

# **Environmental Management Science Program**

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## **Mechanisms, Chemistry, and Kinetics of Anaerobic Biodegradation of Cis-Dichloroethylene and Vinyl Chloride**

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### Research Objective

The objectives of this study are to: (1) determine the biochemical pathways for reductive dehalogenation of cis-1,2-dichloroethene (cDCE) and vinyl chloride (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 and Implications

Progress has been made under each of the three primary objectives. One manuscript related to the first objective has been published. Manuscripts related to the other two objectives have been submitted for publication. 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  $0.019 \text{ h}^{-1}$ . 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  $76 \mu\text{M}$ ;  $V_{\text{max}}$  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, trichloroethene (TCE). Tetrachloroethene (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 1.0 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, 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 higher hydrogen concentrations (> 400 nM), conversion to acetate by acetogens dominated the reaction. At hydrogen concentration somewhat above 12 nM, methanogens were highly competitive. However, with hydrogen concentration between 2 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  $\mu\text{M}$ ) is much lower than that for TCE (1.4  $\mu\text{M}$ ), cDCE (3.4  $\mu\text{M}$ ), and VC (2.7  $\mu\text{M}$ ). 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 One 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 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  $\mu\text{M}$  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 slow organism death. A more detailed study of this phenomenon indicated that cDCE concentration must be below about 20  $\mu\text{M}$  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.

### **Planned Activities**

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.

### **Other Access To Information**

Rosner B., McCarty P.L., 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., McCarty, P.L.: Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture, submitted for publication.

Haston, Z.C., McCarty, P.L.: Chlorinated Ethene Half-Velocity Coefficients ( $K_s$ ) for Reductive Dehalogenation, submitted for publication.