

Report for 2005NJ91B: Resistance of Fractured Rock Dechlorinating Bacteria to Pressure From Heavy Metals

Publications

- Conference Proceedings:
 - Son, E-K.; Lee, K. Y.; Fennell, D. E. Characterization of tetrachloroethene- and vinyl chloride-dechlorinating bacteria enriched from a tetrachloroethene- contaminated site in the Newark basin. Poster presented at the 105th ASM general meeting, Atlanta, GA, June 5-9, 2005.
 - Fennell, D.E.; F. Liu; E.-K. Son; A. Zarnadze; U. Krogmann; L.Totten. Biotransformation of halogenated contaminants in sludges and enrichments from municipal anaerobic digesters. Poster presented at the SETAC North America 26th Annual Meeting, Baltimore, MD, November 13 – 17, 2005.
 - Son, E.-K., Fennell, D.E. : Identification of tetrachloroethene- and vinyl chloride-dechlorinating bacteria enriched from tetrachloroethene-contaminated groundwater and sediments . The 21st SSW Annual Meeting, Amherst, MA, October 17-20, 2005.

Report Follows

Problem and Research Objectives

Because of the extensive use as degreasers, chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are the most common groundwater contaminants (1). The remediation of chlorinated ethenes-contaminated aquifers is a difficult proposition that is further complicated by the presence of heavy metal co-contaminants. Up to 60% of CERCLA sites contain heavy metals along with other contaminants (15) and the DOE has identified heavy metals and TCE as a common contaminant mixture at their sites (2). Microbial reductive dechlorination which transforms chlorinated ethenes to the benign product ethene (3) is an attractive remedial process for contaminated aquifers. Several genera of bacteria have been identified to have the ability to reductively dehalogenate the chlorinated ethenes (4, 5, 6). However, only one genus of bacteria, *Dehalococcoides*, has been identified which is capable of the complete dehalogenation from tetrachloroethylene (PCE) to ethene through serial reductive dechlorination (PCE → TCE → *cis*-DCE → VC → ethene) (7, 8).

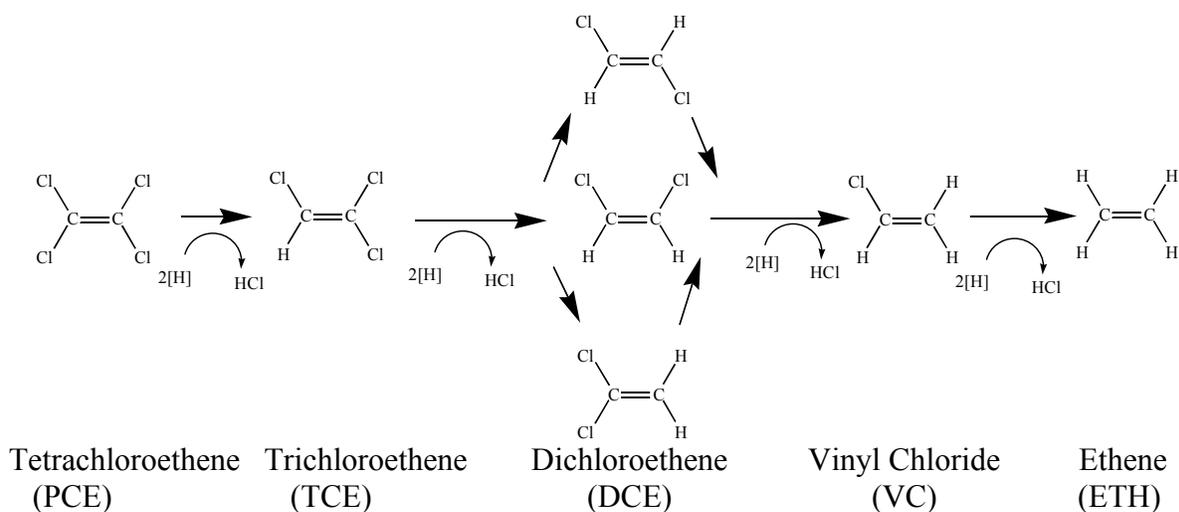


Figure 1. Pathway of anaerobic biological reductive dechlorination of the chloroethenes.

Inhibition of dechlorination of organic compounds in the presence of toxic heavy metal such as Cr, Cd, Pb, Zn, Cu, and Co has been shown in sediments, sludges, and enrichment cultures (12, 13, 17). Though extensive research has been performed on reductive dechlorination of chlorinated ethenes and anaerobic biotransformation of heavy metals respectively, little work has been performed to show how the presence of mixtures of chlorinated ethenes and heavy metal co-pollutant affects dechlorination.

The primary goal of this study is to investigate the effect of heavy metals on the dechlorination potential in groundwater contaminated with both chlorinated ethenes and heavy metals under anaerobic condition using the PCE enrichment culture containing *Dehalococcoides* bacteria. Understanding the interplay between chlorinated ethenes and heavy metal mixtures will allow better description and prediction of their fate and transport.

Methodology

Enrichment culture and growth condition. Highly PCE-enriched cultures which dechlorinated PCE completely to ethene at high rates have been developed in our laboratory using Busch Campus aquifer material (14). The description of enrichment development is as follows; Groundwater and fine sediments were collected from the aquifer. 100 mL of groundwater/sediment fines mixture was distributed to sterile 160 mL serum bottles in a nitrogen-purged glove bag. After determination of initial chloroethene concentrations, the bottles were then divided into different treatment sets. Autoclaved controls, live controls and bottle sets amended with PCE or VC and lactate and butyrate as electron donors were run. PCE or VC were added in corresponding bottles periodically and chlorinated ethenes (PCE, TCE, DCEs and VC), and ethene concentrations were analyzed using gas chromatography with a flame ionization detector (GC-FID). Electron donors and their fermentation products, volatile fatty acids were determined using high performance liquid chromatography (HPLC). To purify the cultures, the enrichments were transferred periodically into anaerobic basal mineral medium. The anaerobic growth medium was described in ref. (18). From the 3rd transfer 1 mg/ml of ampicillin and 5 mM of bromoethanosulfonate(BES) were applied and butyric acid and lactate were substituted with H₂ gas as an electron donor.

Molecular Analysis. By using 16S rRNA gene-based molecular methods such as PCR using primers specific for *Dehalococcoides* or universal bacteria, terminal restriction fragment length polymorphism (TRFLP) and denatured gradient gel electrophoresis (DGGE), we characterized microbial composition of our PCE enrichment culture. To identify microbial community each band from DGGE was excised and DNA sequencing was conducted.

Anaerobic dehalogenation studies in the presence of added metals. The most purified PCE enrichment culture was chosen for determination of metal resistance. To avoid metal sulfur precipitates, Na₂S was deleted from the anaerobic growth medium described above and L-cysteine was used as a reducing agent. 10% transfer (v/v) of the PCE enrichment cultures were conducted into 60 mL serum bottles with the modified growth medium. The cultures were grown with addition of PCE and H₂ gas for 3 weeks for acclimation. All PCE in the cultures was completely transformed to ethene and 10% (v/v) of the adjusted cultures were transferred into the medium containing respective concentrations of heavy metals. Concentrated stock solutions of CdCl₂, CuCl₂·H₂O, and K₂CrO₄ were prepared with sterile water, purged with N₂ gas, sealed in acid-washed serum bottles, and autoclaved. An initial study monitored the dechlorination of the PCE enrichment cultures acclimated with the medium containing L-cysteine in the presence of Cr final concentrations of 0.1 to 5 ppm. Based on the initial study, **Figure 2.**, metal ions solubility and the previous experiment (data not included), three concentrations for each metal were selected (Table 1). About 35 μmole/100ml of PCE was spiked two times in metal amended enrichment bottles and chlorinated ethenes (PCE, TCE, DCEs and VC), and ethene concentrations were determined using gas chromatography with a flame ionization detector (GC-FID). Dose response graphs describing the effect of Cd(II), Cr(IV) and Cu(II) on dechlorination were obtained. All treatment cultures including control were triplicate. The cultures were incubated at room temperature in the absence of light.

Table 1. Heavy metal treatment sets (final concentration)

	Cd(II) : CdCl ₂	Cr(VI) : K ₂ CrO ₄	Cu(II) : CuCl ₂ ·H ₂ O	Control
1	50ppm	50ppm	25ppm	No metal addition
2	100ppm	100ppm	50ppm	
3	200ppm	200ppm	100ppm	

Principal Findings and Significance (Progress Report)

Primary results of the on-going project to date are to characterize the highly purified PCE enrichment which showed complete dechlorination activity and document the effect of three kinds of heavy metals (Cr, Cd and Cu) on dechlorination of the enrichment culture.

Culture description. Microbial enrichment for this study was examined by using 16S rRNA gene-based molecular analysis of genomic DNA of the enrichment culture. On the DGGE gel we had one band and this band was sequenced to turn out *Dehalococcoides*-like microorganism. TRFLP data from the reactions with two different kinds of restriction enzymes (Hae III and Hha I) also gave us one major peak in its chromatograms. To assure the purity of the culture and identify the longer sequence full 16S rRNA gene was amplified and sequenced. We obtained single chromatogram from DNA sequencing of the crude PCR amplicon which meant only one kind of DNA fragment was present in the PCR amplicon sample. From these results, we could speculate tentatively our PCE enrichment culture was highly purified (may be pure) and contained *Dehalococcoides* genus bacteria of which 16S rRNA gene sequence is most similar to *Dehalococcoides* sp. strain CBDB1. This culture showed very fast dechlorination (20 μ mole/100ml of PCE to ethene within 4 days). No methanogenic activity was observed after BES (methanogen inhibitor) application.

Effect of concentrations of metals. Dechlorination of PCE and formation of the intermediates and final product, ethene, in the presence of added metals are presented in **Figure 3.** and **Figure 4.** Chlorinated ethenes and ethene were determined periodically. When complete dechlorination of PCE in the control set was observed, dechlorination degree of each culture in response to the heavy metal presence was monitored. The first spike of about 35 μ mole/100ml of PCE was completely dechlorinated to ethene in 90 days and in the second addition of same amount of PCE complete dechlorination was shortened to 60 days. For comparison of different groups, molar fraction (%) of chloroethenes and ethene among total molar amount was used instead of absolute concentration.

Cadmium. The previous study in our lab revealed that the genome of *Dehalococcoides* contains *zntA/copA*-like genetic elements, known to impart resistance to Cd. Also, the mixed culture with *Dehalococcoides* sp. strain 195 has shown capability to grow under high Cd concentrations (up to 200ppm). Based on these results, relatively higher concentrations of Cd (II) were applied (50ppm, 100ppm, and 200ppm).

Dechlorination intermediates such as TCE, c-DCE and VC were detected first after 30 days incubation in the treatments of Cd (II) concentration of 50ppm and 100ppm. 50ppm of Cd (II) seemed not to affect dechlorination potential of the enrichment culture and all PCE was transformed to ethene and no other intermediates were observed 90 days after PCE addition. At 100ppm and 200ppm of Cd(II) concentrations inhibition effect was obvious and very little ethene formation and large accumulation of intermediates were detected (**Figure 3. (a)**). In the second addition of PCE, dechlorination rates of all Cd (II) treated cultures decreased and no ethene formation was observed at 100ppm and 200ppm (**Figure 4.(a)**). This inhibition effect could be explained by the accumulation of soluble Cd (II) ions inside the dechlorinating bacterial cell (16).

Chromium. In the preliminary study it was observed that neither increase nor decrease in dechlorination occurred by the addition of low concentrations of Cr (VI) (over the range of 0.1ppm to 5ppm) (**Figure 2.**). Thus, higher concentrations were chosen for further inhibition/resistance study for Cr (VI) (50 ppm, 100ppm, and 200ppm). First onset of dechlorination of PCE was observed on day 30th after the first PCE spike at all three concentrations. 90 days incubation data demonstrated that the addition of Cr (VI) concentration of 50ppm to 200ppm resulted in significant inhibition of chloroethenes dechlorination and ethene formation was inversely proportional to the concentrations (**Figure 3. (b)**). In contrast to Cd (II), dechlorination capability was considerably restored at 50ppm and 100ppm of Cr (VI) in incubation with the second PCE spike (**Figure 4. (b)**). It may lead to the speculation that unlike to *Dehalococcoides* sp. strain 195, our culture could have mechanisms responsible for resistance to Cr (VI).

Copper. Because of lower solubility of Cu (II) in the anaerobic growth medium used here, concentrations of 25ppm, 50ppm, and 100ppm were applied. 70% or more recovery in ethene formation was observed at 25ppm and 50ppm of Cu (II) in the first spiked PCE dechlorination (**Figure 3. (c)**). Dechlorination inhibition pattern after the second PCE addition was changed. Overall inhibition pattern was similar to the first spike, but, more PCE was accumulated at all three concentrations and more ethene was produced at the 100ppm (**Figure 4. (c)**).

Discussion. Our data suggest that our culture was highly purified with *Dehalococcoides* bacteria and they are responsible for complete dechlorination of the enrichment culture. Three heavy metals, Cd (II), Cr (VI) and Cu (II) at high concentrations showed inhibition effect on PCE dechlorination of our PCE enrichment culture. However its resistance potential and response pattern to introducing of respective heavy metals were all different. For further elucidation of mechanistic explanation, molecular analysis of metal treated cultures such as PCR specific for heavy metal resistance genes and quantification of the microorganisms interested should be followed. These findings can be directly related to design and evaluation of the remediation options and to assessment of the natural dechlorination potential of contaminated sites.

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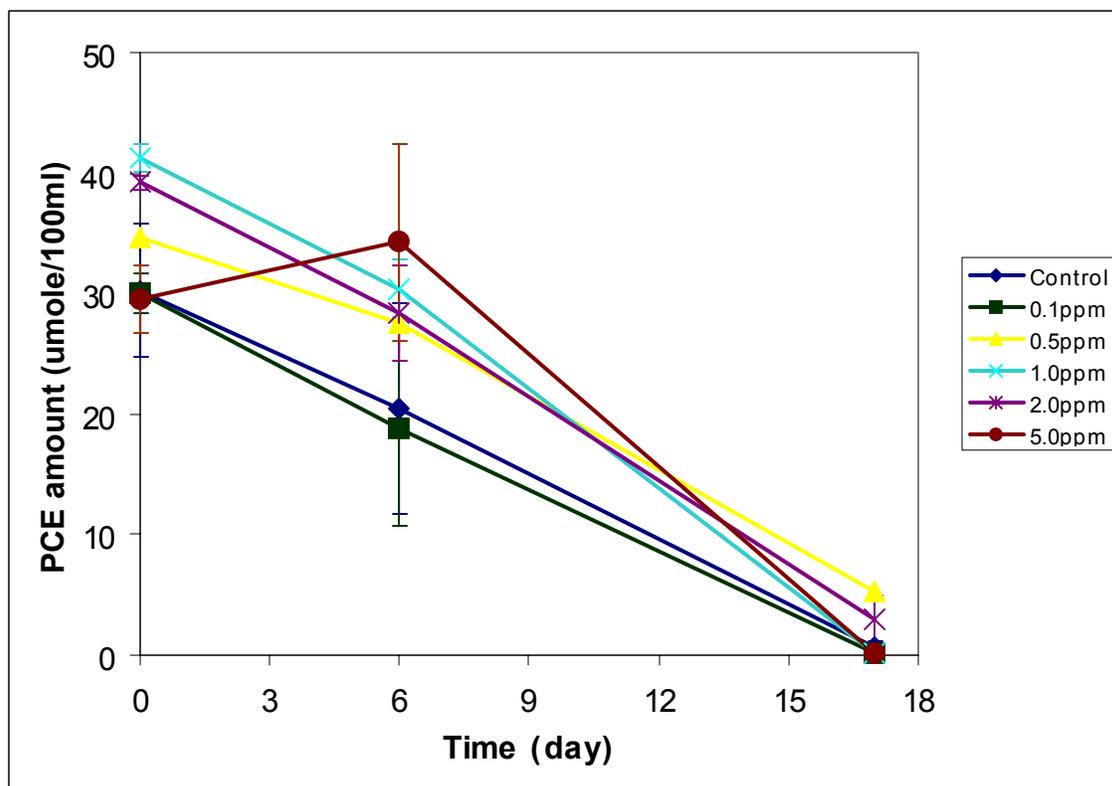
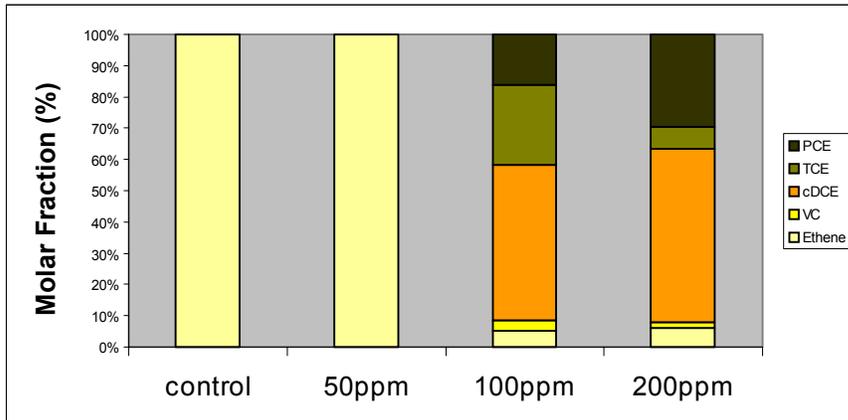
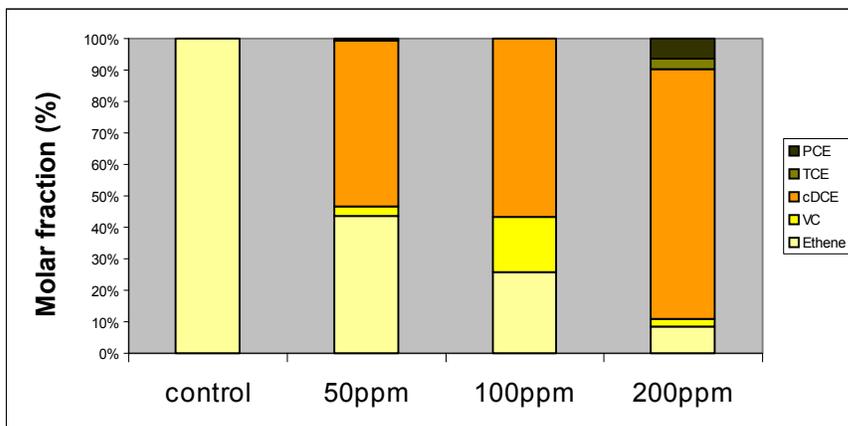


Figure 2. Dechlorination of PCE in the presence of different concentrations of Cr(VI)

(a)



(b)



(c)

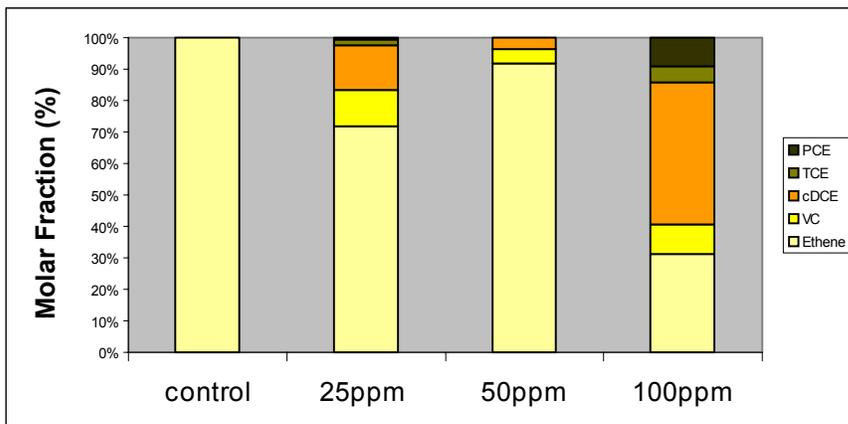
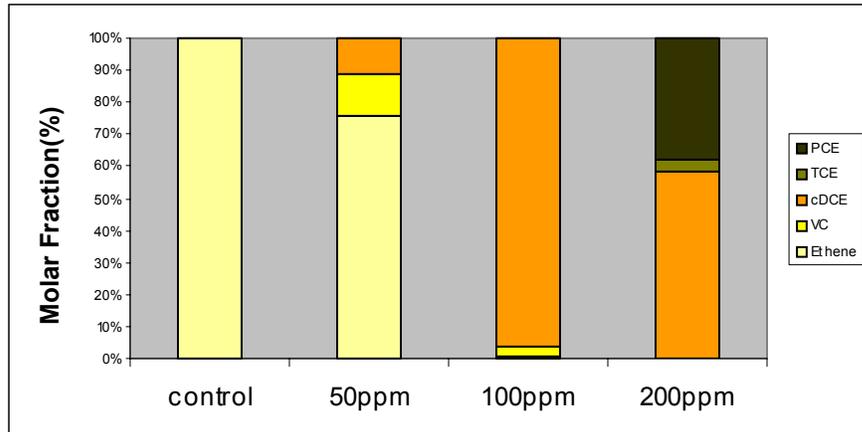
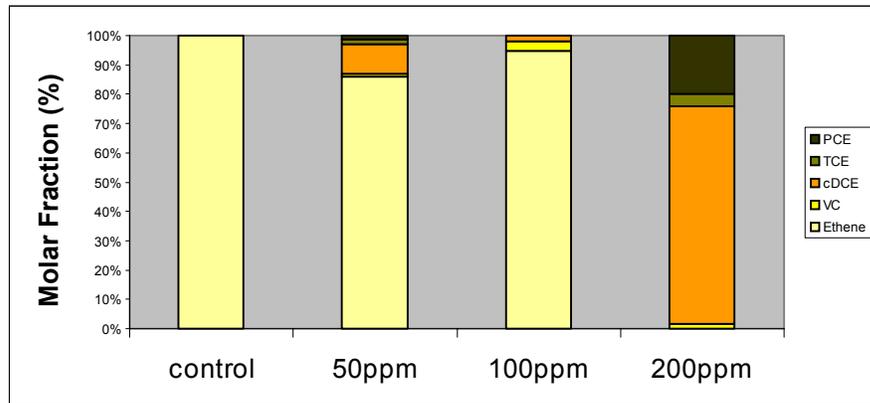


Figure 3. Effect of added Cd(II) ; (a), Cr(VI) ; (b), and Cu(II) ; (c) on dechlorination of the first spiked PCE after 90 days incubation.

(a)



(b)



(c)

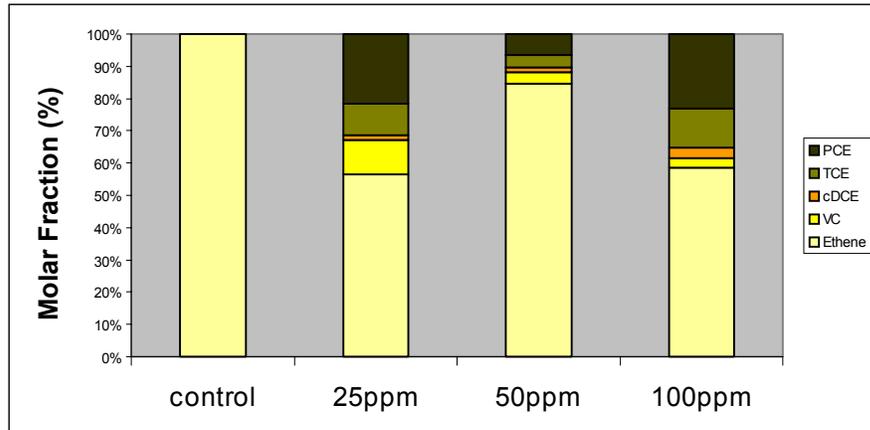


Figure 4. Effect of added Cd(II) ; (a), Cr(VI) ; (b), and Cu(II) ; (c) on dechlorination of the second spiked PCE after 50 days incubation.