

DEHALOGENATION OF TETRACHLOROETHYLENE (PCE) IN ESTUARINE SEDIMENT ENRICHMENTS: CHARACTERIZATION OF THE DECHLORINATING MICROBIAL COMMUNITY AND THE EFFECTS OF SULFATE REDUCTION INHIBITION

Jessi M. Satterberg<sup>1</sup>, Russell P. Herwig<sup>2</sup>, and John F. Ferguson<sup>1</sup>  
Department of Civil and Environmental Engineering<sup>1</sup>, and Aquatic and Fishery Sciences<sup>2</sup>,  
University of Washington, Seattle WA 98105

The emergence of chlorinated solvents into estuarine receiving waters provides a dynamic chemical environment in which the presence of sulfur oxyanions may impact the rate and extent of reductive dechlorination. The reductive dechlorination of PCE in the presence of sulfate and sulfate reduction inhibition was investigated using enrichments of estuarine sediment from four locations. Triplicate enrichments (100ml) of each experimental condition were set up with approximately 40uM PCE and varying sediment inocula of 0.1g, 1.0 g, and 10g. Enrichment conditions included: (1), the addition of 5mM lactate and 3.5% hydrogen in the 60ml headspace as electron donors, (2), the addition of electron donor and 10 mM of sulfate, (3), the addition of electron donor, sulfate and 20mM of the sulfate analog molybdate (inhibition of sulfate reduction), and (4), those that received only molybdate. PCE dechlorination was observed within 16 days in enrichments receiving only electron donor from all four locations: Moss Landing, CA (ML), Orca Island, WA (OI), Sequim, WA (SQ), and the Duwamish waterway Seattle, WA (DW). The extent of PCE transformation varied among the sediment source locations, with sediment inocula size, and with the presence and absence of sulfate and inhibition of sulfate by molybdate. ML, OI, and SQ enrichments have transformed PCE to cDCE, while the extent of PCE dehalogenation in DW enrichments has been primarily to TCE. Increasing rates of PCE and TCE dehalogenation were seen with increasing sediment inocula sizes in nearly all enrichments that received electron donor, and those that received electron donor and sulfate. However, ML and OI enrichments that received only electron donor showed rapid dechlorination at all inocula sizes. PCE dehalogenation has been slower in those enrichments receiving sulfate than those without sulfate. The presence of molybdate appears to have removed the competition between dechlorinating and sulfate reducing bacteria for shared electron donors by inhibiting sulfate reduction. The ML enrichment receiving molybdate and the largest sediment inocula dechlorinated PCE faster than enrichments without molybdate. Genomic DNA was extracted from enrichments and the 16s rDNA gene from enrichments was amplified with PCR using universal bacterial primers 8fm and 1492r-RPH. The various sediment sources required site-specific PCR optimization, which included the addition of Bovine Serum Albumin. The dechlorinating community will be characterized with Terminal Restriction Fragment Length polymorphism (T-RFLP) analysis using the restriction enzymes Msp I, Alu I, and Tse I. Nested PCR will be used to confirm the presence of terminal restriction lengths characteristic of known PCE dehalogenating bacteria. Five sets of specific primers will be used in nested PCR targeting dechlorinating bacteria including: Dehalococcoides sp., D. ethenogenes, Desulfuromonas michiganensis BB1, Sulfurospirillum sp. strain JPD-1, and Desulfitobacterium sp. The results of this study contribute to our understanding of the impact of sulfate on reductive dechlorination and the diversity of dechlorinating bacteria that may exist in estuarine waters. This study was funded by National Institute of Environmental Health Sciences (Grant P42 ES04696).