Validation of Advanced Molecular Biological Tools for Monitoring Chlorinated Solvent Bioremediation and Estimating Degradation Rates

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Background/Objectives: A number of organism- and process-specific biomarker genes for monitoring reductive dechlorination (RD) at chlorinated solvent-impacted groundwater sites have been identified, including the vinyl chloride reductive dehalogenase (RDase) genes *bvcA* and *vcrA* as biomarkers for ethene formation. Whereas RDase gene abundance indicates RD activity *potential*, RDase gene transcript and protein abundance indicate RD activity *expressed*. A previous study (ER-201129, BioPIC tool) sought to link RD activity to RD biomarker gene abundances by regressing field-scale first order RD rate coefficients from multiple field sites versus *Dehalococcoides mccartyi* (*Dhc*) 16S rRNA gene abundances measured in a single groundwater well sample from each site. Results showed a positive correlation but the 1 to 2 orders of magnitude spread in rate coefficient estimates for a given gene abundance failed to yield a useful predictive relationship. The goal of this project is to explore if combined quantitative gene-, transcript-, and protein-centric analyses can provide more accurate estimates of in situ RD rates.

Approach/Activities: This study will quantify and constrain uncertainties for both rate coefficient estimates and biomarker abundances, which have limited the rate-predictive power in previous studies. Further, in contrast to previous efforts where rate coefficient values representative of plumes as a whole were regressed against biomarker abundance values measured in a single location within the plume, this effort will utilize carefully replicated measurements in laboratory microcosms. Triplicate microcosms will be prepared using aquifer materials from contaminated DoD field sites using varied *Dhc* cells densities (10^3 to 10^8 cells/mL) supplied by augmenting with consortium SDC-9™, lactate as the electron donor (500 mg/L), and *cis*-1,2-dichloroethene as the electron acceptor (5 mg/L). Time series samples will be collected from microcosms to assess concentration changes in chlorinated ethenes, volatile gases, anion concentrations, as well as biomarker abundances (RDase genes, transcripts and proteins). Uncertainties in rate coefficient estimates and biomarker abundances will be quantified and accounted for to establish robust linkage between measurable biomarkers and observed rates. Following laboratory validation, the approach will be applied to field sites to determine if meaningful rate estimates can be obtained from quantitative biomarker measurements.

Results/Lessons Learned: Results from initial microcosm tests showing RD rate correlations with biomarker abundance measurements will be presented in context with previous efforts that only quantified *Dh*c gene abundances. A data analysis/model framework, which accounts for uncertainties in both rate coefficients and biomarker abundance values, will be presented. The ongoing demonstration will demonstrate how integrated application of mature qPCR and targeted proteomic measurements can generate meaningful in situ rate estimates and improve bioremediation decisions based on site-specific biological and geochemical constraints.