Annual Progress Report (Grant # DE-FG07-96ER62319)

Complete Detoxification of Short Chain Chlorinated Aliphatics: Isolation of Halorespiring Organisms and Biochemical Studies of the Dehalogenating Enzyme Systems.

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The objectives of our research within this grant are:

1. Isolation and characterization of chlororespiring organisms responsible for the complete dehalogenation of chlorinated ethenes and propanes.
2. Development of conditions that yield high cell densities and induce dechlorinating activity.
3. Development of assay systems to detect the dechlorinating activity in cell-free extracts.
4. Purification and characterization of the dehalogenating enzymes.

Summary of research findings (9/96 through 8/97)

Anaerobic microcosms were obtained from a variety of geographically different sediment samples. In several microcosms complete dechlorination of tetrachloroethene (PCE) to ethene (ETH), and 1,2-dichloropropane (1,2-D) and/or 1,2,3-trichloropropane to propene was observed. Upon subsequent transfers to anaerobic medium, sediment-free, methanogenic enrichment cultures were obtained that dechlorinated PCE to ETH, and 1,2-D to propene, respectively.

2-Bromoethanesulfonate (BES), a well known inhibitor of methanogens, did not inhibit the dechlorination of 1,2-D to propene and the dechlorination of PCE to *cis*-dichloroethene (*cis*-DCE). However, the complete dechlorination of PCE to vinyl chloride (VC) and ETH was severely inhibited. We could show that BES inhibited the dechlorination of chloroethenes in cultures not containing methanogens. Previous to this study, BES was believed to be a specific inhibitor of methanogens and the inhibitory effect of BES on dechlorination was explained by the involvement of methanogens in the dechlorination process.

The non-methanogenic cultures obtained after the BES treatment were subsequently transferred to medium not containing BES and complete dechlorination of PCE to ETH was observed as was in the original microcosms. Subcultures were further enriched with PCE, *cis*-DCE, VC, or 1,2-D as the only available electron acceptor and acetate, or acetate plus hydrogen as the only available electron donor(s). To date these cultures have undergone up to 45 transfers. **Interestingly, two cultures that originally dechlorinated PCE to ETH**, but were then enriched with *cis*-DCE or VC, lost their ability to dechlorinate PCE or TCE. This finding indicates that different populations are involved in the complete dechlorination of PCE to ETH in these cultures. In

contrast, one culture that was enriched with *cis* - DCE and VC, respectively, maintained its ability to dechlorinate PCE. Using molecular tools (16S rDNA targeted PCR, TA cloning of 16S rDNA genes, ARDRA analysis and sequencing) we showed that this culture consisted of three distinct organisms. Two of them could be isolated in pure Culture but neither of them showed any dechlorinating activity, indicating that one organism was responsible for the complete dechlorination of PCE to ETH in the mixed culture. We are currently focusing on the isolation and phylogenetic characterization of this organism.

To date we have isolated two PCE dechlorinating organisms from these enrichment & pure culture. One isolate, a strictly anaerobic organism designated strain BB 1, dechlorinated PCE to *cis* - DCE using acetate, lactate, or pyruvate as electron donors. Phylogenetic analysis showed that this organism belongs to the genus *Desulforomonas* and its closest relative is strain TTB4, another PCE to *cis* - DCE dechlorinating organism. However, strain BB1's 16S rDNA sequence is 5% different to the sequence of strain TTB4 as are several key physiological features, showing that BB1 is a different species from strain TTB4; Strain BB 1's ability to grow by chlororespiration was confirmed by showing the PCE dependent growth with acetate, and by determining the *fe* value. The *fe* value describes the-fractions of electrons going from the electron donor (acetate) to the electron acceptor (PCE). Strain BB 1 revealed an *fe* value, of 0.66 indicating that 66% of the electrons from acetate were consumed in the reductive dechlorination of PCE to *cis* - DCE. The only other electron acceptor besides PCE that supported growth of strain BB1 was fumarate. We optimized the culture conditions and sufficient cell material can be obtained to assess PCE dechlorinating activity in cell-free systems. A test system to measure PCE dechlorination' using gas chromatography was established.

The other isolate we obtained was designated strain Vietl and dechlorinated PCE to TCE. This strictly anaerobic organism was identified as a-new member of the genus ***Desulfitobacterium***. Strain Vietl grew well with pyruvate and in different complex media like LB or TSA, and the dechlorinating activity was induced in the presence-of PCE. Therefore, cell material suitable for the investigation of cell-free dechlorinating activity is available. Like strain BB1, strain Vietl was able to couple the reductive dechlorination of PCE to growth. The ability for chlororespiration was confirmed by hydrogen threshold measurements, by determining the *fe* value, and by showing PCE dependent growth.

Two 1,2-D dechlorinating cultures were obtained that could be indefinitely transferred to mineral salts medium containing acetate as a carbon source and hydrogen as an electron donor. Hydrogen threshold measurements were used to confirm that chlororespiring are responsible for the conversion of 1,2-D to propene. ARDRA analysis of one of the cultures showed seven different patterns. Partial sequence analysis of the cloned 16S rDNA genes revealed that six out of the seven clones had exactly identical sequences and only one sequence was distinct. We are currently analyzing the complete sequences in order to determine the phylogeny of the organisms. Knowing more about the organisms' phylogeny can provide crucial information for an efficient isolation procedure for the dechlorinator(s).

Our work within this grant has resulted in several oral and poster presentations at national and international meetings. One peer reviewed paper was published in 1997 and a second one is in press. Two more publications are currently in preparation (see below). We feel confident that we will be obtaining other significant results in the near future that will further improve our

understanding of the physiology and phylogeny of halorespiring organisms and their dechlorinating enzyme systems.

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