Expansion of the of the Molecular Biological Tool Box: Environmental Proteomics Predicts In Situ Degradation Rates

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Background/Objectives. Several organism- and process-specific biomarker genes for monitoring reductive dechlorination (RD) have been identified. The vinyl chloride reductive dehalogenase (RDase) genes *bvcA* and *vcrA* serve as biomarkers for ethene formation at sites impacted with chlorinated ethenes. Whereas the abundance of RDase genes alone provides a measure of RD *potential*, the quantitative assessment of RDase gene transcripts and proteins can provide information about RD *activity,* and potentially in situ RD rates. The objective of this project is to demonstrate (1) that proteomics workflows can quantify biomarker and proteins over a wide range of *Dehalococcoides* (DHC) cell densities, and (2) that peptide biomarker abundances can be a more reliable predictor of in situ RD rates than gene- and transcript-centric approaches.

Approach/Activities. The link between RDase biomarker abundances and RD rates were established in laboratory microcosms prepared using aquifer materials from Joint Base Lewis-McChord near Tacoma, Washington. Each microcosm was inoculated with the reductive dechlorinating microbial consortium SDC-9™ to achieve a range of DHC cell abundances (10^3 to 10^8 cells/mL), as well as 500 mg/L lactate, 10 g/L *cis*DCE, and calcium carbonate to buffer groundwater pH. Biomarker abundances, chlorinated ethene concentrations, and geochemical conditions in the microcosms were monitored over time. Rate coefficients and their uncertainties for *cis*DCE and VC biodegradation were estimated by fitting a numerical approximation of the first order RD reaction equations to observed data. The abundances of DHC RD biomarker proteins (FdhA, TceA, and VcrA) and genes (*fdhA, tceA, vcrA*) were quantified using targeted proteomics and quantitative PCR assays, respectively. Each biomarker analysis for each sample was performed in triplicate to estimate the uncertainty of the measurement. Biomarker abundances and rate coefficient data were subject to correlation and regression analysis.

Results/Lessons Learned. Results confirmed that biomarkers can be quantified over a range of DHC cell abundances relevant for bioremediation at contaminated sites, i.e. 10^3 cells/mL for monitored natural attenuation and up to 10^8 cells/mL for enhanced bioremediation (biostimulation, bioaugmentation) sites. Biomarker abundances were positively correlated with degradation rates and regression results demonstrated their rate-predictive power. A follow-on field evaluation using a push-pull test format to estimate in situ biodegradation rates in a bioaugmented aquifer is planned. Outcomes will validate an advanced MBT platform combining qPCR technology with targeted proteomic measurements to generate rate estimates and enable bioremediation decisions based on site-specific biological and geochemical constraints.