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# Benzene and toluene biodegradation down gradient of a zero-valent iron permeable reactive barrier

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ABSTRACT

This study simulated benzene and toluene biodegradation down gradient of a zero-valent iron permeable reactive barrier (ZVI PRB) that reduces trichloroethylene (TCE). The effects of elevated pH (10.5) and the presence of a common TCE dechlorination by product [*cis*-1,2-dichloroethene (*cis*-1,2-DCE)] on benzene and toluene biodegradation were evaluated in batch experiments. The data suggest that alkaline pH (pH 10.5), often observed down gradient of ZVI PRBs, inhibits Fe(III)-mediated biotransformation of both benzene and toluene. Removal was reduced by 43% for benzene and 26% for toluene as compared to the controls. The effect of the addition of *cis*-1,2-DCE on benzene and toluene biodegradation was positive and resulted in removal that was greater than or equal to the controls. These results suggest that, at least for *cis*-1,2-DCE, its formation may not be toxic to iron-reducing benzene and toluene degrading bacteria; however, for microbial benzene and toluene removal down gradient of a ZVI PRB, it may be necessary to provide pH control, especially in the case of a biological PRB that is downstream from a ZVI PRB.



## 1. Introduction

With dwindling supplies of groundwater, treatment of contaminated water sources is becoming more critical, especially those containing mixed plumes comprised of petroleum hydrocarbons and volatile chlorinated hydrocarbons. Permeable reactive barriers (PRBs) are an effective way to treat these contaminants by reducing chlorinated hydrocarbons with passage through zero-valent iron (ZVI) and by degrading petroleum hydrocarbons with bacteria that colonize the PRB [1].

There have been more than 200 zero-valent iron permeable reactive barriers (ZVI PRBs) used worldwide since the first field demonstration of the technology in 1995. These PRBs have mainly been used to remove chlorinated hydrocarbons and some other pollutants; the U.S. Environmental Protection Agency designated the ZVI PRB a standard remediation technique in 2002 [2–5].

Remediation of benzene, toluene, ethylbenzene, and xylene (BTEX) is mainly accomplished by microbial degradation. Lenka et al. [6] studied BTEX and naphthalene degradation using a ZVI-free biological permeable reactive barrier (bio-PRB). They achieved BTEX and naphthalene removal rates of 57.3% and 99.9%, respectively. Biodegradation of BTEX has been investigated widely and deemed an efficient and environmentally compatible way to treat contaminated groundwater [7–12].

Previous studies have largely focused on using one method to treat a single pollutant; few effective methods have been established for the removal of mixed contaminants in groundwater, mainly because of the variation in chemical properties of mixed plumes. BTEX is easily degraded by microorganisms under aerobic conditions, but is unaffected by ZVI [13–15]. Chlorinated hydrocarbons like trichloroethylene (TCE) are readily reduced by ZVI, but are thought to be toxic to BTEX-degrading bacteria [16–18]. The challenge then is to design a two-stage PRB system that segregates the two processes: ZVI designed for reductive dechlorination of TCE followed by a bio-PRB for removal of BTEX. Concerns that must be addressed in such a configuration include the potential toxicity of the chlorinated intermediate compounds. Halogenated intermediates like *cis*-1,2-dichloroethene (*cis*-1,2-DCE) and vinyl chloride (VC) may penetrate the ZVI PRB, move into the bio-PRB, and inhibit BTEX removal [19–21]. In addition, most microorganisms have an optimal pH near 7 [22–24]; however, pH may be a concern as well since pH down gradient from ZVI PRBs is always high (>9.0; [4,25–27]). Microbial degradation of BTEX in the subsurface is affected by many other factors as well, including the concentration of BTEX, redox potential (Eh), dissolved oxygen (DO) in the case of aerobic degradation, and availability of other electron acceptors (i.e., $NO_3^-$, $Fe^{3+}$, $SO_4^{2-}$) [19,28].

In this study, we focused on changes that might occur in the groundwater chemistry down gradient of a two-stage ZVI PRB designed to sequentially treat TCE, benzene and toluene. Labora-

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tory batch experiments were conducted with natural groundwater and ZVI added to investigate the effects of increased pH and the presence of *cis*-1,2-DCE on the Fe(III)-mediated biodegradation of benzene and toluene.

## 2. Materials and methods

### 2.1. Groundwater and soil

Groundwater was obtained from a well located on the campus of China University of Geosciences in Beijing, PRC. The concentrations (mg/L) of $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Cl^-$, $NO_3{}^-$, $SO_4{}^{2-}$ in the groundwater were 1.92, 18.01, 73.96, 33.3, 0.21, 47.29, 9.12, 69.78, respectively. The groundwater used in all experiments was first purged with nitrogen to reduce the DO to less than 1 mg/L to simulate the low redox conditions commonly found down gradient of ZVI PRBs. After purging, iron filings were added on a 20% (v/v) basis. The groundwater was stored for 15 d to allow the iron filings to react completely with the groundwater and release some Fe(III) for use as electron acceptor during biodegradation.

Dissolved oxygen was monitored by a HQ-30d Dissolved Oxygen Meter (Hach, Loveland, CO, USA). An o-phenanthroline spectrophotometric method was used to determine the $Fe^{2+}$ and total iron concentrations. Other ions ($NO_3{}^-$ and $SO_4{}^{2-}$) were determined by ion chromatography (Dionex 120, Dionex Corporation, Bannockburn, IL, USA).

Petroleum contaminated soil was obtained from an oilfield in Liaoning Province, PRC. The concentration of organic carbon was 48.53 g/kg, and the C:N was 112.3 [29]. This soil was used in the batch experiments as the source of bacteria for benzene and toluene degradation.

### 2.2. Enrichment, cultivation and inoculation procedures

An enrichment process was employed to obtain benzene and toluene degrading bacteria from soils that had a long history of petroleum contamination. Soil samples (300 g) were added to 500-mL wide-mouth, brown glass jars and closed with butyl rubber plugs. Groundwater was added leaving a small void space. The bottles were then sealed, shaken, and incubated in the dark at 23 °C for 5–7 days.

About 300 mL of water from the initial enrichments were transferred to 1-L amber glass bottles fitted with polytetrafluoroethylene-lined screw cap closures. Fresh groundwater was added and purged with $N_2$ for 1 h to reduce the DO, followed by addition of benzene and toluene for an initial concentration of ~200 μg/L of each. After 5–7 days of incubation under conditions described previously for the initial enrichments, 500 mL of the solution phase was transferred to a new 1-L amber glass bottle. More groundwater, benzene and toluene (~500 μg each) were added. Control bottles, containing groundwater, benzene and toluene but no bacteria from the soil enrichments, showed no evidence of benzene and toluene removal. Analysis of the benzene and toluene from the bottles that contained the soil inocula indicated that they were removed with time, suggesting that the desired bacterial population had been successfully enriched.

Laboratory batch experiments were used to investigate the effects of elevated pH and *cis*-1,2-DCE on benzene and toluene biodegradation. A PB-10 Precision pH meter (Sartorius, Göttingen, Germany) was used to measure pH.

Three separate experiments were conducted with increased pH and/or with *cis*-1,2-DCE added; experiments were designated E-1, E-2 or E-3. Because the pH of groundwater down gradient of ZVI permeable reactor barriers is often greater than 9.0 (e.g., Puls et al. [30] has reported a maximum pH of 10.7), pH 10.5 was chosen as the elevated pH in our experiments. Since the Maximum Contaminant Level (MCL) for *cis*-1,2-DCE established by the U.S. EPA is 70 μg/L [4,31], and the concentration of *cis*-1,2-DCE in groundwater after ZVI PRBs does not normally exceed the MCL [32,33], the concentration of *cis*-1,2-DCE added in our experiments was 90 μg/L.

E-1 were experiments conducted at pH 7.9 and then modified to pH 10.5 by the addition of NaOH. E-2 were experiments conducted at pH 7.9 with or without *cis*-1,2-DCE. E-3 were experiments performed at pH 10.5 with or without *cis*-1,2-DCE. The concentration of benzene and toluene in bacterial and control samples was 2.0 mg/L and 3.0 mg/L, respectively. All experiments were performed in duplicate with deviations not exceeding 10%.

### 2.3. Gas chromatographic analyses

Headspace-gas chromatography–mass spectrometry (HS-GC–MS) with external standards was used to quantify benzene, toluene and cis-1,2-DCE (G1888 Headspace Auto Sampler, Hewlett-Packard; 6890 gas chromatograph, Agilent, Santa Clara, CA, USA; HP-5 MS 30 m × 0.25 m × 0.25 μm capillary column, Hewlett-Packard; 5975 MS, Agilent). Headspace conditions: vial, loop and transfer line temperatures 85 °C, 95 °C and 150 °C, respectively. GC oven temperature: initially 40 °C held for 5 min, gradually increased to 200 °C at the rate of 10 °C/min. Injection temperature: 150 °C. Column flow rate: 1.0 mL/min. Split ratio: 1:1. MS conditions: Ion Source: EI 70 eV. Acquisition Mode: SIM. Sample preparation: a 10 mL glass vial was sealed after 1 mL $NaN_3$ solution and 4 mL of sample were added. The concentration of benzene and toluene was measured at 0 and 0.5 days and every 24 h thereafter. Samples were normally analyzed immediately or stored at 4 °C until analysis.

### 2.4. Calculation of biodegradation half-life and inhibition coefficient

Mehrdad et al. [12] have reported that biodegradation of benzene and toluene corresponds to a quasi-first order kinetic equation. The half-life of benzene and toluene biodegradation in our study was calculated following the equation below:

$$\frac{-dC}{dt} = k_b C \tag{1}$$

$$t_{1/2} = \frac{1}{k_b} \ln 2 \tag{2}$$

In Eqs. (1) and (2), $C$ is the concentration of benzene and toluene in solution; $t$ is the time of benzene and toluene biodegradation; $k_b$ is the rate constant of benzene and toluene removal; $t_{1/2}$ is the half-life of benzene and toluene in solution. In order to account for differences due to variations in bacterial activity and/or bacterial population sizes, the inhibition coefficients (IC) due to effects of increased pH or the presence of *cis*-1,2-DCE on the half-life of benzene and toluene biodegradation was calculated following the equation below:

$$\mathrm{IC} = \frac{t'_{1/2}}{t''_{1/2}} \tag{3}$$

In Eq. (3), $t'_{1/2}$ is the half-life of benzene and toluene at pH 7.9 and/or without *cis*-1,2-DCE; $t''_{1/2}$ is the half-life of benzene and toluene at pH 10.5 and/or with *cis*-1,2-DCE. If IC > 1, the pH increase or presence of *cis*-1,2-DCE inhibited biodegradation of benzene and toluene; at values of IC < 1, these factors accelerated the process.

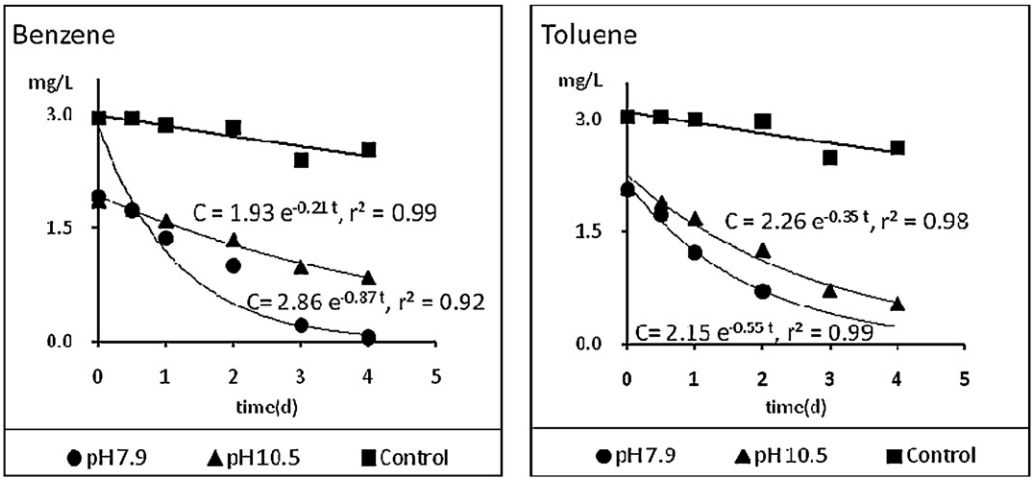


**Fig. 1.** Biodegradation of benzene and toluene at pH 7.9 and 10.5 in the absence of added *cis*-1,2-DCE. Control experiments contained no bacteria.

## 3. Results and discussion

### 3.1. Oxygen, nitrate, sulfate and iron concentrations

Oxygen levels in all experiments were consistently lower than 1 mg/L. Sulfate levels were unchanged throughout all experiments. Nitrate was never detected, presumably because as intended it was reduced by the ZVI before the experiments were initiated. Fe(III) concentrations increased due to some oxidation of the ZVI, thereby providing a reliable source of electron acceptors for the benzene and toluene degraders in the experiments. It was difficult to quantify iron, particularly Fe(II), because of complex interactions with the matrix; however, the iron data support our conclusion that benzene and toluene were degraded under iron-reducing conditions in the enrichment cultures.

### 3.2. Effect of alkaline pH on benzene and toluene biodegradation

The rates of benzene and toluene removal under elevated pH conditions (E-1) are shown in Fig. 1. The removal rate for 2.00 mg/L benzene and toluene at pH 7.9 over 4 days was 97% and 100%, respectively. When the pH was adjusted to 10.5, the removal rate over the same period of time was reduced by 43% and 26%, respectively. Based on the observed removal rates obtained for benzene and toluene in E-1 and the half-life estimates shown in Fig. 2, the inhibition coefficients (IC) of pH 10.5 on benzene and toluene biodegradation was calculated to be 4.2 and 1.6, respectively.

These results clearly suggest that elevated pH inhibits both benzene and toluene biodegradation. This effect was likely due to the bioenergetic problems associated with the surface of a cell membrane populated with hydroxyl ions; in other words, inhibition of the continued formation of a proton gradient across the bacterial membrane that drives ATP synthesis [34]. Our results are consistent with those of Tas and Pavlostathis [35] who reported a two-fold decrease in perchloroethylene (PCE) dechlorination by a *Dehalococcoides*-containing enrichment culture when the pH was increased from 7 to 8.

The inhibition of benzene biodegradation due to high pH was about 2.6 times greater than for toluene. There are two possible explanations for these results. First, it may be due to the different intermediate products formed during biodegradation. The main intermediate of benzene degradation is phenol, while benzoic acid is the main by-product of toluene degradation [36,37]. Ladlie et al. [38] have postulated that the biodegradation rate of any given organic compound corresponds closely with its ionization constant ($pK_a$) and the matrix pH. The $pK_a$ of phenol at 25 °C is 10.0, while the $pK_a$ of benzoic acid is 4.2. Since the $pK_a$ of phenol is about 2.3 times greater than that of benzoic acid, we would expect benzoic acid to be more easily ionized under high pH condition so that electrons are more quickly accepted and transformed into other intermediates (e.g., catechol [11,39]). Secondly, it is possible that the observed inhibition was caused simply by the preferred utilization of toluene over benzene by the bacterial community when both substrates are available, which has been noted by other investigators [12,40].

### 3.3. Effect of culturing conditions on benzene and toluene degradation at pH 10.5

As may be seen in Table 1, significant differences in benzene and toluene removal were observed between E-1 (results just discussed) and E-3. In E-1, the bacteria were cultured at pH 7.9 for 7 days; after this period, the pH of the culture was increased to 10.5 by the addition of NaOH. In E-3, however, the bacteria were cultivated for three successive 7-day periods at pH 10.5. In E-3, benzene and toluene were degraded completely, suggesting that the bacteria adjusted to the higher pH environment when cultured under these conditions. Most bacteria can function within a range of pH values (±3 pH units from an optimum value; [34]). We do not know what the optimum pH was for our iron-reducing bacterial consor-

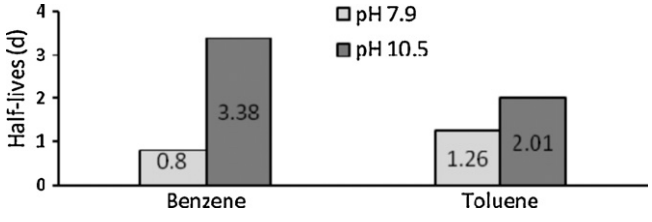


**Fig. 2.** Calculated half-lives of benzene and toluene at pH 7.9 and pH 10.5.

**Table 1**
Effect of culturing conditions on benzene and toluene biodegradation at pH 10.5.

| | | pH | *cis*-1,2-DCE (μg/L) | Benzene | Toluene |
|---|---|---|---|---|---|
| Removal rate (%) | E-1 | 10.5 | 0 | 54 | 74 |
| Half-life (day) | E-1 | 10.5 | 0 | 3.38 | 2.01 |
| Removal rate (%) | E-3 | 10.5 | 0 | 100 | 100 |
| Half-life (day) | E-3 | 10.5 | 0 | 3.17 | 3.93 |

*Note*: Medium pH in E-1 was adjusted from 7.9 to 10.5 directly; bacteria in E-3 were cultivated at pH 10.5.

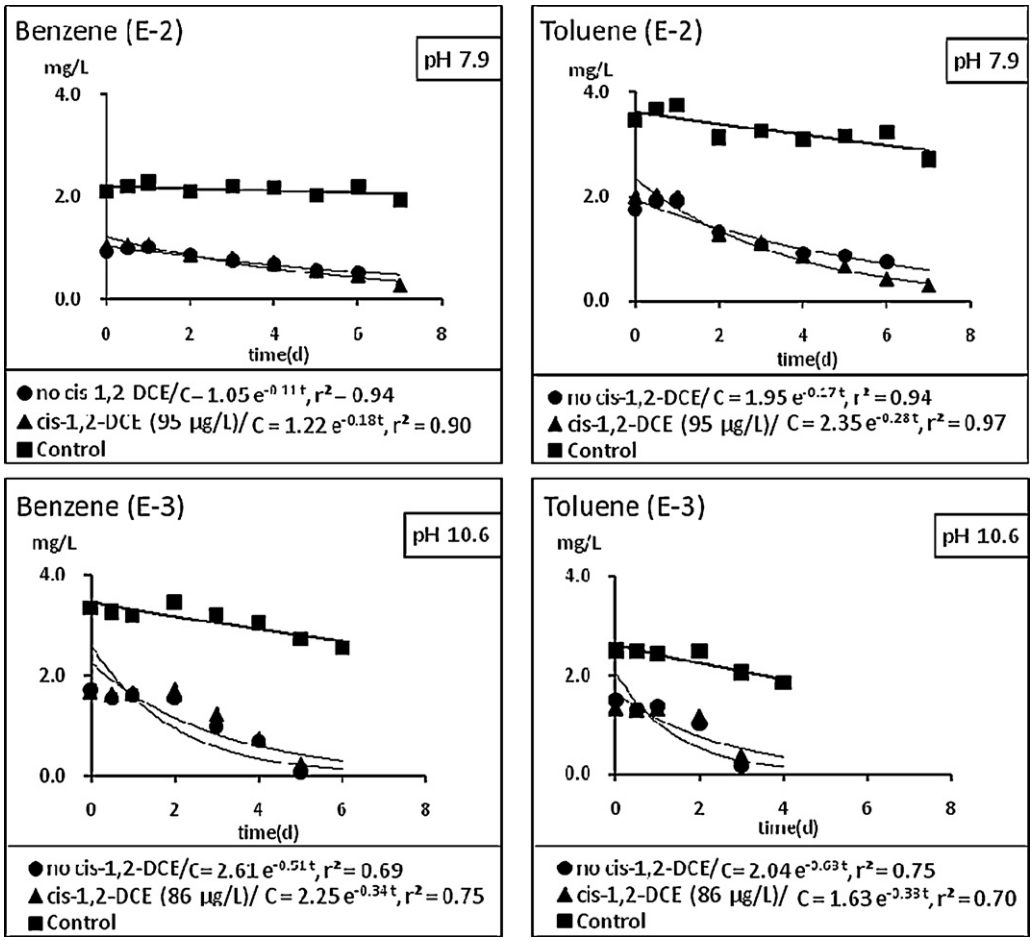


**Fig. 3.** Biodegradation of benzene and toluene with and without *cis*-1,2-DCE. Control experiments contained no bacteria.

tium, although pH 10.5 is well within the expected range of pH variation. We do not have another explanation for the apparent adaptation to pH 10.5 that we observed.

### 3.4. The effect of cis-1,2-DCE on benzene and toluene biodegradation

Fig. 3 illustrates the effect of added *cis*-1,2-DCE on benzene and toluene removal. Table 2 summarizes cumulative benzene and toluene removal in E-2 and E-3 on a percentage basis. The effect of the addition of *cis*-1,2-DCE on removal of both compounds was surprising in that the extent of their removal was greater than or equal to the control cultures. For example, in E-2, the removal of benzene and toluene in 7 days was 44% and 57%, respectively; in contrast, the presence of *cis*-1,2-DCE increased benzene removal to 56% and toluene removal to 79%.

Fig. 4 summarizes the calculated half-lives of benzene and toluene in E-2 and E-3. The E-2 half-lives suggest that the extent of benzene and toluene removal is slightly greater in the presence of *cis*-1,2-DCE (reaching ~60% after 7 days); similarly, the rates of benzene and toluene removal were markedly faster in the presence of added *cis*-1,2-DCE (e.g., 3.9 days vs 6.1 days for benzene). In E-3, similar results were obtained with regard to benzene and toluene half-lives.

These results may be best explained by the possible co-metabolism of benzene and toluene by the *cis*-1,2-DCE dechlorinating bacteria. Co-metabolism of BTEX, chlorinated hydrocarbons and other substances under aerobic/anaerobic conditions has been studied extensively [e.g., 41–44]. Anke and Edward [45] have observed the co-metabolism of *cis*-1,2-DCE with VC and toluene under aerobic conditions. Doong and Wu [46] investigated the anaerobic co-metabolism of chlorinated hydrocarbons with acetic acid, glucose, methanol, and humic acids. Since the metabolism of *cis*-1,2-DCE was not followed systematically in the present study, additional work would need to be done in order to elucidate whether or not co-metabolism occurred during microbial Fe(III) reduction.

**Table 2**
Removal of benzene and toluene with *cis*-1,2-DCE added.

| | E-2 | E-2 + DCE | Control | E-3 | E-3 + DCE | Control |
|---|---|---|---|---|---|---|
| pH | 7.9 | 7.9 | 7.9 | 10.5 | 10.5 | 10.5 |
| *cis*-1,2-DCE (μg/L) | 0 | 95 | 152 | 0 | 86 | 139 |
| Benzene removal (%) | 44 | 56 | 0 | 100 | 100 | 0 |
| Toluene removal (%) | 57 | 79 | 0 | 100 | 100 | 0 |
| *cis*-1,2-DCE removal (%) | 0 | 89 | 39 | 0 | 89 | 39 |

*Note*: Bacteria in E-2 were cultivated at pH 7.9. Bacteria in E-3 were cultivated at pH 10.5. The controls contained no bacteria.

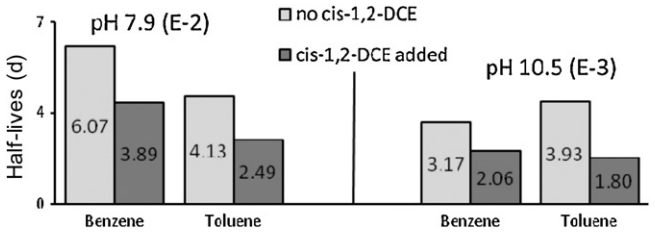


**Fig. 4.** Half-lives of benzene and toluene in the presence and absence of *cis*-1,2-DCE.

Based on Fig. 4, there appears to be a greater effect of *cis*-1,2-DCE addition on toluene removal, which may again be explained by the preferential use of toluene over benzene; however, as evident in Fig. 2, elevated pH appears to have a greater effect on benzene than on toluene. These results suggest that pH and *cis*-1,2-DCE have distinctly different effects on microbial activity; pH directly affects metabolism (e.g., enzymes, cell membranes, and proton motive force; [34,47]), while *cis*-1,2-DCE may be used by the microbial community as a secondary substrate [45,46].

In E-3, elevated pH (10.5) inhibited both benzene and toluene degradation. The average IC of pH on their half-lives was about 2.9, while the IC for *cis*-1,2-DCE was about 0.6. In other words, the effect of alkaline pH on benzene and toluene removal was about five times greater than the effect of added *cis*-1,2-DCE. This leads us to speculate that the effect of elevated pH down gradient of a ZVI PRB is a major factor in the observed inhibition of benzene and toluene biodegradation. Moreover, our experiments suggest that the presence of *cis*-1,2-DCE on benzene and toluene degradation may, in fact, be beneficial.

## 4. Conclusions

This study demonstrates that the Fe(III)-mediated anoxic degradation of benzene and toluene down gradient of a simulated ZVI PRB is adversely affected by elevated pH, while the TCE dechlorination by-product *cis*-1,2-DCE appears to enhance their removal. Alkaline pH (10.5) exerts a larger effect on benzene biodegradation than on toluene removal, which is consistent with differences in the ionization constants of their metabolic intermediates. In a simulated iron-reducing environment, toluene may be preferred over benzene as a substrate when they coexist.

In a sequential ZVI PRB/bio-PRB system, dissolved oxygen in the ground water after passing through the ZVI PRB is usually low (<1.0 mg/L), and the pH always increases (>9.0) [4,25–27]. Moreover, some daughter products may accumulate and flow down gradient and reach the bio-PRB unit [19–21]. All of these factors should be considered in the design of such a sequential groundwater treatment system.

Based on the results of this study, we conclude that increases in pH down gradient of ZVI PRBs need to be considered seriously in both design and operation. Addition of a pH buffer strip is warranted; however, permeation of the bio-PRB with *cis*-1,2-DCE may be neglected. For a containment site with a mixed plume (i.e., TCE and 2 mg/L BTEX), we recommend that the bio-PRB down gradient of the ZVI unit be at least 5 m in thickness if the groundwater flow rate through it is ~1 m/day; this should achieve removal of greater than 98% of BTEX compounds at steady state. These recommendations are based on these study results, so in actual application, several other factors need to be considered, including the activity of appropriate microorganisms, the presence of potentially toxic compounds, the concentration of contaminants of concern, the composition and surface area of the bio-PRB, and other hydrogeologic characteristics of the remediation site.

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## References

[1] A.S. Daniel, Z.H. Li, Synergistic effect of cationic surfactants on perchloroethylene degradation by zero-valent iron, Environ. Sci. Technol. 35 (2001) 3713–3717.
[2] L. Taeyoon, H.B. Craig, R.E. Gerald, Waste green sands as reactive media for groundwater contaminated with trichloroethylene (TCE), J. Hazard. Mater. 109B (2004) 25–36.
[3] S.D. Warner, B.L. Longino, M. Zhang, P. Bennett, F.S. Szerdy, L.A. Hamilton, The first commercial permeable reactive barrier composed of granular iron: Hydraulic and chemical performance at 10 years of operation, IAHS 298 (2005) 32–42.
[4] Interstate Technology and Regulatory Council (ITRC), Permeable Reactive Barriers Team, Permeable reactive barriers: lessons learned/new directions, Washington, DC, USA, 2005, pp.65–80.
[5] U.S.EPA, Field Applications of in-situ remediation technologies: permeable reactive barriers, EPA 68-W-00-084, 2005, Available online at: http://www.clu-in.info/download/rtdf/fieldapp_prb.
[6] V. Lenka, N. Jan, S. Martina, K. Martin, The biofiltration permeable reactive barrier: practical experience from Synthesia, Int. Biodeterior. Biodegrad. 58 (2006) 224–230.
[7] D.R. Lovley, Anaerobic benzene degradation, Biodegradation 11 (2–3) (2001) 107–116.
[8] S.M. Maliyekkal, E.R. Rene, L. Philip, T. Swaminathan, Performance of BTX degraders under substrate versatility conditions, J. Hazard. Mater. 109 (2004) 201–211.
[9] M.K. Jahn, S.B. Haderlein, R.U. Meckenstock, Anaerobic degradation of benzene, toluene, ethylbenzene, and o-xylene in sediment-free iron-reducing enrichment cultures, Appl. Environ. Microbiol. 71 (2005) 3355–3358.
[10] D.M. Mackay, N.R. de Sieyes, M.D. Einarson, K.P. Feris, A.A. Pappas, I.A. Wood, L. Jacobson, L.G. Justice, M.N. Noske, K.M. Scow, J.T. Wilson, Impact of ethanol on the natural attenuation of benzene, toluene and o-xylene in a normally sulfate-reducing aquifer, Environ. Sci. Technol. 40 (19) (2006) 6123–6130.
[11] D.M. Mackay, N.R. de Sieyes, M.D. Einarson, K.P. Feris, A.A. Pappas, I.A. Wood, L. Jacobson, L.G. Justice, M.N. Noske, J.T. Wilson, C.J. Adair, K.M. Scow, Impact of ethanol on the natural attenuation of MTBE in a normally sulfate-reducing aquifer, Environ. Sci. Technol. 41 (2007) 2015–2021.
[12] F. Mehrdad, V.C. Vachelard, D. David, L. Christian, In situ bioremediation of monoaromatic pollutants in groundwater: a review, Bioresour. Technol. 99 (2008) 5296–5308.
[13] P.H. Nielsen, P.L. Bjerg, P. Nielsen, P. Smith, T.H. Christensen, In situ and laboratory determined first-order degradation rate constants of specific organic compounds in an aerobic aquifer, Environ. Sci. Technol. 30 (1996) 31–37.
[14] R.E. Beeman, C.A. Bleckmann, Sequential anaerobic–aerobic treatment of an aquifer contaminated by halogenated organics: field results, J. Contam. Hydrol. 57 (2002) 147–159.
[15] R.A. Doong, Y.J. Lai, Dechlorination of tetrachloroethylene by palladized iron in the presence of humic acid, Water Res. 39 (11) (2005) 2309–2318.
[16] M. Rosy, F. Quintus, K. Nic, A method for the rapid dechlorination of low molecular weight chlorinated hydrocarbons in water, Water Res. 29 (10) (1995) 2434–2439.
[17] J.L. Chen, R. James, A.A. Souhail, Z.B. Li, Effects of pH on dechlorination of trichloroethylene by zero-valent iron, J. Hazard. Mater. 83 (3) (2001) 243–254.
[18] C.J. Lin, S.L. Lo, Y.H. Liou, Dechlorination of trichloroethylene in aqueous solution by noble metal-modified iron, J. Hazard. Mater. 116B (2004) 219–228.
[19] P.J.J. Alvarez, L.A. Cronkhite, C.S. Hunt, Use of benzoate to establish reactive buffer zones for enhanced attenuation of BTX migration: aquifer column experiments, Environ. Sci. Technol. 32 (1998) 509–515.
[20] F. Liu, Study on volatile chlorinated hydrocarbons in groundwater using the permeable reactive barrier of zero valence iron, China University of Geosciences (Beijing), Doctoral Dissertation, 2002, pp. 40–50.
[21] K.L. Straub, M. Benz, B. Schink, Iron metabolism in anoxic environments at near neutral pH, FEMS Microbiol. Ecol. 34 (3) (2001) 181–186.
[22] J.T. McGuire, D.T. Long, D.W. Hyndman, Analysis of recharge induced geochemical change in a contaminated aquifer, Ground Water 43 (2005) 518–530.
[23] V. Andreoni, L. Gianfreda, Bioremediation and monitoring of aromatic-polluted habitats, Appl. Microbiol. Biotechnol. 76 (2007) 287–308.
[24] C.C. Liu, D.H. Tseng, C.Y. Wang, Effects of ferrous ions on the reductive dechlorination of trichloroethylene by zero-valent iron, J. Hazard. Mater. 136B (2006) 706–713.
[25] K. Ritter, M.S. Odziemkowski, R.W. Gillham, An in situ study of the role of surface films on granular iron in the permeable iron wall technology, J. Contam. Hydrol. 55 (2002) 87–111.
[26] K. Ritter, M.S. Odziemkowski, R. Simpgraga, R.W. Gillham, D.E. Irish, An in situ study of the effect of nitrate on the reduction of trichloroethylene by granular iron, J. Contam. Hydrol. 65 (1–2) (2003) 121–136.
[27] F. Xia, Y.L. Liu, F. Liu, H.H. Chen, Column studies on effects of benzene, toluene on reductive dechlorination of PCE by granular iron, Earth Sci. Front. 15 (6) (2008) 142–150.
[28] D.H. Kampbell, T.H. Wiedemeier, J.E. Hansen, Intrinsic bioremediation of fuel contamination in ground water at a field site, J. Hazard. Mater. 49 (1996) 197–204.
[29] X.Y. Liu, F. Liu, H.H. Chen, F. Xia, X.R. Zhang, Film-state under anoxic biodegradation of benzene and toluene in the presence of $Fe(OH)_3$-coated sand, Actual Pathological ET Mineralogical 26 (6) (2007) 529–533.
[30] R.W. Puls, D.W. Blowes, R.W. Gillham, Long-term performance monitoring for a permeable reactive barrier at the U.S. Coast Guard Support Center, Elizabeth City, North Carolina, J. Hazard. Mater. 68 (1996) 109–124.
[31] U.S.EPA, Drinking water contaminants, 2009, http://water.epa.gov/drink/contaminants/index.cfm.

[32] C.B. Wang, W.X. Zhang, Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs, Environ. Sci. Technol. 31 (7) (1997) 2154–2156.
[33] S.W. Jeen, K.U. Mayer, R.W. Gillham, D.W. Blowes, Reactive transport modeling of trichloroethene treatment with declining reactivity of iron, Environ. Sci. Technol. 41 (4) (2007) 1432–1438.
[34] J. Madigan, P. Martinko, D. Dunlap, T.Brock Clark, Brock Biology of Microorganisms, 12th ed., Benjamin Cummings, 2008.
[35] D.O. Tas, S.G. Pavlostathis, Temperature pH effect on the microbial reductive transformation of pentachloronitrobenzene, J. Agric. Food Chem. 55 (2007) 5390–5398.
[36] D. Grbić-Galić, T.M. Vogel, Transformation of toluene and benzene by mixed methanogenic cultures, Appl. Environ. Microbiol. 53 (2) (1987) 254–260.
[37] K.R. Walton, H.S. Janet, M.L. Aaron, The geochemical effects of benzene, toluene and xylene (BTX) biodegradation, Appl. Geochem. 12 (1997) 291–303.
[38] J. Ladlie, W.F. Meggitt, D. Penner, Effect of soil pH on microbial degradation, adsorption, and mobility of metribuzin, Weed Sci. 24 (5) (1976) 477–481.
[39] R Munoz, L.F. Diaz, S. Bordel, S. Villaverde, Inhibitory effects of catechol accumulation on benzene biodegradation in *Pseudomonas putida* F1 cultures, Chemosphere 68 (2007) 244–252.
[40] L.B.M. Da Silva, J.J.P. Alvarez, Enhanced anaerobic biodegradation of benzene–toluene–ethylbenzene–xylene–ethanol mixtures in bioaugmented aquifer columns, Appl. Environ. Microbiol. (2004) 4720–4726.
[41] Y. Steven, C. Kirk, S. Bruce, S. Carl, Multicomponent reactive transport in an in situ zero-valent iron cell, Environ. Sci. Technol. 35 (7) (2001) 1493–1503.
[42] C. Romy, D.C. John, Hydroxylation and carboxylation—two crucial steps of anaerobic benzene degradation by *Dechloromonas strain RCB*, Appl. Environ. Microbiol. 71 (9) (2005) 5427–5432.
[43] F. Dario, P. Davide, N. Massimo, Z. Arianna, F. Stefano, B. Emilia, Z. Davide, F. Angiolo, B. Alfredo, Long-term aerobic cometabolism of a chlorinated solvent mixture by vinyl chloride-, methane- and propane-utilizing biomasses, J. Hazard. Mater. 138 (1) (2006) 29–39.
[44] Interstate Technology and Regulatory Council (ITRC), Bioremediation of DNAPLs Team, In situ bioremediation of chlorinated ethene: DNAPL source zones, Washington, DC, USA, 2008, pp. 65–80.
[45] S. Anke, J.B. Edward, Toluene induced cometabolism of *cis*-1,2-dichloroethylene and vinyl chloride under conditions expected downgradient of a permeable Fe(0) barrier, Water Res. 34 (13) (2000) 3391–3399.
[46] R.A. Doong, S.C. Wu, Substrate effects on the enhanced biotransformation of polychlorinated hydrocarbons under anaerobic condition, Chemosphere 8 (1995) 1499–1511.
[47] X.X. Zhang, H.Q. Wang, J.Q. Liu, Y.J. Chen, Study on benzene degraded by soil microorganisms, Environ. Sci. 26 (6) (2005) 148–152.