fe 0 have shown that the contaminant degradation rates decrease with time (Devlin et al., 1998; Gerlach et al., 2000; Phillips et al., 2000).

Several different iron reducing bacteria using Fe(III) oxyhydroxide and iron oxides as terminal electron acceptors have been identified (Lovley, 1997). These dissimilatory iron reducing bacteria (DIRB) are widely distributed in both pristine and contaminated terrestrial, aquatic, and subsurface environments. DIRB may play an important role in the long-term performance of Fe 0-based PRBs, i.e., respiration by DIRB can convert oxidized Fe(III) to...
Fe(II), and, thus, can reduce more contaminants (Gerlach et al., 2000).

The understanding of the underlying processes is, however, limited by a lack of information on the reactivity of ferrous iron produced during respiration of different ferric compounds with the contaminants. In this study, the reduction of different forms of iron–dissolved ferric citrate, Fe(II,III) oxide, and Fe(III) oxide by the bacterial strain Shewanella alga BrY (S. alga BrY) was investigated with the aim of answering this question. The enhancement of trichloroethylene (TCE) removal by microbially-generated Fe(II) was examined. The influence of the microbially-reduced Fe(II) on the reducing capability of ZVI was also investigated.

2. Experimental section

2.1. Experimental materials

TCE (C₂HCl₃; purity 99.5+ %, ACS grade, Aldrich) was the contaminant used in this research. Iron filings (<0.42 mm, Fisher), Fe(II,III) oxide (Fe₂O₃, powder, <5 μm, 98%, Aldrich), and Fe(III) oxide (Fe₂O₃, powder, <5 μm, 99+%, Aldrich) were used as reactive materials for TCE degradation. Dissolved ferric ions (as ferric citrate), powdered Fe(II,III) oxide, and powdered Fe(III) oxide were provided as electron acceptors during the respiration of the iron reducing bacteria in this study.

S. alga BrY (ATCC number 51181) obtained from American Type Culture Collection was kept aerobically in tryptic soy broth for 15 h in a shaker at 150 rpm and 25 °C. S. alga BrY has been identified and used as a DIRB in a previous research (Gerlach et al., 2000). The cultured cells were washed three times with the experimental medium solution to remove the tryptic soy broth, and then separated by centrifugation at 559g and 4 °C. The composition of the experimental medium was: 30 mM sodium lactate, 56 mM ferrous iron, 30 mM NaHCO₃, 1.3 mM KCl, 28 mM NaH₂PO₄, and 5 mM NaH₂PO₄. The enriched cells were then cultured anaerobically in the experimental medium at 30 °C, following the direction in the Product information sheet for ATCC 51181. All materials such as serum bottles, butyl rubber stoppers, syringes, and pipette tips were sterilized in an autoclave at 121 °C for 15 min.

2.2. Iron reduction by S. alga BrY

Batch experiments with S. alga BrY were conducted using 25 ml glass vials under anaerobic conditions. Three grams of each of two different solid irons (Fe(II,III) oxide and Fe(III) oxide), 27.5 ml of the experimental medium, and 0.5 ml of the enriched S. alga BrY were added to each vial. Ferric citrate was present in the experimental medium, where citrate and ferric ions served as an electron donor and an electron acceptor, respectively. The reduction of iron was determined by monitoring the Fe²⁺ in Fe(II,III) oxide and Fe(III) oxide. Initially, the pH of the experimental medium was adjusted to 7 and the bacterial concentration was 10⁶ CFU ml⁻¹. The vials were kept on a rotary shaker at room temperature and the samples were collected regularly to measure Fe²⁺ and ferrous plus ferric ion concentration (Fe_TOT) in aqueous and solid phases. The bacterial concentrations were also monitored for 160 h.

2.3. TCE degradation kinetics using various iron forms

Batch experiments for TCE degradation by iron filings (ZVI), Fe(II,III) oxide, and Fe(III) oxide were similarly conducted with S. alga BrY in 25 ml glass vials under anaerobic conditions. Three grams of each of three different solid irons, 27.5 ml of the experimental medium, and 0.5 ml of the enriched S. alga BrY were added to each vial. The pH of the experimental medium was initially adjusted to 7, and the solution was spiked with 30 mg l⁻¹ of TCE. The bacterial concentration was 10⁶ CFU ml⁻¹. The vials were capped with Teflon-lined septa in an anaerobic chamber, and then kept on a rotary shaker at room temperature. TCE concentrations were measured at regular sampling intervals. The enhancement in Fe³⁺ production by S. alga BrY addition was assessed by comparing TCE degradation in the aforementioned tests with that in vials with Fe(III) oxide supplemented with 200 mM Fe(II) solution and that in vials containing only the iron filings.

2.4. Analytical procedures

The bacterial concentrations were obtained by measuring adenosine triphosphate (ATP) with a Profile 1-Bioluminometer (New Horizons Diagnostics) and converting the measured ATP value to colony forming unit (CFU). Fifty microliters of the sample and 150 μl of the somatic cell releasing agent were added in a filtravette set placed on a blotter paper, which was then pressurized to separate non-biological ATP. Then 60 μl of the bacterial releasing agent and 50 μl of Luciferin/Luciferase were added. Subsequently, the ATP value was measured with the luminometer.

To measure dissolved Fe(II) and Fe_TOT, the vials containing iron were centrifuged at 559g and the supernatants were filtered through a 0.2 μm pore size filter (Pall). Dissolved Fe(II) and Fe_TOT were measured using the ferrozine method (Violette et al., 2000). Fe(II) and Fe_TOT bound on the solid surface were measured using the ferrozine method after extraction with 10 ml of 0.35 M HNO₃ for 24 h in an anaerobic chamber. Fe(III) was assumed to be Fe_TOT-Fe(II) in both dissolved and bound cases.

For TCE analysis, the supernatant was extracted with hexane (supernatant:hexane = 1:9, v/v) after centrifugation at 559g. TCE concentrations were analyzed by a Shimadzu GC-17A gas chromatograph, equipped with an AT-502.2 column (0.53 mm × 30 m, Altech) and an electron capture detector, operated at oven, injector, and detector temperatures of 100 °C, 250 °C, and 280 °C, respectively.

After 160 h of iron reduction by S. alga BrY, the iron samples were observed with scanning electronic micros-
copy (SEM). Samples for the analysis were prepared by discarding the supernatants in an anaerobic chamber after centrifugation at 559 g and 4 °C. The recovered splits were preserved in a 5% glutaraldehyde solution at 4 °C for 3 h and then washed three times with 0.05 M sodium cacodylate buffer (pH 7.2) for 10 min each at 4 °C. The suspensions were preserved in 1% osmium tetroxide at 4 °C for 2 h, and followed by washing twice with distilled water. Subsequently, the suspensions were pretreated with ethanol and mounted on metal stubs coated with gold for SEM (JSM-5410LV, JEOL, Japan) analysis.

3. Results and discussions

3.1. Iron reduction by *S. alga* BrY

In order to make sure that *S. alga* BrY can reduce Fe(III), the suspended bacteria were mixed with the iron oxides and TCE. Fig. 1 shows the suspended *S. alga* BrY cell growth and dissolved Fe(II) concentrations over 160 h in the vials containing the combinations of *S. alga* BrY, the iron oxides, the ferric citrate medium, and TCE. The aqueous Fe(II) concentrations increased rapidly due to the reduction of Fe(III) in the dissolved ferric citrate, and paralleled the cell concentration increase for initial 25 h. Subsequently, the Fe(II) concentrations decreased to 12–20 mM, following the decrease in the cell concentration.

The changes in Fe(II) and cell concentrations were similar in all cases. From this it is evident that *S. alga* BrY participated in iron reduction. There were no noticeable differences between two different oxides, which indicates that different iron oxides did not affect (from Fig. 1b and d, c and e). From the comparison between Fig. 1b and c, d and e, TCE also did not influence the bacterial reduction of Fe(III).

Typically cell concentrations in microbial experiments reach a peak and a plateau is then maintained for some time followed by a gradual decline. A rather sharp decline in cell population in this research may result from the production of toxic metabolites, but no additional analysis was performed to confirm this. Another possible explanation may be that the bacterial cells show increased adhesion to particulate matter over time, but it is also unlikely since no further decrease was not observed with the iron oxides ((b)–(e)) than without them (a).

The concentration of ferrous ions bound onto the solid phase started to increase after approximately 90 h in the vials containing *S. alga* BrY with ferric citrate medium (Fig. 2) and about 40 h in those with *S. alga* BrY and ferric citrate medium plus crystalline Fe(III) oxide (Fig. 3). Since

![Fig. 1. *Shewanella alga* BrY Cell growth and Fe(II) production in aqueous phase. (a) The bacteria with ferric citrate medium, (b) the bacteria with ferric citrate medium and Fe(III) oxide, (c) the bacteria with ferric citrate medium, Fe(III) oxide, and TCE, (d) the bacteria with ferric citrate medium and Fe(II,III) oxide, (e) the bacteria with ferric citrate medium, Fe(II,III) oxide, and TCE.](image-url)
it is not possible to separate the ferrous ions produced by biological reduction from the ferrous ions inherent in the Fe(II,III) oxide, the experiment with the Fe(II,III) oxide was not performed.

After 160 h, about 56% of the total ferrous iron was bound with *S. alga* BrY and the ferric citrate medium while about 73% was bound with *S. alga* BrY, the Fe(III) oxide, and dissolved ferric citrate. A comparison of the data in Fig. 2 versus Fig. 3 is consistent with the interpretation that reduction of solid Fe(III) oxide is occurring, or with the possibility that Fe(II) produced by reduction of soluble Fe(III) is preferentially sorbed by the solid iron oxide phase. However, the lack of controls that contain only solid phase iron makes it impossible to distinguish between these two possibilities in this study. Reduced Fe(II) can be precipitated as siderite (FeCO$_3$) or vivianite (Fe$_3$(PO$_4$)$_2$·(H$_2$O)$_8$) with HCO$_3^-$ or PO$_4^{3-}$ (Dong et al., 2000). A similar phenomenon involving the precipitation of Fe(II) was observed to occur in the current study, as shown by the complex aggregation of the solids and *S. alga* BrY in the SEM images presented in Fig. 2.

### 3.2. Enhancement of TCE degradation by the microbial reduction of ferric iron

The enhancement of TCE removal due to Fe$^{2+}$ is shown in Fig. 4 and Table 1. The reaction of TCE with iron can be approximated by the pseudo-first-order kinetic model and the reaction rate constants ($k$) can be calculated (Johnson et al., 1996; Cho and Park, 2005). Fig. 4 and Table 1 show that there was little TCE removal in the 200 mM Fe(II) solution only and in the *S. alga* BrY-amended ferric citrate medium without the solid iron oxide powders. The slight difference in TCE removal fractions between the two cases could be caused by the binding of TCE to the bacteria and the experimental medium. This indicates that aqueous...
ferrous ions were not effective in reducing TCE and whether they had been microbially reduced from ferric ion did not make any difference to that. More TCE removal was observed in the presence of the iron oxides. TCE removal after 24 h in the Fe(II,III) oxide only (35%) increased about one order of magnitude from that in the Fe(II) solution only (3%) and that in the S. alga BrY-amended ferric citrate medium (4%). Some portion of TCE removal from the aqueous phase could be attributed to the sorption to the solid iron powder surface, but no efforts for quantification were made in the present study (Cho and Park, 2005).

TCE removal in the Fe(II,III) oxide only (35%) was, interestingly, higher than that in the Fe(III) oxide and Fe(II) solution (15%). Assuming that physically sorbed masses of TCE onto the two different iron oxides in this research were similar, the ferrous ions on the Fe(II,III) oxide effectively reduced TCE while the aqueous ferrous ions did not. Also, electron shuttling to enhance the reduction of TCE between the physically closer Fe(II) and Fe(III) on the Fe(II,III) oxide could be more efficient than that between the aqueous Fe(II) and Fe(III) on the Fe(III) oxide.

Most TCE removal after 24 h was observed in the vials containing the iron oxides and S. alga BrY with ferric citrate medium. The pseudo-first-order reaction rate constant for TCE degradation in the vials with the Fe(II,III) oxide and S. alga BrY (1.3 × 10⁻³ h⁻¹) was higher than that in the vials with the Fe(II,III) oxide only (0.8 × 10⁻³ h⁻¹). TCE removal using the Fe(III) oxide with Fe²⁺ reduced by S. alga BrY (45% after 24 h and 1.1 × 10⁻³ h⁻¹, respectively) was higher than that using the Fe(III) oxide with Fe(II) solution (15% after 24 h and 0.3 × 10⁻³ h⁻¹, respectively). Therefore, biological reduction of Fe³⁺ further enhanced TCE removal than abiotic electron shuttling alone.

Separate TCE toxicity/consumption tests were performed, the result of which is not reported here, in order to make sure that TCE was not microbially degraded by S. alga BrY. The results from those tests indicated that TCE was not used as an electron donor or carbon source during respiration by S. alga BrY.

### 3.3. Effect of S. alga BrY on TCE removal by ZVI

TCE degradation rate by ZVI was compared with that by ZVI, S. alga BrY, and the ferric citrate medium in Fig. 5. ZVI degraded 80% of TCE while ZVI with S. alga BrY degraded only 37% of TCE. The reaction rate constant by ZVI with S. alga BrY (0.8 × 10⁻³ h⁻¹) was less than 28% of that with ZVI only (2.9 × 10⁻³ h⁻¹). From the experiment with ZVI and the ferric citrate medium only, it can be concluded that the effect of the medium on TCE degradation was less than that of the bacteria. It is believed that precipitation of reduced iron hydroxide and attachment of S. alga BrY to the surface of iron filings limited the access of TCE to the reactive sites on the ZVI. This is visually supported by the SEM image shown in Fig. 5.

### 4. Summary and conclusions

Reduction of iron oxides in aqueous and solid phases by S. alga BrY (ATCC number 51181) was investigated in batch mode. The precipitation of reduced Fe(II) hydroxides and attachment of S. alga BrY to the iron oxide was identified by measuring Fe(II) in the solid phase and physical observations of SEM images. In experiments with iron oxides, iron hydroxide and S. alga BrY covered on iron filings

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**Table 1**

TCE removal comparison between iron oxides, iron oxides with S. alga BrY, and iron(III) oxide with Fe(II) solution

<table>
<thead>
<tr>
<th>Contents</th>
<th>TCE removed after 24 h (%)</th>
<th>Reaction rate constant (10⁻³ h⁻¹)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron(II,III)oxide + S. BrY</td>
<td>51</td>
<td>1.3</td>
<td>0.92</td>
</tr>
<tr>
<td>with ferric citrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron(II,III)oxide + S. BrY</td>
<td>45</td>
<td>1.1</td>
<td>0.92</td>
</tr>
<tr>
<td>with ferric citrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron(II,III)oxide</td>
<td>35</td>
<td>0.8</td>
<td>0.90</td>
</tr>
<tr>
<td>with Fe(II) solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. BrY with ferric citrate</td>
<td>15</td>
<td>0.3</td>
<td>0.92</td>
</tr>
<tr>
<td>medium</td>
<td></td>
<td></td>
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<tr>
<td>Fe(II)solution</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Degradation of TCE using ZVI with Shewanella alga BrY (initial TCE concentration: 30 mg l⁻¹) and scanning electron microscopy image of ZVI and Shewanella alga BrY in ferric citrate medium.
des TCE degradation was fastest when S. alga BrY was present. However, TCE removal by S. alga BrY in experiments lacking a solid iron mineral phase was minimal. These results indicated that ferrous ions require the presence of the solid iron oxide surface to reduce TCE. With ZVI, however, the presence of S. alga BrY inhibited the reduction of TCE, possibly due to the accumulation of iron hydroxides and bacteria on the reactive surface of iron filings.

In summary, the presence of DIRB in PRBs can influence their longevity by reducing the oxidized iron particles to ferrous iron ions, which become available for reduction of additional contaminants, such as TCE. The most practical way to apply the findings in this research to the field is to inoculate the DIRB along with the medium to the PRB that is no longer as efficient as before. Alternatively, only proper medium for DIRB may be added to the spent PRB where the microbial activity of iron-reducing bacteria is active. However, the effect of DIRB on contaminant reduction in other less oxidized iron forms, or ZVI, is expected to be minimal, or even inhibitory. Several practical problems, such as the potential decrease in hydraulic conductivity by biomass accumulation and the means for delivering electron donors to the DIRB, need to be addressed for successful field application of this technology in the field.

References


