Comparing Reductase Enzyme Peptides to *Dehalococcoides* DNA as Predictors of Rates of Dechlorination of *cis*-DCE and VC

Paul Hatzinger (Paul.Hatzinger@aptim.com, Aptim, Lawrenceville, NJ, USA), Mandy M. Michalsen (U.S. Army ERDC EL, Vicksburg, MS, USA)**;** Ember Korver (Seattle District USACE, Seattle, WA); Kate Kucharzyk, Craig Bartling, Larry Mullins, Jayda Meisel (Battelle Memorial Institute, Columbus, OH, USA), Frank Löffler, Fadime Kara-Murdoch (University of Tennessee, Knoxville, TN, USA and Oak Ridge National Laboratory, Oak Ridge, TN, USA), John Wilson (Scissortail Environmental Solutions LLC, Ada, OK, USA), Jonathan Istok (Oregon State University, Corvallis, OR, USA)

Background/Objectives: Well-established molecular markers for the microbial reductive dechlorination process include qPCR assays for the *Dehalococcoides mccartyi* (*Dhc*) 16S rRNA gene, the TCE reductive dehalogenase (RDase) gene *tceA*, and the vinyl chloride (VC) RDase genes *bvcA* and *vcrA*. Unfortunately, rate constants for reductive dechlorination are not closely associated with the abundance of DNA or mRNA that is amplified by the well-established reductive dehalogenation biomarkers. As a result, robust predictions of the rate of microbial reductive dechlorination of *cis*-1,2-dichloroethene (cDCE) and VC in contaminated groundwater cannot be made by measuring these nucleic acid biomarkers. In hopes of developing an approach using quantitative measurements of molecular markers that serve as predictors of the rate constants, we are comparing the reductive dechlorination rates observed in dechlorinating microcosms to the abundance of RDases enzymes) in the contaminated groundwater.

Approach/Activities: We are conducting a microcosm study using groundwater from a contaminated site. The microcosms are spiked with 5 mg/L of cDCE, 500 mg/L lactate as an electron donor, and are inoculated with the dechlorinating consortium SDC-9™ at initial densities of 10^8, 10^7, 10^5 and 10^3 *Dhc* cells/mL. Liquid samples are collected over time and analyzed for cDCE and VC concentrations, the abundance of the *Dhc* nucleic acid biomarkers using qPCR, and the abundance of selected peptides that are components of RDase enzymes using a targeted proteomics approach.

A series of peptide-specific pseudo first order rate constants are calculated using an Excel spreadsheet. The spreadsheet provides a best fit estimate of the rate constants by minimizing the sum of squares of the difference between the measured concentrations of cDCE and VC, and the concentrations that would be expected from the RDase-specific rate constants and the abundance of the specific RDase enzyme in the microcosms. The RDase-specific rate constants are equivalent to dividing the bulk rate constant for removal of cDCE or VC in the microcosms by the abundance of the specific RDase enzyme. As a benchmark and a comparison, a best fit estimate of the rate constant specific to the *Dhc* biomarker is extracted. A weighted least squares analysis is used to determine the uncertainty in the fitted rate constants, expressed as the standard deviation of the rate constants.

Results/Lessons Learned: Monitoring of the dechlorinating microcosms is on-going. Samples are analyzed for *Dhc* RDase and nucleic acid biomarkers, which will be compared and evaluated by comparing the uncertainty associated with the estimates of the rate constants. The rate constants that have a lower coefficient of variation are better predictors of reductive dechlorination. If the rate constants estimated based on RDase abundance correlate with the actual measured rates, this approach represents an advance over the nucleic acid-based qPCR approach. If successful, the the predictive power of the targeted proteomics approach will be

further evaluated by conducting a similar experiment at pilot scale with a push-pull test in a contaminated aquifer.