Application of Metagenomic-Guided Proteomics to Assess Degradation of Chlorinated Ethenes in Pure Cultures and Groundwater from Contaminated Sites

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Background/Objectives. In groundwater contaminated with chlorinated ethenes, the dominant biodegradation mechanism is typically reductive dechlorination, whereby tetrachloroethene (PCE) and/or trichloroethene (TCE) are sequentially dehalogenated to cis-1,2-dichloroethene (cis-DCE), vinyl chloride (VC) and finally ethene, which is environmentally benign. A number of different dehalogenating bacteria catalyze one or more steps of this process, with *Dehalococcoides* (DHC) being the only organism known to perform the critical VC-to-ethene dechlorination step. Several commercially available chemical-based products are also available to reduce the mass of chlorinated ethenes in situ.

In this study, sediments from southeastern Washington, D.C. were used to set up microcosm experiments with selected biotic and abiotic amendments. Microcosms were evaluated for the efficiency of CVOC degradation by testing for several groundwater parameters and utilization of TCE and cis-DCE. Additionally, concentration of DHC cells was evaluated for the time zero and end point of the microcosms containing microbial population. Proteomics was further used to assess abundance of reductive dehalogenases (RDases) and link their concentration to the degradation rate in each microcosm.

Approach/Activities. Microcosms evaluated for biotic process were amended with commercially available DHC-containing microbial culture, a slow release emulsified vegetable oil [EVO] used to promote enhanced reductive dichlorination (ERD), and an amendment that provides microbes with soluble electron donors for rapid utilization. Microcosms evaluated for the abiotic degradation of CVOCs consisted of chemical reduction using zero valent iron (ZVI) applied at two doses, 5 and 20 g/L.

Assessment of dehalogenating populations was performed with the use of the targeted proteomic analyses. In general, the rate of an enzymatic reaction depends on the concentration of the substrate(s) and enzyme(s) involved; thus, the abundance of an RDase is directly proportional to the rate of dechlorination of the enzyme's substrate (e.g., VC). Such targeted measurements of specific proteins are made possible through technological advances in mass spectrometry and knowledge about keystone RDases involved in the detoxification of chlorinated ethenes.

Results/Lessons Learned. Three key findings included: 1) identification of a variety of RDase peptides that may serve as biomarkers for degradation of chlorinated ethenes, 2) absolute quantification of a subset of these peptides, and 3) demonstration of a correlation between dechlorination rates in DHC microcosms and RDase concentrations in microcosms with additional amendments. The data presented demonstrate the validity of addition of targeted proteomics to predict degradation potential of specific amendments in the microcosm experiment that can be further extrapolated into field scenarios.