Evaluation of the effects of nanoscale zero-valent iron (nZVI) dispersants on intrinsic biodegradation of trichloroethylene (TCE)

Y. C. Chang, S. C. Huang and K. F. Chen

ABSTRACT

In this study, the biodegradability of nanoscale zero-valent iron (nZVI) dispersants and their effects on the intrinsic biodegradation of trichloroethylene (TCE) were evaluated. Results of a microcosm study show that the biodegradability of three dispersants followed the sequence of: polyvinyl alcohol-co-vinyl acetate-co-itaconic acid (PV3A) > polyoxyethylene (20) sorbitan monolaurate (Tween 20) > polyacrylic acid (PAA) under aerobic conditions, and PV3A > Tween 20 > PAA under anaerobic conditions. Natural biodegradation of TCE was observed under both aerobic and anaerobic conditions. No significant effects were observed on the intrinsic biodegradation of TCE under aerobic conditions with the presence of the dispersants. The addition of PAA seemed to have a slightly adverse impact on anaerobic TCE biodegradation. Higher accumulation of the byproducts of anaerobic TCE biodegradation was detected with the addition of PV3A and Tween 20. The diversity of the microbial community was enhanced under aerobic conditions with the presence of more biodegradable PV3A and Tween 20. The results of this study indicate that it is necessary to select an appropriate dispersant for nZVI to prevent a residual of the dispersant in the subsurface. Additionally, the effects of the dispersant on TCE biodegradation and the accumulation of TCE biodegrading byproducts should also be considered.

Key words | dispersant, intrinsic biodegradation, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), trichloroethylene (TCE)

INTRODUCTION

Contamination of soil and groundwater by pesticides, organic solvents, petroleum hydrocarbons and other organic compounds has become a serious problem. Chlorinated solvents such as trichloroethylene (TCE) are mainly used for degreasing and dry cleaning, and have entered the environment through the leakage of storage tanks and irresponsible disposal (Jencova et al. 2004; Kargi & Eker 2005). Conventional pump-and-treat technology may control spill plumes but it has limited effectiveness in remediating groundwater pollution (Smidt & de Vos 2004; Aulenta et al. 2005; Demnerova et al. 2005). Recently, nanoscale zero-valent iron (nZVI) has been widely used for the treatment of chlorinated compounds due to its high reactivity and strong reducing power (Cundy et al. 2008). Chlorinated compounds can be reductively dechlorinated by nZVI according to the following reaction (Chen et al. 2012a)

$$\text{Fe}^0 + \text{RCl} + \text{H}^+ \rightarrow \text{Fe}^{2+} + \text{RH} + \text{Cl}^-$$

(1)

However, the aggregation and sedimentation of nZVI usually limit the transport of nZVI in the subsurface. Therefore, dispersants such as polyelectrolytes, carboxymethyl cellulose and surfactants are applied to improve the mobility of nZVI in aquifers (Sun et al. 2007; Keane 2009; Jiemvarangkula et al. 2011). In recent years, there has been increased interest in the use of microorganisms for environmental restoration. Chlorinated pollutants such as TCE are generally resistant to biodegradation as microorganisms do not use them as a carbon and energy source (Wilson & Wilson 1985). However, for some anaerobic microbes,
TCE acts as an electron acceptor in the reductive dehalogenation (Lee et al. 2007). Furthermore, some bacteria are able to utilize TCE as a secondary substrate through the process of cometabolism (Hubert et al. 2005).

Although many studies focus on the enhancement of stabilization, dechlorination capacity and transport of nZVI (Keane 2009), information on the effects of nZVI dispersants on the intrinsic biodegradation of contaminants is lacking. In addition, it has been reported that the lifetime of nZVI dispersants, especially following injection into the subsurface, is generally unknown (Grieger et al. 2010). Therefore, the biodegradability of nZVI dispersants at contaminated sites needs to be determined. Additionally, incomplete degradation of contaminants may occur when nZVI is applied in the field (Keane 2009). Thus, intrinsic biodegradation would play an important role in subsequent contaminant removal.

In this study, the biodegradability of nZVI dispersants and their effects on the intrinsic biodegradation of TCE were evaluated. Since polyacrylic acid (PAA) (Kim et al. 2009; Lin et al. 2010), polyvinyl alcohol-co-vinyl acetate-co-itaconic acid (PV3A) (Sun et al. 2007; Jiemvarangkula et al. 2011), and polyoxyethylene (20) sorbitan monolaurate (Tween 20) (Kanel et al. 2007; Dong & Lo 2013) are commonly used to modify the surface of nZVI, these three dispersants were selected for the evaluation. Additionally, bacterial counts and molecular biotechnology were used to evaluate possible adverse effects of the dispersants on native microorganisms. The main purposes of this study were to:

1. evaluate the biodegradability of the dispersants under aerobic and anaerobic conditions;
2. evaluate the effects of the dispersants on TCE natural biodegradation; and
3. determine the effects of the dispersants on microbial numbers and community.

### MATERIALS AND METHODS

#### Materials

The target compound, TCE (C₂HCl₃, >99%), was purchased from J.T. Baker, Germany. The dispersants used were as follows: PAA (MW 8,000–12,000, Showa Corporation, Japan), PV3A (MW 4300–4400, Shimakyu's Pure Chemicals, Osaka, Japan), and Tween 20 (MW 1227.5, Shimakyu's Pure Chemicals, Osaka, Japan).

#### Microcosm study

A microcosm study was conducted using 60 mL serum bottles under aerobic and anaerobic conditions to evaluate the biodegradability of the dispersants and their effects on the intrinsic biodegradation of TCE. Aquifer soil collected from a TCE-contaminated site was used as the source of in situ microorganisms. Each bottle was filled with 10 g of the aquifer soil and 50 mL mineral medium (Chen et al. 2012b) with required TCE and dispersant concentrations. Control bottles contained 250 mg/L of HgCl₂ and inocula used for the control groups were autoclaved before use. All batch experiments were conducted in duplicate and kept at 28 ± 2°C in darkness until analysis. Table 1 shows the experimental parameters of the microcosm study.

<table>
<thead>
<tr>
<th>Redox condition</th>
<th>Group</th>
<th>Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>Dispersant biodegradation</td>
<td>PAA 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PV3A 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tween 20 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td>TCE biodegradation</td>
<td>TCE 10 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + PAA 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + PV3A 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + Tween 20 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Dispersant biodegradation</td>
<td>PAA 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PV3A 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td>TCE biodegradation</td>
<td>TCE 10 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + PAA 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + PV3A 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCE 10 mg/L + Tween 20 1,000 mg/L + aquifer soil 10 g + mineral medium 50 mL</td>
</tr>
</tbody>
</table>
Methods of analysis

Aqueous samples were pretreated using purge and trap equipment and were then analysed for TCE, trans-dichloroethylene (trans-DCE), cis-DCE, 1,1-DCE and vinyl chloride (VC) using a gas chromatograph/flame ionization detector (GC/FID). The GC/FID used a capillary column (GsbP-624, 60 m × 0.32 mm) with nitrogen gas (99.9995% purity) flowing at 10 mL/min as the carrying gas. The operating temperatures were maintained at 180 °C for the injector and 230 °C for the detector. The oven temperature was initially maintained at 35 °C for 5 min, then elevated with a temperature ramp of 11 °C/min to 115 °C, and held at 115 °C for 5 min. Afterwards, the temperature was raised with a temperature ramp of 20 °C/min to 220 °C, and then held at 220 °C for 1 min.

Microbial analysis

Total bacterial count and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analyses were performed during batch experiments to evaluate the effects of different dispersants on the indigenous microbial community. The total heterotrophic count was used to assess an approximate enumeration of the total numbers of bacteria in soil samples using the spread plate method (APHA 2006). Prepared plates were incubated at 30 °C for 48 hours, then counted for colony forming units. DNA extraction was conducted with 0.5 g of soil samples using a PowerSoil® DNA Isolation Kit (Mo Biol, USA). The V6-V8 region of the 16S rDNA was amplified using the primers 968F (5′-AAC GCG AAG AAC CTT AC-3′) and 1401R (5′-CGG TGT GTA CAA GAC CC-3′). PCR amplification was conducted with an initial denaturation at 97 °C for 5 min and then 28 cycles of annealing at 95 °C for 1 min, 54 °C for 40 s and 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. A denaturing DGGE using a Bio-Rad DCode system (Bio-Rad, Hercules, CA, USA) was further performed on each amplified PCR product to monitor the changes in microbial diversity during the experiments. A 10% polyacrylamide gel with a 40–60% denaturant gradient was used and electrophoresis was performed at 60 °C and 65 V for 870 min. After electrophoresis, the gels were stained by the silver-stain method.

RESULTS AND DISCUSSION

Effects of dispersants on intrinsic microorganisms

Figure 1 shows the dispersants’ biodegradation by intrinsic microorganisms. As shown in Figures 1(a) and 1(b), PAA

![Figure 1](https://iwaponline.com/wst/article-pdf/69/11/2357/470816/2357.pdf)

**Figure 1** | Dispersants’ biodegradation by intrinsic microorganisms: (a) aerobic biodegradation, (b) anaerobic biodegradation, (c) total bacterial count under aerobic condition.
was not biodegraded while PV3A and Tween 20 could be removed by in situ microorganisms under both aerobic and anaerobic conditions during 140 days of incubation. Removal of PV3A and Tween 20 reached 100% and 82%, respectively, under aerobic conditions during 140 days of reaction. Aerobic biodegradation of Tween 20 was a little slower than that of PV3A. The biodegradability of the three dispersants followed the sequence of: PV3A > Tween 20 > PAA under aerobic conditions. Complete degradation of PV3A was achieved under anaerobic conditions during a 130-day incubation. Although Tween 20 was also biodegraded under an anaerobic environment, the biodegradation of Tween 20 seemed to be limited. The biodegradability of the three dispersants followed the sequence of: PV3A > Tween 20 > PAA under anaerobic conditions. The results indicate that PAA was not biodegradable in the subsurface, probably due to its high molecular weight (MW 8,000–12,000).

Figure 1(c) shows the total bacterial counts during the aerobic microcosm study. The results reveal that the biodegradability of the dispersants did not seem to be consistent with the growth of in situ microorganisms. Total bacteria counts with the three dispersants were: Tween 20 > PV3A ≧ PAA under aerobic conditions. Since Tween 20 was biodegradable, a better growth of native microorganisms was observed. Although PV3A was also biodegradable, the growth of in situ microbes with PV3A was not as good as that with Tween 20. This may be due to the fact that PV3A has a higher molecular weight and more complex structure than Tween 20. Therefore, most energy produced from bacterial metabolism might be used to break PV3A molecular, resulting in less reproduction of in situ bacteria. Since PAA was not biodegraded, intrinsic bacteria may use natural organic matters as carbon sources to support their growth.

**TCE removal by intrinsic biodegradation**

The biodegradability of TCE at the polluted site was investigated with an initial TCE concentration of 10 mg/L. Figure 2(a) shows the degradation of TCE under aerobic conditions. In situ microorganisms decomposed 83% of TCE during approximately 140 days of incubation. Native bacteria may use natural organic matters of aquifer sediments to cometabolize TCE under aerobic conditions. Figure 2(b) presents the degradation of TCE under anaerobic conditions. Biodegradation of TCE reached 77%
during approximately 140 days of incubation. The byproducts of TCE anaerobic biodegradation including cis-DCE and trans-DCE were formed with highest concentrations of 0.018 and 0.007 mg/L, respectively, after 5 days of incubation (Figure 2(c)). Therefore, the removal of TCE under anaerobic conditions was mainly attributed to reductive dechlorination. The results indicate that intrinsic microorganisms were able to degrade TCE under both aerobic and anaerobic conditions.

Effects of dispersants on TCE removal by intrinsic microorganisms

Since dispersants may affect the biodegradation of TCE, the effects of the three selected dispersants on TCE biodegradation were evaluated. Figure 3 shows TCE biodegradation with the presence of dispersants. Results show that the removal of TCE with the addition of the dispersants was similar to that of TCE alone (Figure 3(a)). Although PV3A and Tween 20 could be biodegraded under aerobic conditions, TCE removal was not enhanced with the addition of these two dispersants. This indicates that intrinsic microorganisms mainly used natural organic matter as a primary carbon source to cometabolize TCE. As shown in Figure 3(b), the addition of PAA seemed to have a slightly adverse impact on TCE biodegradation under anaerobic conditions while TCE anaerobic biodegradation was not affected by PV3A and Tween 20. However, higher accumulation of TCE anaerobic biodegrading byproducts was detected with the addition of PV3A and Tween 20, probably due to the preference of native microbes for the more biodegradable dispersants. In microcosms constructed with PAA, only cis-DCE and trans-DCE were observed, with highest concentrations of 0.016 and 0.010 mg/L, respectively. However, in addition to cis-DCE and trans-DCE, 1,1-DCE and VC were also produced with the addition of PV3A. The highest concentrations of cis-DCE, trans-DCE, 1,1-DCE and VC reached 0.020, 0.056, 0.015 and 0.025 mg/L, respectively. With the presence of Tween 20, the highest concentrations of cis-DCE, trans-DCE and VC reached 0.020, 0.021 and 0.023 mg/L, respectively. Results from the microcosm study indicate that no significant effects were observed on the intrinsic biodegradation of TCE under aerobic conditions with the presence of the three selected dispersants. However, the addition of PAA caused a little decrease of TCE removal under anaerobic conditions. The addition of PV3A and Tween 20 would cause higher accumulation of the byproducts of TCE anaerobic biodegradation.

DGGE analysis of bacterial community

DGGE analysis of 16S rDNA fragments was applied to investigate the bacterial community changes during microcosm study. Figure 4 presents the profiles of DGGE analysis under aerobic conditions. As shown in Figure 4(a), the increase of microbial diversity was consistent with the biodegradability of the dispersants. The addition of PAA seemed to have a slightly adverse impact on TCE biodegradation under anaerobic conditions while TCE anaerobic biodegradation was not affected by PV3A and Tween 20. However, higher accumulation of TCE anaerobic biodegrading byproducts was detected with the addition of PV3A and Tween 20, probably due to the preference of native microbes for the more biodegradable dispersants. In microcosms constructed with PAA, only cis-DCE and trans-DCE were observed, with highest concentrations of 0.016 and 0.010 mg/L, respectively. However, in addition to cis-DCE and trans-DCE, 1,1-DCE and VC were also produced with the addition of PV3A. The highest concentrations of cis-DCE, trans-DCE, 1,1-DCE and VC reached 0.020, 0.056, 0.015 and 0.025 mg/L, respectively. With the presence of Tween 20, the highest concentrations of cis-DCE, trans-DCE and VC reached 0.020, 0.021 and 0.023 mg/L, respectively. Results from the microcosm study indicate that no significant effects were observed on the intrinsic biodegradation of TCE under aerobic conditions with the presence of the three selected dispersants. However, the addition of PAA caused a little decrease of TCE removal under anaerobic conditions. The addition of PV3A and Tween 20 would cause higher accumulation of the byproducts of TCE anaerobic biodegradation.

Figure 3 | TCE biodegradation with the presence of dispersants under (a) aerobic conditions and (b) anaerobic conditions.
was not utilized by *in situ* microbes, the increase of the strains should be due to the biodegradation of TCE and natural organic matters.

Figure 5 shows the profiles of DGGE analysis under anaerobic conditions. Since PAA was not biodegraded under anaerobic conditions, significant microbial community changes were not observed (Figure 5(a), Lanes 1–3). Anaerobic degradation of PV3A caused a slight increase of microbial diversity (Figure 5(a), Lanes 4–6). Although biodegradation of Tween 20 was limited under anaerobic conditions, the microbial diversity with Tween 20 addition was also enhanced (Figure 5(a), Lanes 7–9). The addition of the three selected dispersants caused significant changes to the microbial community during TCE anaerobic biodegradation. However, the variation of the microbial community did not affect TCE removal except for the addition of PAA in the microcosms.

CONCLUSIONS

In this study, the biodegradability of three nZVI dispersants (PAA, PV3A and Tween 20) and their effects on the intrinsic biodegradation of TCE were evaluated. The conclusions of this study are described as follows:

1. The biodegradability of the three dispersants followed the sequence of PV3A > Tween 20 > PAA under aerobic conditions and PV3A > Tween 20 > PAA under anaerobic conditions. PAA was not biodegradable under both aerobic and anaerobic conditions.
2. Intrinsic microorganisms were able to degrade TCE under both aerobic and anaerobic conditions. TCE could be removed by cometabolism using soil organic matters under aerobic conditions while microbial reductive dechlorination of TCE occurred with the
production of less chlorinated byproducts under anaerobic conditions.

3. No significant effects were observed on the intrinsic biodegradation of TCE under aerobic conditions with the presence of the three selected dispersants. The addition of PAA seemed to have slightly adverse impact on TCE biodegradation under anaerobic conditions while TCE anaerobic biodegradation was not affected by PV3A and Tween 20.

4. Higher accumulation of TCE biodegrading byproducts was detected with the addition of PV3A and Tween 20 under anaerobic conditions, probably due to the preference of native microbes for the more biodegradable dispersants.

5. The increase of microbial diversity was positively correlated with the biodegradability of the dispersants under aerobic conditions.

6. It is necessary to select an appropriate dispersant for nZVI to prevent the residual of the dispersant in the subsurface. Additionally, the effects of the dispersant on TCE biodegradation and the accumulation of TCE biodegrading byproducts should also be considered.

**REFERENCES**


Keane, E. 2009 * Fate, Transport, and Toxicity of Nanoscale Zero-valent Iron (nZVI) used during Superfund Remediation*. US Environmental Protection Agency, Washington, DC.

Kim, H. J., Phenrat, T., Tilton, R. D. & Lowry, G. V. 2009 *Fe0 Nanoparticles remain mobile in porous media after aging due to slow desorption of polymeric surface modifiers*. Environmental Science & Technology 43 (10), 3824–3830.


First received 11 November 2013; accepted in revised form 18 March 2014. Available online 31 March 2014.