EXECUTIVE SUMMARY

Validation of Advanced Molecular Biological Tools to Monitor Chlorinated Solvent Bioremediation and Estimate CVOC Degradation Rates

ESTCP Project ER-201726

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ACRONYMS AND ABBREVIATIONS

cis-DCE	cis-1,2-dichloroethene
cVOC	chlorinated volatile organic compound
Dhc	dehalococcoides
DoD	Department of Defense
MBT	molecular biological tool
MNA	monitored natural attenuation
qPCR	quantitative polymerase chain reaction
qProt	quantitative proteomics
RDases	reductive dehalogenases
RPM	remediation project managers
VC	vinyl chloride

1.0 INTRODUCTION

Knowledge about the rates of *in situ* contaminant degradation is crucial for optimizing remedial design and supporting site management decisions. Despite progress understanding the factors influencing microbial degradation of chlorinated ethenes, determining rates of microbial contaminant degradation at field sites remains challenging. Molecular biological tool (MBTs) for quantifying *Dehalococcoides mccartyi* (*Dhc)* nucleic biomarkers are available and guide site management decision making; however, these measurements have not been useful to generate good estimates of contaminant degradation rates. Quantification of reductive dehalogenases (RDases) may provide a more direct measure of activity (as these are the actual enzymes/proteins that catalyze biodegradation of chlorinated ethenes), and technological advances in mass spectrometry instrumentation allow the sensitive, quantitative determination of RDase proteins of interest in groundwater. This project explores if RDase gene and protein biomarker abundances, alone or in combination, may be used to estimate degradation rates.

2.0 OBJECTIVES

This demonstration had three specific objectives. The first objective was to demonstrate the utility of quantitative proteomics (qProt) to measure the absolute abundance of *Dhc* reductive dechlorination biomarker proteins in laboratory-controlled microcosms with various *Dhc* cell titers. Contaminant concentration and ethene measurements over time were used to determine cis-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC) reductive dechlorination rates. The second objective was to correlate observed degradation rates with *Dhc* biomarker gene and protein abundances. The successful completion of objectives 1 and 2 led to the third objective: a go/no-go decision point before conducting demonstration/validation efforts of the qProt approach at military sites impacted with chlorinated ethenes.

3.0 TECHNOLOGY DESCRIPTION

The sensitive and quantitative measurement of proteins in environmental matrices is now possible, and process-specific biomarker proteins such as the *Dhc* RDases TceA, BvcA and VcrA can be measured in groundwater samples. Since the abundances of the catalysts (i.e., the specific RDase enzymes) control the rate of *cis*-DCE and VC reductive dechlorination, the quantitative measurement of these catalysts may be useful for estimating *in situ* degradation rates. Accurate assessment of *in situ* degradation rates often requires *in situ* test design, execution and appropriate data interpretation, which can be costly and time consuming to complete. Demonstration/ validation of this qProt tool has significant potential to establish (1) the predictive link between *in situ* RDase enzyme abundances and corresponding *in situ* reductive dechlorination rates at multiple Department of Defense (DoD) field sites, (2) a framework remediation project managers (RPMs) may use to convert RDase enzyme abundances directly into a rate estimates, and (3) enhanced/expedited site management decisions that can result in substantial cost savings to the DoD and even early site closure.

4.0 PERFORMANCE ASSESSMENT

The quantitative and qualitative performance metrics were met through demonstration in defined laboratory microcosm systems prepared using DoD site aquifer materials and the development of a model that predicts chlorinated volatile organic compound (cVOC) degradation rates based on RDase biomarker abundances. Bioaugmentation with the SDC-9™ consortium was used to obtain the desired range of *Dhc* cell abundances and reductive dechlorination rates. Correlation and regression analyses results confirmed that RDase biomarker abundances were significantly and positively correlated with rate coefficients. Regression analysis results were used to test the rate-predictive power of the RDase biomarker abundances. RDase proteins predicted rate constants k_{cisDCE} and k_{VC} values within one order of magnitude; using RDase proteins and genes combined further improved predictions.

5.0 COST ASSESSMENT

During the long-term monitoring and assessment phase of the project, implementation of advanced MBTs, such as metagenome sequencing or proteomics, are impacted by a multitude of factors, such as: the size of the site, proximity of the site to nearby receptors, regulatory requirements, and nature and diversity of contaminant of concern. Although there are currently no regulatory requirements that specifically mandate advanced MBTs be used to assess a site, the data provided by the advanced MBTs are meant to supplement and possibly replace other forms of data that provide lines of evidence that monitored natural attenuation (MNA) is occurring and to estimate a removal rate. Hence, the total sampling and analytical cost is driven by the number of sample locations at a site and the total number of samples collected (a greater number of samples equates to a higher cost). It should be noted however that the individual cost per sample may decrease based on a greater number of total samples requiring analyses, since the lab work is highly specialized and cost efficiencies generally can be realized for a greater number of analyses.

Many of the advanced MBTs, such as qProt, have only limited commercial availability and/or are available through a university or other research laboratory. As such, application costs remain relatively high. It is expected as these techniques mature, they will become more widely available and the analytical cost per sample will decrease substantially. For comparison purposes, the cost of the metagenomics and metaproteomic analyses based on cost data collected during the commencement of ER-201726 in 2017 were $300 and $1,500 per sample, respectively, assuming analysis of a batch of 10 samples. These costs decreased to $150 and $1,000 (for cVOCs) when evaluated in 2019. These costs are anticipated to decrease further as the technologies mature.

6.0 IMPLEMENTATION ISSUES

The primary end users of qProt are expected to be DoD site managers, consultants, and their contractors. The general concerns of these end users are likely to include the following: (1) regulatory acceptance; (2) insufficient confidence in results and limited access to specialized laboratories; and (3) technology cost compared to other more conventional monitoring options. Proteomics is a new tool in environmental assessment and one which requires further validation.

It is anticipated that, as for many technologies such as quantitative polymerase chain reaction (qPCR), regulatory acceptance will occur as the technology is field-validated, its benefits over existing approaches (e.g., ability to predict cVOC degradation rates) are realized, and the regulatory community is educated regarding its field application. As noted in the previous section, the issues of limited commercial availability of the technique and relatively high cost are also likely to improve over time (i.e., more availability and lower cost) as the qProt technology matures.