**MECHANISMS, CHEMISTRY, AND KINETICS OF THE
ANAEROBIC BIODEGRADATION OF CIS-DICHLOROETHYLENE AND
VINYL CHLORIDE**

First Annual Progress Report

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Background and Significance

This three-year project is to study the anaerobic biological conversion of cis-1,2-dichloroethene (cDCE) and vinyl Chloride (VC) to ethene. The study is being conducted in three separate phases, the first to better understand the mechanisms involved in cDCE and VC biodegradation, the second to evaluate the chemistry of the processes involved, and the third, to study factors affecting reaction kinetics. Major funding is being provided by the U.S. Department of Energy, but the DuPont Chemical Company has also agreed to directly cost-share on the project at a rate of \$75,000 per year for the three year period.

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are solvents that are among the most widely occurring organic groundwater contaminants. The biological anaerobic reduction of chlorinated aliphatic hydrocarbons (CAHs) such as PCE and TCE to cDCE and VC in groundwater was reported in the early 1980s. Further reduction of PCE and its intermediates to ethene was reported in 1989. Several pure cultures of anaerobic bacteria have been found to reductively dehalogenate PCE to cDCE. Rates of reduction of PCE and TCE to cDCE are high and the need for electron donor addition for the reactions is small. However, the subsequent reduction of cDCE to VC, and then of VC to the harmless end product, ethene, is much slower and only recently has a pure culture been reported that is capable of reducing cDCE to VC or VC to ethene. There are numerous reports of such conversions in mixed cultures. The reduction of cDCE and VC to ethene is where basic research is most needed and is the subject of this study.

Research Objectives

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 effect of concentration of both the electron donors and the electron acceptors (cDCE and VC).

Progress to Date

Progress has been made under each of the three primary objectives. One manuscript related to the first objective has been submitted and is now in press. Drafts of manuscripts related to the other two objectives are currently available and will be submitted for publication soon. Findings related to the three objectives are summarized in the following

Objective One - Biochemical Pathways and Enzymes

Reductive dehalogenation of VC was studied with an anaerobic mixed culture enriched on VC. In growth experiments, ethene formation from VC increased exponentially at a rate of about 0.019 h^{-1} . Reductive VC dehalogenation was measured in vitro using cell-free extracts of the mixed culture. The apparent K_m for VC was determined to be about $76 \text{ }\mu\text{M}$; V_{max} was about $28 \text{ nmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$. The VC dehalogenating activity was membrane-associated. Propyl iodide had an inhibitory effect on the VC dehalogenating activity in the in vitro assay. However, this inhibition could not be reversed by illumination as is generally the case when vitamin B-12 is the transforming factor involved. Cell-free extracts also catalyzed the reductive dehalogenation of cDCE and, at a much lower rate, TCE. PCE was not transformed. The results indicate that the VC dehalogenating microorganism(s) in the enrichment culture could also dehalogenate cDCE, but not the more chlorinated ethenes. Thus, the reductive dehalogenation by this culture is different from previously reported reductive dehalogenations of PCE and TCE.

Objective Two - Chemical Requirements

A continuously stirred tank reactor (CSTR) was used to develop a PCE-dehalogenating enrichment culture, and was originally seeded with sediment from a PCE-contaminated groundwater aquifer in Victoria, Texas, where complete reduction to ethene was occurring following addition of benzoate to the groundwater. A study using several potential electron donors, including benzoate, sucrose, lactate, ethanol, and acetate indicated most complete conversion of PCE to ethene was obtained with benzoate. Benzoate was thus selected as the donor and was fed at a constant concentration of 1.7 mM along with a mM PCE to provide a 36 day detention time. Near complete conversion of PCE to ethene was obtained. A mass balance indicated 70 % of the benzoate electron equivalents were converted to acetate, 13 % to methane, and 9 % to dehalogenation, and 4 % to biomass.

Anaerobically, one mole benzoate is theoretically fermented to 3 moles of acetate and 3 moles of hydrogen. Our laboratory study results indicate hydrogen is the major electron donor for cDCE and VC dehalogenation. Batch studies were conducted to evaluate the competitors for available hydrogen, and indicated these were homoacetogens, methanogens, and dehalogenators. At high hydrogen concentration, conversion to acetate by acetogens dominated the reaction. At hydrogen concentration somewhat above 12 nM, methanogens were highly competitive. However, with hydrogen concentration between 3 and 11 nM, dehalogenation was the most competitive process. Low hydrogen concentrations that favored dehalogenation could be maintained with the CSTR, but were difficult to obtain with batch donor addition. This suggests a useful approach for reductive dehalogenation of PCE.

Objective Three - Process Kinetics

Preliminary studies suggest the affinity constant for hydrogen used in dehalogenation of cDCE and VC to be about 20 nM, which is consistent with values provided by others. Studies on the affinity constants (K_s) for the chlorinated ethenes themselves, while serving as electron acceptors, indicate the value for PCE (0.06 μM) is much lower than that for ICE (1.4 μM), cDCE (3.4 μM), and VC (2.7 μM). The maximum rate for PCE dehalogenation was about 2 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$. That for TCE was about the same while the values for cDCE and VC were about one-fifth of the value for PCE. These rates, which are based upon total population concentration rather than the compound specific population, are low compared with that for the VC enrichment noted under Objective Three where the culture was much more enriched for the specific organism of interest. The relative rates, however, do fit within the general observation that PCE and TCE dehalogenations are much faster than for cDCE and VC.

Another observation made during this first year was the particular toxicity to methanogens and dehalogenators of cDCE. A phenomenon was found where cDCE could be dehalogenated with initial concentrations as high as 400 μM as long as the microorganism population started at a sufficiently high concentration so that dehalogenation was complete within a few days. However, at lower population levels where dehalogenation may take longer than about 10 days, the dehalogenation slowed down and eventually stopped. Further studies indicated that inhibition with high cDCE concentration was a time dependent factor. The inhibition resulted in organism death slowly. A more detailed study of this phenomenon indicated that cDCE-concentration must be below about 20 μM for

inhibition to be absent to small initial dehalogenating populations. This is an important finding for groundwater systems where concentrations of cDCE, are often higher than this level for extended periods of time. Initial studies with high cDCE and population concentrations missed this phenomena.

Future Studies

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.