Quantitative Proteomics Approach to Monitor cVOC Bioremediation and Degradation Rates

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Background/Objectives. Several organism- and process-specific biomarker genes for monitoring reductive dechlorination (RD) have been identified. The reductive dehalogenase (RDase) genes *tceA*, *bvcA* and *vcrA* serve as degradation biomarkers 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 study is to demonstrate that: (1) targeted proteomics can quantify protein biomarkers over a wide range of *Dehalococcoides mccartyi* (*Dhc*) cell densities, and (2) current quantitative proteomics (qProt) assays are suitable for quantifying *Dehalococcoides* (*Dhc*) proteins in samples from chlorinated volatile organic compound- (cVOC-) contaminated field sites under monitored natural attenuation (MNA) conditions.

Approach/Activities. NASNI Site 5, NASNI Site 9 and FLOD, Lordstown sites have been selected to demonstrate application of quantitative proteomics to monitor the sites and estimate degradation rates. Shotgun metagenome sequencing and bioinformatics analysis of the selected wells from Site 5 were performed to identify relevant RDase genes and their allelic variations (e.g., the sequence variants of the *tceA* and *vcrA* genes) potentially present in indigenous *Dhc* strains. The current qProt assays were compared with site-specific data and qProt analyses were performed for samples from a series of groundwater wells at Site 5, Site 9 and FLOD. The wells were also sampled for cVOC concentrations and geochemical parameters. The provided site data were used to estimate first-order rate constants for cDCE and VC applicable to the portion of the aquifer that is sampled by each monitoring well. The estimated rate constants and corresponding biomarker abundances from the field site were used to extend and validate the existing predictive model for application of qProt to field sites.

Results/Lessons Learned. At NASNI Site 5, results demonstrated that *kVC* estimates based on VcrA protein abundance measurements were within a factor of 2 for *kVC* estimates based on groundwater concentration data. For Site 9, in locations where both biomarker genes and proteins were quantified, predicted rates were within the same order of magnitude –within a factor of 4 for k*cDCE* and a factor of 20 for k*VC*. For Site FLOD, genes and proteins typical for enhanced biological reductive dechlorination were not detected. The information provided by advanced MBT application will support site management and decision making and would give the regulatory community another line of evidence for contaminant attenuation, which will result in more site closures and realize substantial cost savings for site owners and the U.S. taxpayer.