

2000 Annual Progress Report
Mechanisms, Chemistry, and Kinetics of Anaerobic Biodegradation of cDCE and VC

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Research Objectives

Biological reductive dehalogenation of the chlorinated ethenes, tetrachloroethene (PCE) and trichloroethene (TCE) to cis-1,2-dichloroethene (cDCE), vinyl chloride (VC) and then ethene is of great interest both for natural attenuation and engineered remediation of these hazardous contaminants in groundwater. This study was directed towards a better understanding of the factors affecting the rate and extent of conversions of cDCE and VC to ethene, which are generally considered the rate limiting steps in the overall process. The objectives of this study are to (1) determine the biochemical pathways for reductive dehalogenation of cDCE and VC, including identification of the enzymes involved, (2) determine the chemical requirements, especially the type and quantity of electron donors needed by the microorganisms for reductive dehalogenation, and (3) evaluate the kinetics of the process with respect to the concentration of both the electron donors and the electron acceptors (cDCE and VC).

Research Progress

Four manuscripts have been published, one is in press, and another is under review (see Project Publications). This report summarizes results from the last year of this three year project.

Objective One - Biochemical Pathways and Enzymes We reported a novel cDCE and VC reductive dehalogenase activity in a highly active, mixed culture (Rosner, McCarty & Spormann, 1997). We are currently purifying the VC dehalogenase. The VC dehalogenating activity is found associated with the membrane fraction of the cell extract and is partially released by treatment with zwitterionic detergents. Using anion exchange chromatography, the dehalogenating activity elutes as a single fraction which is further purified by hydrophobic interaction chromatography and gel filtration. Analysis of the active fraction reveals at least 3 protein bands as determined by Coomassie-stained SDS-polyacrylamide gels. From 2D-protein gel electrophoresis, several peptides were identified, some of which have the same molecular mass as the peptides from the highly enriched VC dehalogenase fraction. We have determined the N-terminal amino acid sequence of the three peptides and designed degenerate oligonucleotide probes in order to identify the genes encoding these peptides. We expect that only one additional purification step is required to obtain a pure preparation of the VC dehalogenase. Further research with the purified enzyme will be directed towards the development of methods for quantifying the presence of the VC dehalogenating organisms at contaminated sites.

Objective Two - Chemical Requirements Use of an appropriate hydrogen level is necessary to favor dehalogenation of chlorinated solvents, such as PCE and TCE in substrate competition with other microorganisms. Batch studies were conducted to evaluate different possible electron donors for dehalogenation: benzoate, propionate, oleate, tetrabutylorthosilicate (TBOS), and biomass. Both benzoate and propionate are soluble and move with the ground water once mixed with it. The other

three donors can be rendered non mobile, and thus can feed into groundwater as it passes by. They fall into the category of slow release compounds. TBOS is insoluble and can be placed into a well or a barrier system. It hydrolyzes slowly, releasing butanol at a fixed rate. Oleate has been used as a hand soap in the past and is soluble in the sodium form, but insoluble in the calcium form. It is toxic to microorganism in the low milligram per liter level, but not when precipitated with calcium. It can be distributed in an aquifer in the sodium form, and precipitated by following its introduction with calcium chloride. Dispersion mixes the oleate and calcium, causing precipitation in a wall across a plume. The slow decomposition of oleate leads to hydrogen production for reductive dehalogenation. Biomass can serve a similar purpose as oleate. In the absence of additional donor, the biomass will decompose, releasing hydrogen for reductive dehalogenation. All five substrates supported dehalogenation indicated (Yang and McCarty, 2000). Three times more ethene was produced from dehalogenation of cDCE using propionate than benzoate as electron donor, while benzoate produced three times more methane than propionate. More cDCE was dehalogenated with TBOS than with benzoate, although TBOS initially had an inhibitory effect. The most efficient dehalogenation was associated with biomass, 20% of which was used for dehalogenation, even higher than the 17% obtained with propionate. Oleate was found to be efficient at dehalogenation and an excellent substrate with which to establish a curtain of slow-hydrogen-release substrate across a contaminated plume. Plans for use of oleate at a field site have now been formulated.

Objective Three - Process Kinetics Biological reduction of the chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) completely to ethene is of interest for engineered or intrinsic destruction of these prevalent groundwater contaminants. However, the transformations are frequently not complete, leading to the production of vinyl chloride (VC), a more hazardous compound. A model of reductive dehalogenation that can be used to predict organism growth, rates of reductive dehalogenation of PCE and TCE to ethene, and the extent of dehalogenation at a site that can be expected has been formulated. During the past year, the validity of the dual limitation model for the combination of the chlorinated ethenes (cDCE and VC) and hydrogen has been under evaluation. A new experimental method to maintain low concentrations of hydrogen and the chlorinated solvent has been devised and evaluated. We are now conducted the dual-limitation experiments required for model validation. The results will be important for evaluating the long term potential and effectiveness of both natural attenuation and engineered in-situ bioremediation of chlorinated solvents.

Planned Activities

A request for a one-year extension of this grant without additional funds was approved. With the short time remaining, ongoing experiments will be completed and further work will be carried on through new grants received. The completion date for this project is now September 14, 2000.

Project Publications

- Haston, Z. C. and McCarty, P. L. 1999. Chlorinated Ethene Half-Velocity Coefficients (K_s) for Reductive Dehalogenation, *Environmental Science and Technology*, **33**(2), 223-226.
- Haston, Z. C., Yang, Y., and McCarty, P. L. 2000. Organism Growth and Substrate Utilization Kinetics for the Anaerobic Dehalogenation of cis-Dichloroethene and Vinyl Chloride, submitted for publication.
- Rosner B, McCarty PL, Spormann AM 1997: In vitro studies on reductive vinyl chloride dehalogenation by an anaerobic mixed culture. *Appl. Environ. Microbiol.*, **63** (11):4139-4144.
- Yang, Y. and McCarty, P. L. 1998. Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Mixed Culture, *Environmental Science & Technology*, **32**(22), 3591-3597.

- Yang, Y. and McCarty, P. L., 1999. Response to “Comment on ‘Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture,’” *Environmental Science & Technology*, **33**(12), 2128.
- Yang, Y. and McCarty, P. L. 2000. Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation, *Journal of Bioremediation*, in press.