Bioremediation and Sustainable Environmental Technologies—2017. Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies (Miami, FL; May 2017). ©2017 Battelle Memorial Institute, Columbus, OH, www.battelle.org/biosymp. A. Barton and S. Rosansky (Chairs)

Proteomic Tools to Monitor Chlorinated Solvent Bioremediation and Estimate In Situ Degradation Rates

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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. Assessment of dehalogenating populations at a site is usually based on the enumeration of biomarker genes using quantitative polymerase chain reaction (qPCR). A number of qPCR assays have been designed to enumerate specific reductive dehalogenase (RDase) genes such as the DHC TCE RDase gene *tceA* and the VC RDase genes *bvcA* and *vcrA*. In addition, specific qPCR assays are available to enumerate the 16S rRNA genes of DHC and other dechlorinators. While 16S rRNA gene and RDases gene copy numbers provide useful abundance information, these measures do not necessarily inform about dechlorination activity. In light of this limitation and in an effort to provide a more robust and specific measurement that is directly correlated to degradation rates, a proteomics approach to quantify specific RDase proteins has been developed. 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.

Approach/Activities. We introduce a protein-based diagnostic tool that directly quantifies RDases from the commercially available bioaugmentation culture SDC-9™. Based on the SDC-9™ metagenome, specific RDase gene sequences were identified to develop a database that allowed us to identify and quantify RDase proteins using bottom-up proteomic techniques. Using SDC-9™ microcosms, we sought to link RDase levels to dechlorination rates through quantitative proteomic measurements. Additionally, a first attempt to link protein concentrations to rates of natural attenuation or *in situ* bioremediation will be described.

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 SDC-9™ microcosms and RDase concentrations. Further, the methods developed during this work aid in the detection and quantification of RDases in environmental samples, a measurement that can potentially be linked to *in situ* dechlorination rates. The data presented demonstrate the validity of a stepwise approach, in which metagenome sequencing aids quantitative targeted proteomics in the identification of key peptides involved in the biodegradation of chlorinated ethenes.