Project ID: **54666**

Mechanisms, Chemistry, and Kinetics of Anaerobic Biodegradation of cDCE and VC

THIRD ANNUAL REPORT
for period from June 1, 1998 to May 31, 1999

DOE Project Number DE-FG07-96ER62300

Perry L. McCarty (PI)
Department of Civil and Environmental Engineering
Stanford University
Stanford, CA 94305-4020
telephone: (650).723-4131
e-mail: mccarty@ce.stanford.edu

Alfred M. Spormann (Co-PI)
Department of Civil and Environmental Engineering
Stanford University
Stanford, CA 94305-4020
telephone: (650).723-3668
e-mail: spormann@ce.stanford.edu

Prepared for the Environmental Management Science Program
U.S. Department of Energy

June 14, 1999

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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

Progress has been made under each of the three primary objectives. One manuscript related to the first objective, two related to the second objective, and one related to the third objective have been published or are in press. An additional manuscript relating to each of the second and third objectives have been submitted for publication. Pertinent information is provided in the following under "Project Publications." Findings related to the three objectives are summarized below.

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). This enzymatic activity appears to be different from previously observed corrinoid-based reductive PCE dehalogenases because 1) the VC dehalogenase activity can not be inhibited with iodopropane, iodoethane, or iodomethane, inhibitors specific for corrinoid-catalyzed reactions, and 2) the higher chlorinated ethene PCE and TCE are reduced by cell-free extracts of this culture only at rates that are orders of magnitude lower than VC dehalogenation (Rosner, McCarty & Spormann, 1997). We are currently purifying the VC dehalogenase. As starting material, we use cells of the mixed culture that reduces VC to ethene (Rosner, McCarty & Spormann, 1997, see above). Cells of this culture are grown in several 15 liter batches of anaerobic medium containing hydrogen, acetate, yeast extract and VC. Cells are harvested under anoxic conditions, broken by passage through a French pressure cell, and unbroken cells are separated from the cell extract by centrifugation. The VC dehalogenating activity is found associated with the membrane fraction of the cell extract and is partially released by treatment with zwitterionic detergents. The first purification step of the VC dehalogenase is anion exchange chromatography. The VC dehalogenating activity elutes as a single fraction which is further purified by hydrophobic interaction chromatography and gel filtration. Analysis of the proteins

present in the active fraction of this purification step reveals at least 3 protein bands as determined by Coomassie-stained SDS-polyacrylamide gels. We expect that only one additional purification step is required to obtain a pure preparation of the VC dehalogenase. Currently, studies to isolate the VC dehalogenating microorganism are under way.

Objective Two - Chemical Requirements

Use of an appropriate hydrogen level is necessary to favor dehalogenation of chlorinated solvents, such as PCE andTCE in substrate competition with other microorganisms. We examined the competition between dehalogenators and other microorganisms occurring in a benzoate-acclimated dehalogenating methanogenic mixed culture (Yang and McCarty, 1998, 1999a). Results show that the dehalogenators competed best against methanogens and homoacetogens when the hydrogen level was maintained between 2 and 11 nM. The 2 nM hydrogen concentration represents the lower threshold value found here for cDCE dehalogenation. The usefulness of this hydrogen range was further confirmed with both batch fed and continuously fed reactors. In batch studies, 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. A three times greater hydrogen utilization efficiency for dehalogenation was obtained with a semi-continuous CSTR than with batch reactors when benzoate was used as substrate because a constant hydrogen concentration in the appropriate range could be maintained with the CSTR. These results suggest different approaches that might be used to favor dehalogenators in competition with other microorganisms.

We also conducted comparative studies with benzoate, propionate, oleate, tetrabutyl orthosilicate (TBOS) and biomass as substrates for dehalogenation of cDCE (Yang and McCarty, 1999b). All five substrates supported dehalogenation. Sufficient calcium was required to precipitate oleate and thus reduce its toxicity to the dehalogenating microorganisms. 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. Use of biomass as a reaction medium in a permeable reaction barrier system is suggested.

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. Factors affecting the relative rates of destruction of the solvents and their intermediate products are thus of interest. We examined the maximum degradation rates (kX) and half-velocity coefficients (K_S) for these chlorinated ethenes used as electron acceptors in reductive dehalogenation with hydrogen in excess using an enrichment culture grown on benzoate, hydrogen, and PCE (Haston and McCarty, 1999). Initial dehalogenation rates were measured at various chlorinated ethene concentrations in batch studies. With 38 mg/L volatile suspended solids of this culture, the kX and 95% confidence intervals for PCE, TCE, cis-dichloroethene (cDCE), and VC at 25ºC were found to be 77 ± 5, 59 ± 11, 14 ± 3, and 13 ± 3 µM/day with K_S values of 0.11 ± 0.04, 1.4 ± 0.9, 3.3 ± 2.2, and 2.6 ± 1.9 µM, respectively. The lower maximum transformation rates and higher K_S values for cDCE and VC partly explain why incomplete transformation of PCE and TCE often occurs in the field.

In order to predict the time required for onset of dechlorination following bioaugmentation of a field site , information is needed about the growth rates for the microorganisms involved and the factors affecting dechlorination rates. Studies we recently completed using a mixed PCE-dehalogenating culture indicated that the same species was involved in both cDCE and VC dechlorination, and that cDCE and VC competitively inhibited each other's dechlorination rate (Haston, Yang, and McCarty, 1999). The inhibition coefficients for each were similar to their cDCE and VC half-velocity coefficients of 3.3 and 2.6 µM, respectively. Using a Monod-type growth model, the maximum specific growth rate on VC was determined to be 0.21 ± 0.02 /day. Commercial cDCE was found to inhibit growth of the culture in proportion to the amount added. Biologically generated cDCE, however, exhibited no such adverse affect. Correcting for this inhibition, the maximum specific growth rate for cDCE dechlorination was 0.30 ± 0.06 /day.

Planned Activities

A request for a one-year extension of this grant without additional funds was approved internally by Stanford University. The completion date will now be September 14, 2000. Research under all three objectives is continuing. Under objective one, efforts are being made to purify the

dehalogenating enzymes. Under objective two, studies are being conducted to find ways to increase the efficiency of electron donor utilization for dehalogenation. Under phase three, further efforts are being made to quantify the kinetics of dehalogenation, especially for high concentrations of chlorinated solvents where potential toxicity is of great importance.

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. 1999. 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 and Technology* , **32**(22), 3591-3597.

Yang, Y. and McCarty, P. L., 1999a. Response to "Comment on 'Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture," *Environmental Science & Technology,* In Press.

Yang, Y. and McCarty, P. L. 1999b. Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation, submitted for publication.