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Enhanced dissolution of TCE in NAPL by TCE-degrading bacteria in wetland soils

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Abstract

The influence of trichloroethene (TCE) dechlorinating mixed cultures in dissolution of TCE in nonaqueous phase liquid (NAPL) via biodegradation was observed. Experiments were conducted in batch reactor system with and without marsh soils under 10 and 20 °C for 2 months. The dissolution phenomenon in biotic reactors containing mixed cultures was showed temporal increases compared to abiotic reactors treated with biocide. Effective NAPL-water transfer rate (K_m) calculated in this study showed more than four times higher in biotic reactors than that in abiotic reactors. The results might be attributed to the biologically enhanced dissolution process via dechlorination in reactors. Temperature would be a factor to determine the dissolution rate by controlling bacterial activity. The TCE dechlorination occurred even in an interface of TCE-NAPL that demonstrated no previous TCE biodegradation, suggesting that microbes may be useful in developing source-zone bioremediation system. In conclusion, dechlorinating mixed culture could enhance dissolution in NAPL that may be useful in the application of source zone bioremediation.

Keywords: TCE; Dechlorination; Biodegradation; NAPL (nonaqueous phase liquid); Dissolution

1. Introduction

Hydrophobic organic pollutants (HOPs) and spilled or leaking fuels are often present at waste disposal sites and often found in the form of nonaqueous phases in polluted environments. These nonaqueous phase liquids (NAPLs) in soils or aquifers frequently are long-term sources and/or reservoirs of toxic contaminants. Because they frequently contain many toxic constituents that partition into the aqueous phase, NAPLs pose a risk to humans, animals and other organisms. Pump-and-treat remediation of soils and aquifers polluted with such mixtures is difficult due to the tendency of the constituents to remain in the separate phase.

Trichloroethene (TCE) is among the most frequently detected contaminants in groundwater because of their limited aqueous solubility and miscibility [1]. The reductive dechlorination of TCE by anaerobic bacteria has been widely studied for nearly two decades. TCE has known as most prevalent contaminant in groundwater and well documented by findings of byproducts to be degraded under anaerobic condition into dichloroethene isomers (DCEs), vinyl chloride (VC), and ethene [2–5], although ethene and carbon dioxide has also been observed [6–8]. With these findings, the emergence of bioremediation treating chlorinated solvent-contaminated aquifers has been widely appealed through the dechlorination process of the dehalorespiring organisms [9].

TCE is often associated with mixed organic NAPLs. Their low aqueous solubility and miscibility in organic contaminants often lead to TCE at contaminated sites characterized by the presence of nonaqueous phase liquid (NAPL) source zones that present unique remediation challenges.

Extensive research has focused on means of biodegrading NAPL constituents [12] and biosurfactant produced from bacteria [10] to reduce the exposure of susceptible populations. Due to low rate of partitioning to water, biodegradation of HOPs is limited by the rate of partitioning of the pollutant from NAPL to water. The biodegradation of PAH present in NAPLs has been found to be severely limited by the slow kinetics of abiotic mass transfer or partitioning of HOPs into the water phase [11]. Efroymson and Alexander [12] found out that biodegradation of xenobiotics dissolved in NAPL occurs frequently at a rate higher than the rate of partitioning from nonaqueous to aqueous

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phases, evaluated in the absence of biodegrading microorganisms. Ortega-Calvo and Alexander [13] postulated that bacteria growing at the NAPL-water interface has physiologically distinctive characteristic from that of bacteria suspended in the aqueous phase.

The effect of bacterial activity in dissolution process of contaminant from NAPL phase was studied with naphthalene [14], and tetrachloroethene [15]. Marx and Aitken explained an influence of bacterial chemotaxis in biodegradation in systems containing small-scale heterogeneity in contaminant distribution and limitation of mixing. Yang and McCarthy [15] found out that dense nonaqueous phase liquid (DNAPL) dissolution rate was significantly enhanced when directly coupled with biological dehalogenation in a column study.

However, little studies have been conducted to elucidate potential bacterial effects coupled with mass transfer in NAPL with consideration of rate of spontaneous partitioning. In this study, we investigated the influence of microorganisms in dissolution via partitioning from NAPL to aqueous phase in biotic and abiotic conditions containing wetland soils.

2. Materials and methods

2.1. Chemicals and soils

Trichloroethylene (TCE, 99.5% purity, spectrophotometric grade), Sudan III used as a dye, formaldehyde (37%, ACS grade) used as a biocide, and all constituents for nutrient media for bacteria were purchased from Aldrich Chemical (Milwaukee, WI, USA) and used as supplied. Soils in this study were collected from Madisonville, LA. Soil properties were determined according to standard methods in the Huffman laboratories Inc., Golden, CO. Briefly, soils contained 27.58% organic carbon, 32.44% carbon, 3.93% hydrogen, 23.32% oxygen, 2.06% nitrogen, 1.00% sulfur, and 42.40% ash. Moist soils were stored in refrigerator maintained temperature under 4 °C prior to use. Moist contents were calculated as 45 ± 2% as a dry weight basis.

2.2. Bacteria, media, and cultivation

The bacteria used in this study were originated from TCE-contaminated marsh soils previously used for TCE dechlorination study. Preparation of soil-free and soil-slurry reactors were described in details in a section named "reactor set-up".

Prior to transfer, the samples were analyzed in GC–MS and flame ionization detector (FID) to ensure no residual amounts of chlorinated ethenes via dechlorination of TCE with only ethene left in gaseous phase.

For the preparation of nutrient medium, reagent-grade chemicals were used by following the previous study [16]. Briefly, the media consisted of the following constituents: 400 mg/L NH_4Cl, 400 mg/L KCl, 400 mg/L $MgCl_2{\cdot}6H_2O$, 140 mg/L KH_2PO_4, 25 mg/L $CaCl_2{\cdot}2H_2O$, 10 mg/L $(NaPO_3)_{16}$, 2.5 mg/L KI, 2.5 mg/L $CoCl_2{\cdot}6H_2O$, 0.5 mg/L $ZnCl_2$, 0.5 mg/L $MnCl_2{\cdot}4H_2O$, 0.5 mg/L H_3BO_3, 0.5 mg/L $NiCl_2{\cdot}6H_2O$, 0.5 mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.5 mg/L NH_4VO_3, 200 mg/L yeast extract, and $NaHCO_3$ as needed to buffer the systems (ranging in concentration from 3.0 to 5.5 g/L). The prepared nutrients were purged with N_2 for 10 min to remove potential residual amounts oxygen dissolved in nutrients and maintain anaerobic condition. To sustain anaerobic dechlorinating activity, addition of H_2 as an electron donor was applied.

2.3. Analytical methods

Gas chromatography (GC) was used to determine concentrations of chlorinated ethenes in both aqueous and gaseous phases. For TCE, *cis*-DCE (Dichloroethene) and VC quantification, aqueous samples (20 μL) were injected into a GC (Hewlett-Packard, Model 6890) equipped with a mass spectrophotometer (MS) and purge-trap autosampler (Tekmar, Model 2016) by following EPA standard method 8260. Detailed information regarding this method was described in a previous study (Lee et al., 2002).

Gaseous samples were collected to quantify ethene and methane. Three milliliter of headspace samples were directly injected into GC (Hewlett-Packard, Model 5890 Series II) equipped with flame ionization detector and a capillary column (30 m × 0.25 mm, 0.25 μm in O.D.) purchased from J&W Scientific.

2.4. Reactor set-up

One hundred and sixty milliliter capacity Serum bottles purchased from Nalgene Inc. (Rochester, NY) were used as reactors. As capillary tubes, 200 μL capacity micropipettes were used (Drummond Scientific, Broomall, PA, USA). One hundred milliliter of prepared nutrient solution purged with H_2 and N_2 gas for 10 min to provide electron donor source and sustain anaerobic condition was transferred into each bottle and put butyl-coated septa on can-crimp sealed under glove box filled with N_2 gas (99.5% purity). A capillary tube was filled with 100 μL of pure TCE dyed with Sudan III and immersed into nutrient solutions via pierced septa, open-side down after treating tip of tube flame-sealed.

Treatments were applied to investigate the bacterial effect on dissolution rates of TCE-NAPL in both soil-free and soil-slurry phase under 10 and 20 °C. First, 100 mL of nutrient solution prepared as described above was added with 10 mL of precultured TCE-degrading bacteria in soil free phase. Additional 5 mL of formaldehyde (37%) representing total 16 mg/L was added to inhibit bacterial activity in abiotic treatment which was autoclaved as well. Second, to evaluate of the presence of marsh soil in effect on dissolution rate, biotic reactors were contained with 10 mL of soil-slurry representing approximately 5.2 mg/L of dry soil basis. Meanwhile, for abiotic reactors, additional 5 mL of formaldehyde (37%) was added into reactors previously autoclaved to inhibit bacterial activity for possible bacterial activity. Third, temperature effect on dissolution process was also evaluated by incubating all reactors under 10 and 20 °C.

Brief measurements of aqueous concentrations for chlorinated ethenes at vicinity as well as in a distance of NAPL were conducted to understand whether degrading bacteria could occur dechlorination process. The subsample inside tube was

collected, analyzed and then compared with that collected from a distance.

2.5. NAPL dissolution-biotransformation model

A simple mass transfer-biodegradation model developed by [19] was applied to investigate the potential biological enhancement in dissolution. Partitioning into aqueous phase from NAPL was described by the relationship as follows:

$$\frac{dC}{dt} = K_m(C_s - C)$$

where C is the aqueous phase concentration (mM), C_s is aqueous solubility (mM), t is time (day), and K_m is the effective NAPL-water transfer rate (day^{-1}).

3. Results

Dechlorinated ethenes from TCE exhibited in biotic reactors incubated at 20 °C. Distinct difference in dissolution pattern in both reactors with and without soil-slurry was observed (Fig. 1). TCE dissolution in bioreactor containing aqueous phase only bypassed after 52 days of incubation compared to 27 days in soil-slurry reactors. More dechlorinated ethenes were produced in soil-slurry reactors as well. Meanwhile, for the bioreactors incubated at 10 °C, no production of chlorinated ethenes in reactors containing only aqueous phase was occurred, however, residual amounts of *cis*-DCE and VC were monitored in reactors with soil slurry after 40 days (Fig. 2).

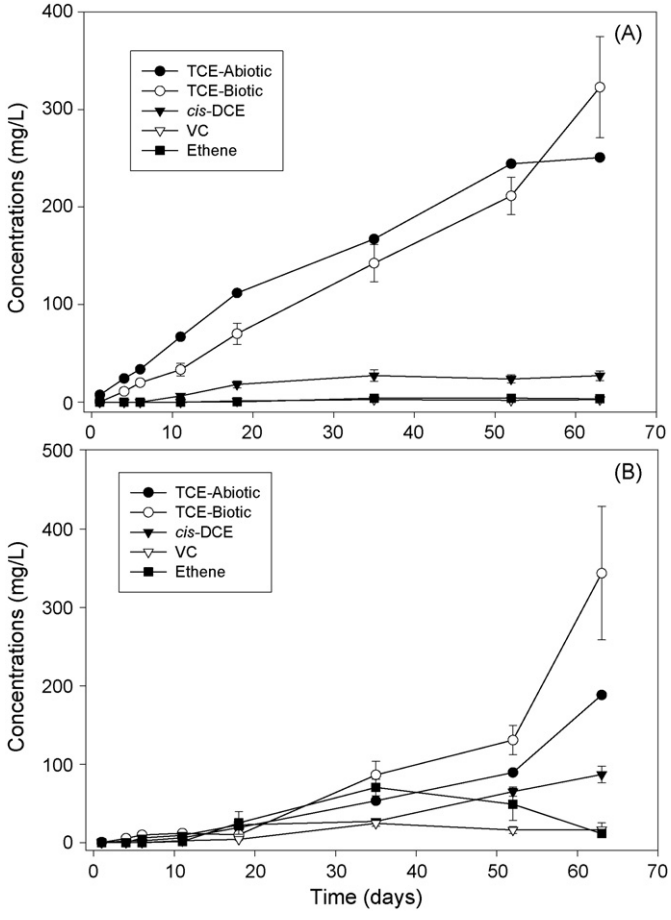

Fig. 1. Temporal changes of concentrations of chlorinated ethenes in reactors maintained at 20 °C: (A) soil-free phase; (B) soil-slurry phase.

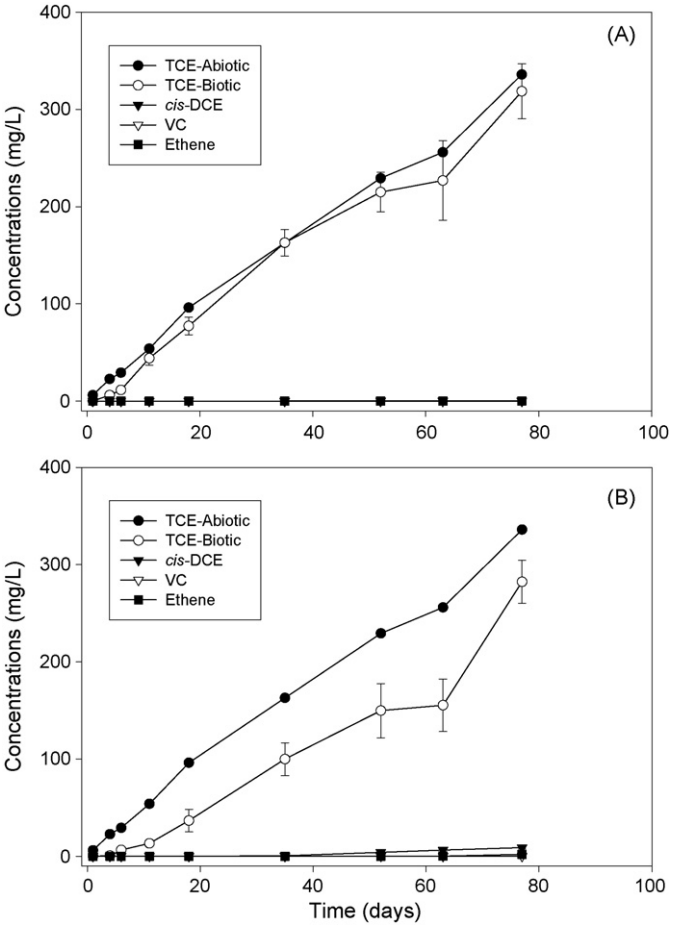

Fig. 2. Temporal changes of concentrations of chlorinated ethenes in reactors maintained at 10 °C: (A) soil-free phase; (B) soil-slurry phase.

Temporal aqueous concentrations of TCE and its dechlorinated products calculated as molar basis were presented for both abiotic and biotic reactors (Fig. 3). Fig. 3 presents the aqueous concentrations of chlorinated ethenes combined with TCE, DCE, and VC and ethene calculated in molar basis in soil-free phase. For the abiotic reactor, the temporal aqueous concentrations describe the dissolution of TCE in NAPL. No significant effect of temperature maintained at 10 and 20 °C for dissolution in abiotic reactor was observed (Fig. 3). However, slight increase of dissolution in biotic reactor maintained at 20 °C was observed over the 30 days, then rapid increase after 60 days.

For the reactors containing soil-slurry as shown in Fig. 4, temporal changes in aqueous concentration from NAPL were observed as same as those in soil-free reactors. Compared to soil-free reactors, relatively lower aqueous concentration was observed, which might be attributed to sorption to soils. As shown in Fig. 3, the presentation of biologically enhanced dissolution was observed in biotic reactor maintained at 20 °C with significant difference. The extent of dissolution in biotic reactor

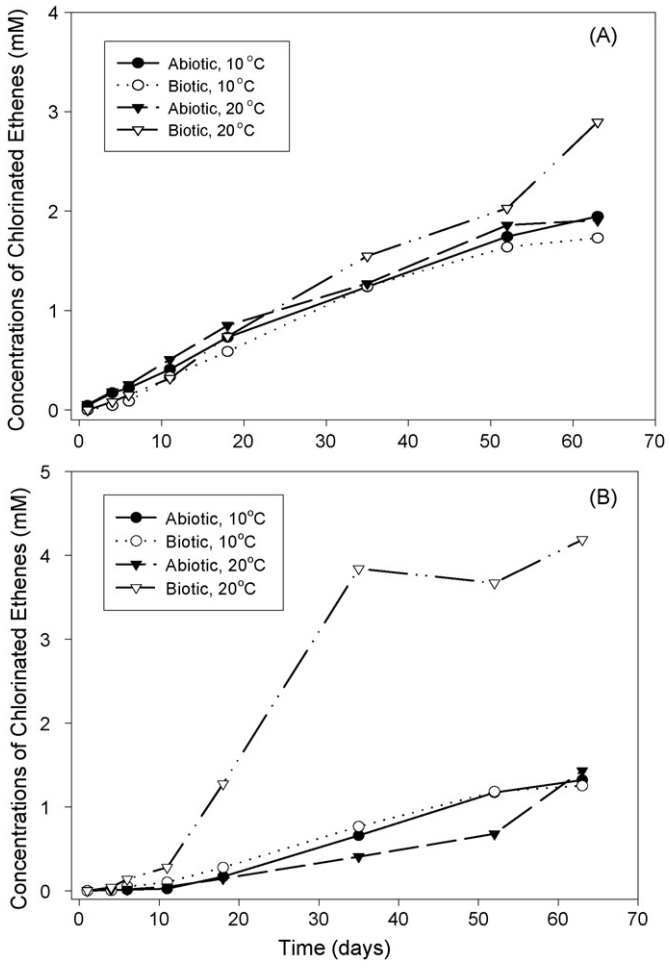

Fig. 3. Concentrations of chlorinated ethenes dissolved from NAPL phase by an influence of micro-organisms: (A) soil-free phase; (B) soil slurry phase.

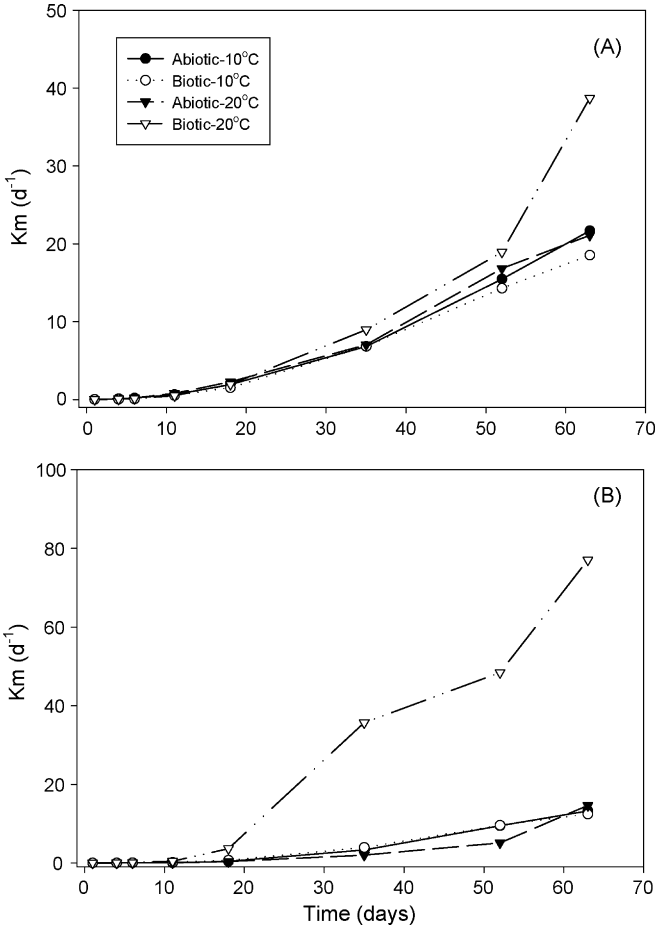

Fig. 4. Changes in effective NAPL-water mass transfer rate: (A) soil free phase; (B) soil-slurry phase.

was as much as two times of that in abiotic reactor. The dissolution in biotic reactor was sharply increased in day 10 until day 35.

Chlorinated ethenes such as TCE, *cis*-DCE and VC were observed in both fractions with some differences in concentrations showing TCE (320 mg/L), *cis*-DCE (23 mg/L) and VC (3 mg/L) in NAPL-water interface compared to 226 mg/L TCE, 33 mg/L *cis*-DCE and 5 mg/L VC in aqueous phase, respectively.

With an aid of NAPL dissolution biotransformation model, biotransformation of TCE to *cis*-DCE to VC to ethene was calculated and presented as dissolution patterns in reactors incubated at 10 and 20 °C (Fig. 4). No significant difference in biotic and abiotic reactors without containing soils appeared; however, a gradual increase in biotic reactor at 20 °C was occurred with rapid increase after day 52 (Fig. 4A). Dissolution in biotic soil-slurry reactor maintained at 20 °C increased after day 20 and exceeded that of the abiotic reactor four times over 2 months (Fig. 4B). No significant differences in dissolution process were observed in both abiotic and biotic reactors maintained in 10 °C. However, a slight higher dissolution process in soil-free reactors was observed than that in soil-slurry reactors.

4. Discussion

The goal of this study is to evaluate the possibility of applying anaerobic dechlorination of TCE from NAPL phase into aqueous phase to increase mass transfer via dissolution process in the presence of wetland soil. Previous studies suggest that there are challenges to applying reductive biological dechlorination directly to the DNAPL source zone. Removal rates of tetrachloroethane (PCE) from NAPL were well documented in continuous flow stirred-tank reactor (CFSTR) by Carr et al. [17]. They found that 14-fold increases in PCE removal rates were observed in the presence of mixed dechlorinating bacteria. In this study, static batch reactor system was used to investigate the effect of bacterial activity in dissolution process of TCE in NAPL phase in the presence of soil containing high organic matter. The results suggest that there is significant increment of dissolution rates by bacterial activity, which transform TCE into dechlorinated byproducts containing higher water solubility.

First, the TCE degrading bacteria precultured in Louisiana marsh soils were able to transform TCE into its dechlorinated ethenes, such as *cis*-DCE, VC, and ethene. Higher and faster dechlorination process was monitored in bioreactor containing wetland soils with a finding of shorter lag time than that in soil free reactor. This also implies that first; the dechlorina-

tion process was in process when microbial populations were added, second; TCE degrading bacteria stimulated the dissolution of TCE as well as dechlorinated ethenes produced via biodegradation.

The degradation was investigated in aqueous phase even at adjacent to interface of NAPL indicating that dechlorination process is still in process even under high concentrations. Relatively high concentration of TCE near NAPL was also observed compared to that in aqueous phase showing lower TCE, but higher concentrations of byproducts.

Second, biologically enhanced dissolution rates were observed with a significant increase in the dissolution process influenced by bacterial activity. It may be attributed to the production of byproducts containing higher solubility that drives more soluble in aqueous phase by partitioning. Much higher dissolution rates were investigated in biotic reactor containing soil-slurry maintained at 20 °C. This may also be attributed to the much higher population of TCE-degrading bacteria in soil-slurry than that in soil-free phase by showing shorter lag time in reactor containing soil-slurry. Much shorter time to reach methanogenic condition by producing methane inside reactors might provide appropriate environment for TCE degrading bacteria. Mass transfer rates of chlorinated ethenes increased in the reactor treated with soil slurry at 20 °C which enhance dissolution rates. The dissolution of TCE into aqueous phase was relatively slow in abiotic reactor that might be explained by diffusion that appears to control the movement of TCE from NAPL phase. Production of dechlorinated ethenes enhances the dissolution rates because of the solubility of DCE, VC and ethene having higher solubility than that of TCE. It is known that the *cis*-DCE is three times hydrophilic than TCE. VC and ethene are certainly more hydrophilic than even *cis*-DCE. The low affinity of *cis*-DCE, VC and ethene to NAPL remaining in source zone results in dechlorination in regions down-gradient from the source.

Third, it was apparent that detection of the dechlorinated ethenes such as VC and ethane in the vicinity of NAPL in this study was somewhat not in accordance with the finding by Adamson et al. [18] showing no findings of VC and ethenes in a vicinity of DNAPL source zone after inoculation of *Dehalococcoides* species in a DNAPL source zone contaminated with PCE. This finding may be attributed to the longer biotransformation rates from PCE to less-chlorinated ethenes than that of TCE probably shorter rates than PCE.

A slight higher dissolution processes in soil-free reactors was observed than that in soil-slurry reactors until day 50 indicating sorption might affect more significantly than those containing low bacterial activity derived from low temperature.

5. Conclusions

This study provides information of implications with respect to the feasibility of anaerobic bioremediation technology in NAPL source zone by finding of biologically enhanced dissolution process in wetlands. It was demonstrated that TCE dechlorination was occurred in biotic reactors incubated at 20 °C. The response of dechlorinating bacteria was relatively faster (within 3 weeks) in biotic reactors containing soil-slurry indicating that signs of community establishment and development along with methanogenic condition trigger the dechlorination faster. Temperature would be a factor to determine the dissolution rate by stimulating bacterial activity. Furthermore, the TCE dechlorination could occur in a TCE-NAPL phase that demonstrated no previous TCE biodegradation, suggesting that microbes may be useful in developing source-zone bioremediation system. This phenomenon might be explained by the influence of bacterial chemotaxis previously studied by Marx and Aitken [14] for naphthalene. Our study is also in agreement with findings that DNAPL dissolution rate was significantly enhanced when directly coupled with biological dehalogenation in a column study [15].

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