

Report for 2004MD68B: Using Bioaugmentation to Improve the Biodegradation of Chlorinated Compounds in Wetlands -- Summer Fellowship

There are no reported publications resulting from this project.

Report Follows

Evaluation of the Use of Bioaugmentation and Biostimulation to Improve the Degradation of Chlorinated Compounds in Natural Freshwater Tidal Wetlands

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Contamination of groundwater with chlorinated solvents, specifically, 1,1,2,2-tetrachloroethane (TeCA), tetrachloroethene (PCE), trichloroethene (TCE), trichloromethane (chloroform, CF), and carbon tetrachloride (CT), is a serious problem at Aberdeen Proving Grounds (APG). Of particular concern are numerous seep sites at APG where groundwater is presumably discharging to the surface water at a relatively high rate and, more importantly, significant levels of the above parent chlorinated compounds and/or daughter degradation products are detected in the surface waters.

The objectives for Summer 2004 were to test the hypothesis that contaminant-degrading bacteria are absent from, or present in insufficient numbers, at seeps, and to evaluate the use of bioaugmentation to improve the degradation of chlorinated compounds. The culture used for the bioaugmentation experiment, WBC2, was obtained from another West Branch Creek site where successful degradation has been observed. Reductive dehalogenation can only be sustained if sufficient electron donors are present. In addition, in past studies, it has been shown that chlorinated compounds, such as TeCA and TCE, degrade more quickly in the presence of methanogenic conditions, rather than under nitrate or sulfate-reducing conditions. Therefore, in addition to bioaugmentation, it was also hypothesized that biostimulation through the addition of limiting growth substrates, i.e., electron donors, might also be necessary to promote biodegradation of the chlorinated solvents at the seep sites.

A preliminary methane experiment, conducted for 23 days, evaluated the availability of methanogenic substrates in the unamended seep site sediment and groundwater. The results showed that low concentrations of methane (less than 0.05 μM) were present, suggesting that the levels of endogenous degradable substrates were low and addition of electron donors to the seep site samples would be needed to sustain reductive dechlorination. Therefore, the bioaugmentation experiments included treatments that were designed to evaluate the effect of exogenous electron donors on chlorinated solvent biodegradation. Ethanol and lactate were the exogenous donors selected for use in various bioaugmentation experiment treatments because previous studies conducted with batch and column studies have shown that these compounds are effective at sustaining reductive dechlorination of the parent compounds and potential daughter products in APG sediments.

The effectiveness of bioaugmentation and biostimulation at enhancing chlorinated solvent biodegradation at a single seep site, 3-4W, is being evaluated in two experiments, which are referred to as the small-scale and large-scale experiments and are described below.

Small-Scale Experiment

In the small-scale microcosm experiment, the concentrations of the parent chlorinated solvents and their daughter products were monitored using gas chromatographic analysis of headspace samples. This precluded the use of TeCA, which is much less volatile compared to the other parent chlorinated compounds, but it meant that the same microcosms could be repeatedly sampled at each sampling interval and limited the number of microcosms that had to be prepared. Four anaerobic microcosms were prepared in serum bottles. Each contained a slurry of sediment collected from site 3-4W and groundwater and a headspace of N_2/CO_2 . Since PCE, TCE, CT, and CF are soluble in ethanol, an ethanol stock solution of PCE, TCE, CT, and CF was added to each microcosm to provide the appropriate concentration of ethanol (as an electron donor) and chlorinated solvents. Lactate was also added to each microcosm. To two of the microcosms, the bacterial culture, WBC2, was added, in addition to the electron donors. Two water controls were also prepared to monitor abiotic losses. The results of this experiment collected to date appear in Figures 1 and 2.

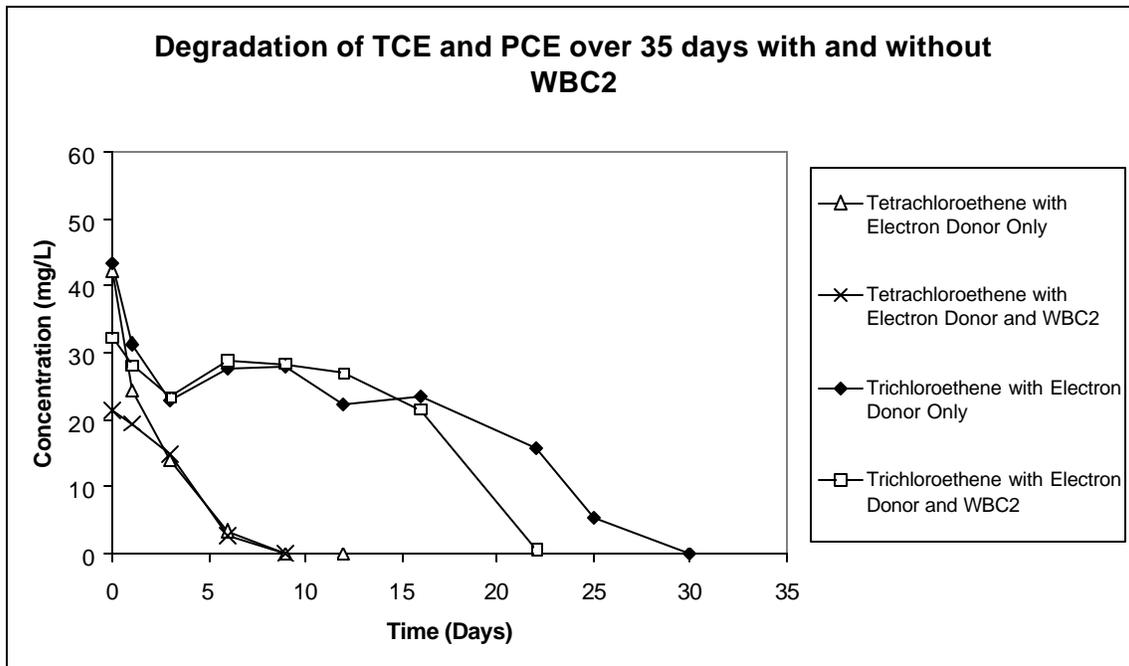


Figure 1. Concentrations of TCE and PCE in seep site (3-4W) sediment slurries with and without addition of WBC2 culture. Each data point represents the average concentration for the sampling day.

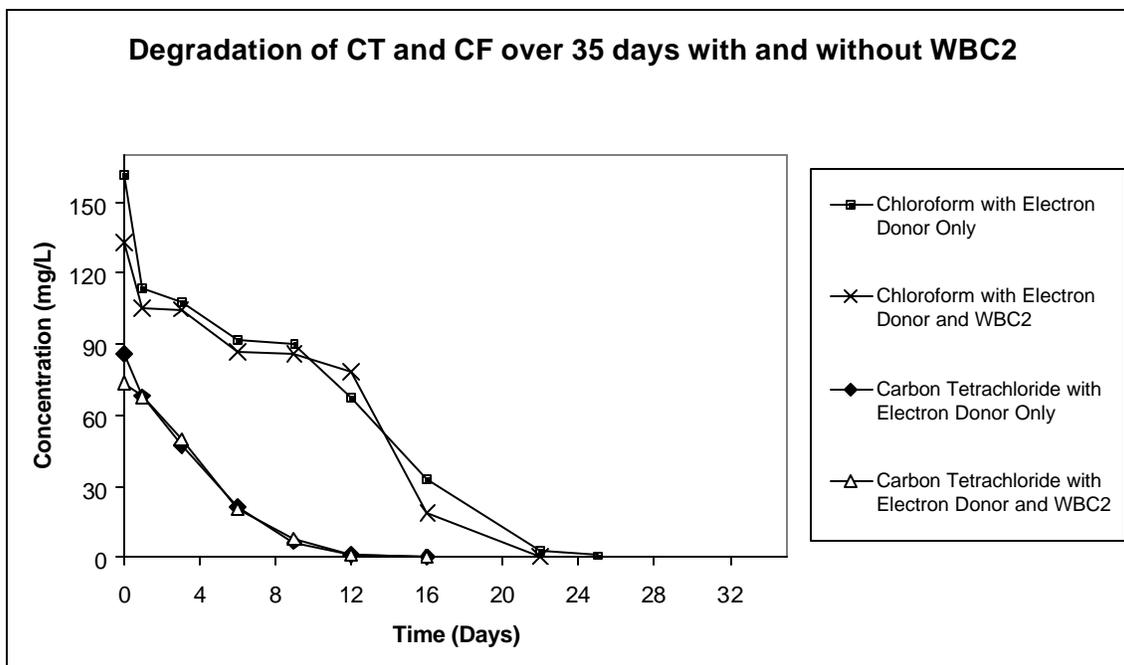


Figure 2. Concentrations of CF and CT in seep site (3-4W) sediment slurries with and without addition of WBC2 culture. Each data point represents the average concentration for the sampling day.

In particular, removal of TCE appeared to be significantly enhanced by the addition of WBC2 (Fig.1). CF in WBC2 augmented samples also appeared to occur at slightly faster rates compared to unaugmented samples (Fig. 2). CT and PCE displayed no change in its degradation rate between augmented and unaugmented samples. Additional analyses will evaluate the effect of WBC2 on the accumulation and biodegradation of daughter products, in order to determine whether bioaugmentation or biostimulation improves the complete degradation of the parent compounds.

At the conclusion of the experiment, the community DNA in each microcosm will be extracted and analyzed using a fingerprinting technique, to observe significant differences in the structures of the bacterial communities due to the addition of WBC2 and/or the biodegradation of the chlorinated solvents.

A larger-scale experiment was also conducted in order to evaluate the effectiveness of biostimulation, with and without bioaugmentation, at enhancing the removal of TeCA, as well as the other parent chlorinated solvents, in the seep sediment.

Large-Scale Experiment

Because of the need to analyze TeCA in the large-scale experiment, it differed in several respects compared to the small-scale experiment. Specifically, the chlorinated solvents had to be extracted from the sediment slurry before being quantified using gas chromatography. This meant that individual microcosms could not be repeatedly sampled, and, instead, duplicate microcosms were sacrificed at each sampling event. All

microcosms were amended with a mixture of chlorinated solvents. One set of viable microcosms did not receive any other amendments. In addition to the chlorinated solvents, three other sets of viable microcosms were amended with electron donors only, WBC2 culture only, or electron donors plus WBC2 culture. Several different types of abiotic controls were also prepared for sacrificing at different time points. Therefore, a very large number of microcosms were established for this experiment.

In addition to observing the removal of chlorinated compounds, other tests are being done to observe the changes in the microcosm biogeochemistry over time. A colorimetric method using bipyridine is used to monitor iron reduction by detecting the change in ferrous iron concentration. The amount of methane produced in the microcosms is being tested using gas chromatography. At the conclusion of this experiment, we should have a better understanding of how biostimulation through the addition of electron donors and bioaugmentation with the enrichment culture affects biodegradation of the chlorinated solvents in the seep sediment.