

FINAL REPORT

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

ER 201726

March 24, 2020

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REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

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1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE			3. DATES COVERED (From - To)
03/24/2020	ESTCP Final Report			
4. TITLE AND SUBTITLE			5a. CC	NTRACT NUMBER
Validation of Advanced Molecular Biological Tools to Monitor Chlorinated				
Solvent Bioremediation and Es			Eh CE	RANT NUMBER
	•		SD. Gr	KANT NUMBER
			5c. PR	OGRAM ELEMENT NUMBER
6. AUTHOR(S)			5d. PR	OJECT NUMBER
Mandy Michalsen, U.S. Army E	•	•	ER-20	01726
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Jonathan Istok, Oregon State U		University of Tennessee	5f. WC	ORK UNIT NUMBER
Fadime Kara Murdoch, University		and Oak Ridge National		
		Laboratory		
 PERFORMING ORGANIZATION N U.S. Army Corps of Engineers 				8. PERFORMING ORGANIZATION REPORT NUMBER
4735 E. Marginal Way S.				ER-201726
Seattle, WA 98134				EN 201720
Geattle, WA 30104				
9. SPONSORING/MONITORING AGE	ENCY NAME(S) AND ADDRE	SS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)
Environmental Security Technology Certification Program				ESTCP
4800 Mark Center Drive, Suite	17D03			
Alexandria, VA 22350-3605				11. SPONSOR/MONITOR'S REPORT NUMBER(S)
				ER-201726
12. DISTRIBUTION/AVAILABILITY S	TATEMENT			
DISTRIBUTION STATEMENT	A. Approved for public re	elease: distribution unli	mited.	

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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-DCE and 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 lead to a go/no-go decision point before conducting demonstration/validation efforts of the qProt approach at military sites impacted withchlorinated ethenes.

15. SUBJECT TERMS

Validation, Advanced Molecular Biological Tools, Monitor Chlorinated Solvent Bioremediation, cVOC Degradation Rates

16. SECURITY CLASSIFICATION OF:		=		19a. NAME OF RESPONSIBLE PERSON	
a. REPORT	b. ABSTRACT	c. THIS PAGE	ABSTRACT	OF PAGES	Mandy Michalsen
					19b. TELEPHONE NUMBER (Include area code)
UNCLASS	UNCLASS	UNCLASS	UNCLASS	134	206-764-3324

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LIST OF ACRONYMS

CE collision energies

CID MS/MS collision induced dissociation tandem mass spectrometry

cis-DCE cis-1,2-dichloroethene
CO₂ carbon dioxide
CTC cost to complete

cVOC chlorinated volatile organic compound

Dhc Dehalococcoides mccartyi
DoD Department of Defense

EISB enhanced *in situ* bioremediation EPA Environmental Protection Agency

ESTCP Environmental Security and Technology Certification Program

IDA information dependent acquisitions

IDL instrument detection limit

JBLM Joint Base Lewis-McChord

LC liquid chromatography

LC-MS/MS liquid chromatography tandem mass spectrometry

LOD level of detection LOQ level of quantitation

MBT molecular biological tool
MCL maximum contaminant level
MDL method detection limit
MNA monitored natural attenuation

MRM monitored natural attenuation MRM multiple reaction monitoring

MS mass spectrometry

NBK Naval Base Kitsap

O&M operation and maintenance

PRM parallel reaction monitoring

PCE tetrachloroethene PI Principle Investigator

QA quality assurance QC quality control

qPCR quantitative polymerase chain reaction

qProt quantitative proteomics

QTOF-MS quadrupole time-of-flight tandem mass spectrometer

RDase reductive dehalogenase
ROD Record of Decision
RPD relative percent difference
RPM remediation project manager

LIST OF ACRONYMS (continued)

RT-qPCR reverse transcriptase qPCR

TCE trichloroethene

trans-1,2-dichloroethene trans-DCE

United States Army Corps of Engineers USACE

Vandenberg Air Force Base vinyl chloride VAFB

VC

ACKNOWLEDGEMENTS

This report represents the results and conclusions of a collaborative effort between scientists and engineers at U.S. Army Engineer Research Development Center, Battelle Memorial Institute, Aptim Federal Services, LLC (Aptim), Scissortail Environmental Solutions, LLC, Oregon State University and University of Tennessee, Knoxville. This laboratory phase of the demonstration project was funded by the Environmental Security Technology Certification Program (ESTCP), with the goal of defining and validating correlations between *in situ* degradation rates of chlorinated volatile organic compounds (cVOCs) and quantities of biomarker genes and key reductive dehalogenase proteins (RDase).

Researchers for this project included Dr. Mandy Michalsen (Principal Investigator, U.S. Army Engineer Research Development Center), Dr. Kate Kucharzyk, Dr. Craig Bartling and Dr. Jayda Meisel (Battelle Memorial Institute), Dr. Paul Hatzinger (Aptim), Dr. John Wilson (Scissortail Environmental Solutions, LLC), Dr. Jonathan Istok (Oregon State University), Fadime Kara Murdoch (University of Tennessee, Knoxville) and Dr. Frank Löffler (University of Tennessee, Knoxville and Oak Ridge National Laboratory, Oak Ridge, TN). Several personnel at Battelle Memorial Institute, including Larry Mullins, Amy Hill and Angela Minard-Smith, were instrumental in assisting with metagenomic and metaproteomic data analysis and interpretation. Dr. Fadime Kara Murdoch from the University of Tennessee, Knoxville participated in analysis and interpretation of qPCR and transcript-related data. Other site personnel that provided significant project support included Charles Condee, Anthony Soto, Sheryl Streger, and Simon Vainberg from Aptim.

Finally, the project team wishes to thank Dr. Andrea Leeson and the support staff from the ESTCP program office for their help and guidance throughout this demonstration.

EXECUTIVE SUMMARY

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.

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*-DCE and 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 lead to a go/no-go decision point before conducting demonstration/validation efforts of the qProt approach at military sites impacted with chlorinated ethenes.

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 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.

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 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.

Cost Assessment. Implementation of advanced molecular biological tools (MBTs) such as metagenome sequencing or proteomics, during the long-term monitoring and assessment phase of the project are impacted by 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 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 number of sample locations at a site and total number of samples collected (i.e., 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 larger quantity 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.

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 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 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 be improve over time (i.e., more availability and lower cost) as the qProt technology matures.

1.0 INTRODUCTION

1.1 BACKGROUND

The Department of Defense (DoD) is responsible for over 26,000 contaminated groundwater sites with cost to complete (CTC) values estimated at \$12.8 billion (in 2010 dollars) [1]. A majority of these sites are contaminated with chlorinated volatile organic compounds (cVOCs). With over 25% of the remedies in place using enhanced *in situ* bioremediation (EISB) and over 50% of remedies using monitored natural attenuation (MNA) either as a sole remedy or as a final phase after EISB and/or other treatment approaches, a significant portion of the CTC dollars will be spent on EISB and MNA remedy monitoring. For both EISB and MNA, monitoring of a wide range of chemical, geochemical, and microbial parameters is required to demonstrate that biodegradation of cVOCs is occurring and/or progressing as expected. What is currently missing is a monitoring technology that could directly confirm active contaminant degradation and provide *in situ* degradation rate estimates. A direct measure of reductive dechlorination activity and information about degradation rates would be marked improvements for supporting both EISB and MNA approaches for site remediation. Such advances in monitoring strategies are needed to optimize remedy implementation and monitoring, and to develop predictive understanding about the trajectory of a contaminant plume, which will ultimately accelerate site closures.

In groundwater contaminated with chlorinated ethenes, the dominant and productive biodegradation mechanism is typically reductive dechlorination, whereby the parent tetrachloroethene (PCE) and/or trichloroethene (TCE) are sequentially dehalogenated to cis-1,2dichloroethene (cis-DCE), vinyl chloride (VC) and finally ethene and/or ethane, which are considered environmentally benign [2]. A number of different dehalogenating bacteria catalyze one or more steps of this process, with Dehalococcoides mccartyi (Dhc) being the only microbial group known to complete the entire pathway [3]. Assessment of dehalogenating populations at a site is usually based on the enumeration of 16S rRNA genes using quantitative polymerase chain reaction (qPCR) [4]. A number of qPCR assays have been designed to enumerate specific reductive dehalogenase (RDase) genes such as the Dhc TCE RDase gene tceA and the VC RDase genes bvcA and vcrA [5-8]. In addition, specific qPCR assays are available to enumerate the 16S rRNA genes of *Dhc* and other dechlorinators. While the number of copies of 16S rRNA genes and RDases can provide useful abundance information, these measures do not necessarily correlate with dechlorination activity. In light of this limitation and in an effort to provide a more robust and specific measurement that directly correlates to degradation rates, a proteomic approach that quantifies specific RDase proteins has been developed. In general, the rate of an enzymatic reaction depends on the concentration of the substrate(s) and enzyme(s) involved; thus, the abundance of an RDase is directly proportional to the rate of dechlorination of the enzyme's substrate (e.g., VC). Such targeted measurements of specific proteins are made possible through technological advances in mass spectrometry and knowledge about keystone RDases involved in the detoxification of chlorinated ethenes. The overarching goal of this project was to validate the utility of quantitative proteomics (qProt), and to demonstrate that the integrated, quantitative analysis of biomarker genes and proteins provides estimates of cVOC degradation rates.

1.2 OBJECTIVES OF THE LABORATORY DEMONSTRATION

The value of molecular biological tools (MBTs) has been demonstrated; however, current tools fall short of providing information about contaminant degradation rates. The overarching goal of this demonstration was to validate a platform combining mature qPCR technology with targeted qProt measurements to generate rate estimates and enhance site-specific bioremediation decision making. The specific objectives were to: (1) demonstrate that proteomics can substantially increase the value of currently accepted MBTs for cVOC biodegradation monitoring, and (2) demonstrate the utility of integrated quantitative nucleic acid- and protein-based biomarker analysis applications to estimate cVOC degradation rates. The ultimate demonstration/validation approach for this technology will be to quantify the predictive relationship between RDase proteins and reductive dechlorination rates at multiple field sites. However, because this qProt technology has not yet been demonstrated for this purpose, the initial demonstration was performed in defined laboratory microcosms established with aquifer materials collected from military sites. A validated approach to assess in situ contaminant degradation rates that provides predictive understanding of the longevity of a contaminant plume would be a major advance over the current state-of-the art. The extrapolation of meaningful rate information from MBT data promises more efficient (i.e., lower costs and reduced environmental impact) implementation of EISB, as well as the more frequent implementation of MNA, which will accelerate site closures with substantial cost-savings realized for the DoD.

1.3 REGULATORY DRIVERS

Federal Safe Drinking Water Act Maximum Contaminant Levels (MCLs) for common cVOCs are summarized in Table 1-1. Persistence of cVOCs in groundwater, their prevalence at DoD hazardous waste sites, and their concentrations far in excess of health-based levels drive the need for cost-effective remediation technologies. DoD field sites featured in this demonstration (Section 4.0) all have MCL-based groundwater cleanup objectives.

Table 1-1. Safe Drinking Water Act Maximum Contaminant Levels (MCLs) for Key cVOCs

Compound	MCLs, μg/L*
Tetraloroethene (PCE)	5
Trichlorethene (TCE)	5
cis-Dichloroethene (cis-DCE)	70
trans-Dichloroethene (trans-DCE)	100
Vinyl Chloride (VC)	2
*40 CFR 141.61	

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

Conventional Molecular Biological Tools (MBTs). The use of MBTs for detection and quantification of biomarkers, especially genes and transcripts, in environmental samples has been rapidly increasing over the last decade. MBTs are used by remediation professionals to aid remedial design, assess remedial performance, and perform long-term monitoring of biodegradation. The goal of MBT application is to measure the abundance of microorganisms of interest and their activities over temporal and spatial scales.

The most widely used MBT for environmental applications is qPCR, which allows absolute abundance measurements of genes or transcripts of interest within a sample. In the case of reductive dechlorination, qPCR assays that specifically quantify 16S rRNA and RDase genes are employed. The nucleic acid-based biomarkers for detoxification at sites impacted with chlorinated ethenes are the *vcrA* and the *bvcA* genes, which both encode VC RDases, as well as *pceA* and *tceA*, which encode PCE RDases, and TCE/DCE RDases, respectively.

The key organisms (i.e., *Dhc*) that aid in detoxification of chlorinated solvents as well as their relevant RDase genes involved in the reductive dechlorination of chlorinated ethenes have been identified, [6, 7, 9] and sensitive qPCR assays for detection and quantification of key biomarker genes have been developed and tested in multiple laboratories [3, 10, 11]. Further, refined protocols for extraction of nucleic acids from groundwater samples are available [8, 12-15]. Thus, qPCR tools that enumerate *Dhc* 16S rRNA genes and RDase genes can provide information about specific cVOC dechlorination steps [3, 6, 10, 16].

To date, efforts have been made to correlate cVOC degradation rates to *Dhc* and/or RDase gene or transcript abundance. The application of Monod-based equations showed that cVOC degradation kinetics can be roughly correlated to *Dhc* cell abundances, as determined with qPCR; however, there were large differences in activity per cell based on qPCR data collected from batch versus column studies [17]. Importantly, these correlations are based on qPCR assays that quantify all *Dhc*-like sequences, not just those responsible for cVOC degradation (i.e., those encoding and expressing RDase genes). In other words, the gene-centric qPCR approach also measures *Dhc* cells that are not contributing to the dechlorination of the target contaminant(s).

Advanced MBTs – Quantitative Proteomics (qProt). In contrast to nucleic acid based MBTs, quantitative proteomics (qProt) involves the identification and quantification of proteins (i.e., enzymes) within a sample. That is, nucleic acid-based tools generate information about potential activity, whereas protein-based measurements generate information about *actual* (i.e., functional) activity.

In general, a shotgun proteomic workflow for protein identification includes protein extraction, digestion with a protease (typically trypsin) to create tryptic peptides, and liquid chromatography tandem mass spectrometry (LC-MS/MS) for peptide separation and generation of mass-resolved spectra (Figure 2-1). Peptide identification involves querying the resulting spectra against a representative protein sequence database using search engines such as Mascot or ProteinPilot [18]. Ideally, these sequence databases are specific to the analyzed samples such that the highest

numbers of proteins can be accurately identified. Once identified using shotgun proteomics, peptides from proteins of interest (e.g., RDases) can be confirmed and quantified through the use of commercially available isotopically labeled peptides of the same sequence using multiple reaction monitoring (MRM) mass spectrometry.

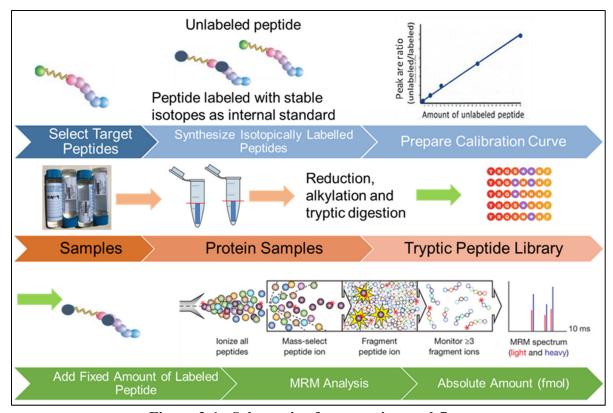


Figure 2-1. Schematic of proteomics workflow

The MRM proteomic analysis allows multiplexing of protein assay and generation of highly accurate results with near absolute specificity. This proteomic strategy is a proven and widely accepted technique for quantification of proteins [18] and has been used for decades in various matrices such as serum [19] and recently in environmental samples of groundwater and sediments [20-23]. Quantitative MRM proteomic techniques rely on targeting specific precursor peptide ions and the resulting fragment ions produced from these precursors during the analysis. Moreover, the LC-MS/MS settings can be optimized to maximize the number of precursor ions that are fragmented and scanned. Thus, in the MRM assay, specific transitions (precursor → fragment ions) for individual peptides are targeted and monitored as a function of LC retention time, which provides a highly selective, sensitive and reliable approach for quantitative analysis through integration of reproducible chromatographic peaks (Figure 2-1). With MRM, a suitable instrument such as triple-quadrupole mass spectrometer can be a priori configured to scan for a defined set of target peptides, and a selected subset of fragment ions.

Specific to this demonstration, MRM proteomic techniques have been recently used in microbial cultures to identify and quantify RDases from dechlorinators [24]. Thus, proteomics shows high potential for absolute quantification of RDases within a sample that contains mixed microbial communities. However, proteomics has not yet been exploited for the purpose of correlating cVOC

degradation rate to RDase absolute abundance. To this end, optimized protocols exist to extract proteins from biomass associated with aquifer solids or groundwater and detect and quantify key cVOC RDases with LC-MS/MS approaches [21-23].

MBTs in Assessment of cVOC Degradation Rates. Conventional nucleic-acid based MBTs can provide evidence for biodegradation, but do not aid site remediation project managers (RPMs) in prediction of contaminant longevity due to the lack of linkage to actual degradation rates. While models that include a microbial biomass, based on qPCR or total protein measurements exist [2, 17, 25, 26], their predictive power is limited. For example, batch culture/microcosm studies used biomass measurements to model cVOC degradation rates, but such models have a number of limitations and their application in support of *in situ* remediation decision making remains challenging. This limitation is due to the fact that the specific components of the microbial biomass responsible for the cVOC degradation (i.e., the RDase proteins) are not currently measured. Thus, while nucleic acid based MBTs or total biomass measurements are widely used and represent mature technologies, they may have limited value for inferring degradation rates unless combined with a more direct measure of activity (i.e., that provided by qProt). More specifically, nucleic acid-based MTBs provide a sensitive and routine means to detect and quantify DNA and transcripts of RDases, but without proteomic-based measurements, a defined correlation to cVOC degradation rate is difficult to achieve with environmental samples.

2.2 TECHNOLOGY DEVELOPMENT

At sites contaminated with chlorinated ethenes, biostimulation of indigenous dechlorinating bacteria or bioaugmentation with dechlorinating microbial consortia can achieve detoxification and environmental restoration. Contemporary bioremediation performance monitoring tools rely on nucleic acid biomarkers targeting key organohalide-respiring bacteria such as Dhc. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) allows the selective quantification of Dhc reductive dehalogenase (RDase) proteins that catalyze reductive dechlorination of chlorinated ethenes. This work applied LC-MS/MS to detect and quantify RDase peptides in the commercial bioaugmentation consortium SDC-9 comprising *Dhc* strains capable of reductive dechlorination of chlorinated ethenes and vinyl chloride to non-toxic ethene. Metagenome sequencing of the SDC-9 consortium provided a reference database for the accurate identification of target RDase peptide sequences. Shotgun proteomics workflow identified 143 RDase peptides and proteome characterization resulted in 36 distinct peptides corresponding to PceA, TceA and VcrA proteins that covered 99-100% of the annotated protein-coding sequences. From the 14 annotated RDase genes, two distinct pceA genes, one vcrA and one tceA gene were identified. Twelve of the 14 RDase genes were associated with RDase B. Quantification using parallel reaction monitoring (PRM) assays with ¹³C-labeled peptides determined 1.8x10³ for TceA, and 1.2x10² VcrA molecules per *Dhc* cell. This approach allowed for sensitive detection and accurate quantification of relevant Dhc RDases and has potential utility in bioremediation monitoring regimes.

qProt has now reached a maturity level that justifies its inclusion in environmental monitoring regimes. The combined gene-, transcript-, and protein-centric approach could reveal gene presence (functional potential), transcript abundance (gene activity), and protein abundance (actual catalytic activity). The integrated analysis of these biomarkers, together with geochemical parameters, can

be used to estimate degradation rates of specific contaminants of interest (e.g., cVOCs). In an effort to move this approach into field practice, the project team completed the following tasks to generate initial qProt data and to illustrate the reductive dechlorination rate-predictive potential in the ER-201726 project proposal.

- 1. Metagenomic sequencing and bioinformatics analysis enabled identification of a total of 14 RDase gene sequences in the SDC-9 consortium (Table 2-1).
- 2. The proteomic analysis revealed more than 14 unique RDase peptides as well as peptides from accessory proteins potentially involved in transferring electrons during the reductive dechlorination process (Table 2-1).
- 3. RDase peptides were identified and quantified in microcosm experiments using qProt procedures.
- 4. Utility of the MRM proteomics approach for quantifying RDase proteins was demonstrated in microcosm studies with the commercially available cVOC biodegradation consortium SDC-9 [27].

Results of the SDC-9 metagenomic sequencing suggested that the RDase peptides were derived from three RDase proteins (highlighted in grey, Table 2-1). Results also demonstrated that only three of the 14 RDases were identified to be expressed and presumably active, even though all 14 corresponding genes would probably be detected and enumerated with qPCR.

Table 2-1. RDase genes identified in SDC-9 metagenome

RDaseA gene locus ^a	RDaseB gene locus	Number of transmembrane helices in RDaseB	Putative taxonomy	TAT signal ^b	Percent amino acid identity	Accession number of best NCBI alignment	Predicted gene
scaffold-6337_195	scaffold-6337_193	3	Dehalococcoides	Yes	99%	WP_081042195.1	ND
scaffold-6337_194	scaffold-6337_193	3	Dehalococcoides	Yes	100%	WP_081042194.1	ND
scaffold-352_158	ND	3	Dehalococcoides	Yes	100%	BAZ97963.1	ND
scaffold-6337_252	scaffold-6337_251	3	Dehalococcoides	Yes	100%	WP_010935983.1	ND
scaffold-352_212	scaffold-352_213	3	Dehalococcoides	Yes	99%	AEI59454.1	vcrA
scaffold-178_59	scaffold-178_58	3	Dehalococcoides	Yes	99%	WP_062900263.1	tceA
scaffold-3176_24	scaffold-3176_25	3	Dehalobacter	Yes	94%	CAD28790.2	рсеА
scaffold-6337_160	ND	3	Dehalococcoides	Yes	100%	BAZ97963.1	ND
scaffold- 133_66	scaffold-133_67	3	Dehalobacter	Yes	40%	WP_015043198.1	ND
scaffold-2271_52	scaffold-2271_51	3	Dehalococcoides	Yes	100%	WP_010935983.1	ND
scaffold-352_192	scaffold-352_191	3	Dehalococcoides	Yes	100%	WP_081042194.1	ND
scaffold-3175_18	scaffold-3175_19	3	Desulfitobacterium	Yes	100%	CDX01551.1	ND
scaffold-3176_29	scaffold-3176_30	3	Dehalobacter/ Desulfitobacterium	Yes	82%	WP_025206074.1/CDX02974.1	рсеА
scaffold-352_193	scaffold-352_191	3	Dehalococcoides	Yes	99%	WP_081042195.1	ND

^aThe amino acid sequence encoded by scaffold-133_66 possessed a query coverage of 99% against reference sequence WP_015043198.1. All other amino acid sequences from RDase loci reported had query coverages of 100%.

^bNo SEC signal peptides were detected in any RDase amino acid sequences examined.

ND – not determined

To date, several RDase peptides have shown good calibration linearity ($R^2 = 0.9$) and a broad dynamic range, allowing quantification of RDases from sample extracts and a comparison of their absolute abundances to dechlorination activity. In fact, quantitative analysis of two RDase peptides (TceA and PceA) in the initial proof-of-concept microcosm experiments performed using SDC-9 cell suspensions yielded good correlations between dechlorination rate and RDase concentrations (Figure 2-2). In addition to these initial proof-of-concept microcosm studies, other similar experiments have shown that RDase peptides can be identified from environmental samples [21-23]. Specifically, BvcA, VcrA and TceA peptides were identified in samples from a cVOC-contaminated site.

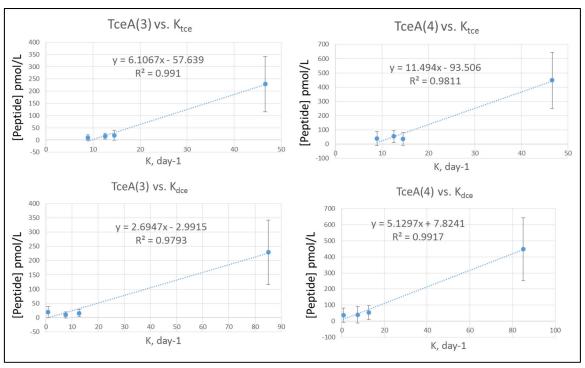


Figure 2-2. RDase peptide concentrations versus degradation rate constants RDase peptide abundance vs. first order for TCE and cisDCE in SDC-9 microcosms.

The utility of qProt as an advanced MBT for environmental monitoring has been demonstrated. However, a quantitative link between RDase peptide abundance in environmental samples and reductive dechlorination rates has yet to be established in microcosm experiments. This demonstration (1) validated the qProt method for measuring RDase peptide abundances in environmental samples, and (2) established a quantitative link between biomarker abundance (RDase peptides and genes) and rates of *cis*-DCE and VC reductive dechlorination. This was accomplished through a series of microcosm studies performed using aquifer material from a cVOC-contaminated DoD site. A detailed description of the microcosm study design is provided in Section 5.0. Briefly, *Dhc* cell abundances were varied in each set of incubation vessels over six orders of magnitude (10³ – 10⁹ cells/mL) and the rate of *cis*-DCE and VC reductive dechlorination was measured in each microcosm replicate. Live and killed controls were included and all biomarker and cVOC measurements were made in triplicate. This well-controlled and replicated microcosm study used real-world aquifer materials and provided data required to establish the

quantitative link between abundances of peptides and nucleic acid biomarkers with reductive dechlorination rates. The results presented herein can now be applied in the field to validate the link between biomarker abundances and *in situ* reductive dechlorination rates at one or more cVOC-contaminated DoD sites.

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The main advantage of proteomic techniques for the determination of *in situ* degradation rates is that the absolute amount of the reaction catalysts (e.g., RDase enzymes) are measured. Proteomicsbased techniques are limited by the amount of biomass (more specifically, the amount of proteins of interest) collected in the sample. This limitation is due to the fact that in contrast to nucleic acids, proteins cannot be amplified, so their quantification is inherently limited by the instrument detection limit (IDL) and overall method detection limit (MDL). The IDL is a function of the LC-MS/MS instrumentation and the concentration of specific peptides to be quantified. These detection limits can approach the low attomol (10⁻¹⁸) range for quadrupole time-of-flight tandem mass spectrometers (QTOF-MS) [28]. The MDL considers the sensitivity of the overall method, including any loss associated with extraction and purification from interfering substances (e.g., detergents that can suppress MS signals), which can reduce sensitivity to high fmol to attomol (10⁻¹⁵ - 10⁻¹⁸) levels for environmental samples. Prior work at Battelle [21-23] and under SERDP ER-2312 [13] demonstrated that the overall MDL for the quantification of *Dhc* RDases approaches 3,000 fmol (3 pmol) RDase per liter of groundwater, which corresponds to an approximate Dhc biomass of 10⁶ total cells. Although *Dhc* abundances are generally low at MNA sites, the qProt assay may still be reliably used at these locations by collecting suitably large sample volumes, which will ensure the sample contains sufficient RDase mass to be above the quantitation limit. Because quantitation of RDases is paramount to the proteomics method being demonstrated, additional IDL and MDL studies were completed as part of this demonstration as described in Section 5.0.

The targeted nature of qProt is both an advantage and a limitation. The main limitation is that RDases (and other proteins of interest) from indigenous (native) dechlorinating organisms may respond to biostimulation and contribute to the observed degradation activity. These native RDases may have slightly different RDase sequences and may thus not be detected in the MRM proteomic assay (i.e., false-negative results). While this is a legitimate concern, the issue can be addressed by metagenome sequencing of DNA extracted from biomass collected from site groundwater. Metagenome sequencing has become a routine procedure and can be accomplished at reasonable cost (e.g., <\$1,000). Bioinformatics pipelines to extract RDase gene fragments from metagenome datasets are available and this information can then be used to determine the exact sequences of native RDase genes of interest. With this information, the peptides of native RDases can be predicted and therefore detected and quantified with the qProt approach.

Environmental distribution of the trichloroethene reductive dehalogenase gene (tceA) suggests lateral gene transfer among Dehalococcoides [29]. Therefore, a suite of qProt assays can be developed that will be applicable to the majority of sites. Prior studies have also demonstrated allelic sequence variations of RDases genes such as tceA; however, we expect that the sequence variability of tceA gene sequences will be limited at contaminated sites and we will not find new tceA sequences at every site investigated. Thus, as our study demonstrates that the development of

site-specific qProt assay will be cost feasible at most sites, our knowledge of RDase gene sequence variations suggests it may be possible to design a multiplexed RDase qProt assay which will encompass several target peptides that will be useful at the majority of sites.

3.0 PERFORMANCE OBJECTIVES

This project demonstrated the utility of advanced MBTs for prediction of cVOC degradation rates in laboratory microcosms. Demonstration results highlighted the utility of this approach for estimating *in situ* reductive dechlorination rates at field sites. The following section describes quantitative and qualitative performance objectives (Table 3.1) specific to the laboratory microcosm phase of the demonstration.

Table 3-1. Demonstration performance objectives for the microcosm study

Performance Objective	Data Paguiraments	Success Criteria
r eriormance Objective	Data Requirements	
	Quantitative Performance Obj	
Quantify rate coefficients for <i>cis</i> -DCE and VC degradation in aquifer microcosms bioaugmented with reductive dechlorinating consortium SDC-9 at varied initial cell densities. Rates of production of VC from <i>cis</i> -DCE and ethene and ethane from VC will also be quantified.	Concentrations of <i>cis</i> -DCE, VC, ethene and ethane over a minimum of six time periods in triplicate microcosms. Data will be used to estimate rate coefficients and corresponding uncertainties for each test.	Initial cell densities in the bioaugmented microcosms will be varied by 4 orders of magnitude (10^5 , 10^6 , 10^7 and 10^8 cells/mL) to ensure we obtain a range of rate coefficients and reductive dechlorination activity levels. Rate coefficients estimated using the microcosm data will be of sufficient quality if the global \mathbb{R}^2 of the kinetic model is ≥ 0.75 , and if the average ratio of the 95% confidence interval to the rate coefficient value for both k_{cisDCE} and k_{VC} (i.e. the average of ratios in brackets $\left[\frac{95\% CI \ k_{cisDCE}}{k_{cisDCE}}, \frac{95\% CI \ k_{VC}}{k_{VC}}\right]) \text{ is } \leq 125\%$
RDase biomarkers (RDase peptides, genes, and RNA transcripts) are quantifiable at microcosm-required and environmentally-relevant concentration levels.	Quantify initial and final RDase biomarker abundance and corresponding uncertainty for each treatment within each microcosm test.	RDase biomarker abundance measurments meet the Data Quality Objectives for this analysis. Ability to measure one or more of these RDase biomarkers at environmentally relevant <i>Dhc</i> concentrations (i.e., 10 ⁵ to 10 ⁶ cells/mL).
One or more of the RDase biomarkers exhibits a quantifiable, predictive association with <i>cis</i> -DCE (and/or VC) degradation rates in the microcosms.	Rate constants for each microcosm that meet objectives described above. RDase biomarker abundance (peptides, genes, and RNA transcripts) measurements that meet objectives described above.	The association between RDase biomarker abundance (RDase peptides, genes, and RNA transcripts) and the rate constants is positive and significantly different from zero at the 95% confidence level.
	Qualitative Performance Obje	ectives
RDase biomarker abundance input to multivariate regression (or other suitable) model predicts reductive dechlorination rates with equal or better confidence than using conventional DNA-based MBTs alone.	Rate constants for each microcosm that meet objectives described above. RDase biomarker abundance (peptides, genes, and RNA transcripts) measurements that meet objectives described above.	Utility of RDase biomarkers (RDase peptides, genes, and RNA transcripts) – alone and in combination – will be quantified and documented.
Effectively communicate benefits of advance MBTs to end users — particularly managers of cVOC-contaminated DoD groundwater sites — through multiple technology transfer platforms.	Rate constants for each microcosm that meet objectives described above. RDase biomarker abundance (peptides, genes, and RNA transcripts) measurements that meet objectives described above.	At the conclusion of the 2-year microcosm test, at least one manuscript will have been submitted to a top-quality, peer-reviewed journal.

3.1 Quantify rate constants for cis-DCE and VC degradation in aquifer microcosms.

Rate constants were calculated for *cis*-DCE and VC degradation in each of the different microcosm treatments (Section 5.5.2). The procedure for calculating rate constants is provided in Section 5.7.

<u>Data Required</u>: The data required included concentrations of *cis*-DCE and VC as a function of incubation time in the microcosms. Concentrations of *cis*-DCE and VC were measured at a minimum of 8 time points in each microcosm treatment, which were prepared in triplicate. Analysis was conducted by EPA Method 8260 (Gas-Chromatography – Mass Spectrometry; GC-MS) using liquid 2-mL samples as described in Section 5.6.1.

<u>Success Criteria</u>: The first order rate constants were fit to the microcosm data and were considered to be of acceptable quality if the global R² of the kinetic model was ≥ 0.75 , and if the average ratio of the 95% confidence interval on the rate constant to the rate constant value itself for both k_{CISDCE} and k_{VC} (i.e. the average of ratios in brackets $\left[\frac{95\% CI k_{CISDCE}}{k_{CISDCE}}, \frac{95\% CI k_{VC}}{k_{VC}}\right]$) was $\leq 125\%$.

3.2 Measure target RDase biomarkers (RDase genes and proteins) at environmentally relevant abundance levels.

Abundance of RDase proteins was linked to abundance of the reductive dechlorinating microbes expressing them. The microcosm test was designed to quantify RDase biomarkers associated with Dhc cell densities in the $< 10^6$ cells/mL range, which is relevant to MNA sites, and up to $> 10^8$ cells/mL range, which is relevant to biostimulated and bioaugmented sites.

<u>Data Required</u>: Required data include abundances of RDase biomarkers (genes and proteins) in each microcosm at the time corresponding to the beginning, middle and end of the incubation used to determine the rate constants for *cis*-DCE and VC degradation. The RDase biomarkers were quantified using methods described in Section 5.6.2 and 5.6.3.

<u>Success Criteria</u>: Abundance of RDase biomarkers met the method detection limits and other data quality objectives summarized in Section 5. Microcosm study results were utilized to establish a lower limit of detection for quantifying peptides of interest. Results showed that single RDase protein biomarkers in the $2x10^6$ *Dhc* cells/mL or more range corresponded to k_{cis} and k_{VC} rates in the range of 0.0001 day⁻¹ (0.04 year⁻¹), which is relevant to sites pursuing or managing MNA remedies.

3.3 Quantify relationship between target RDase biomarker abundances and reductive dechlorination (RD) activity in aquifer materials

Abundance of RDase biomarkers (genes and proteins) were compared with the rate constants for biodegradation of *cis*-DCE and VC collected from the microcosms.

<u>Data Required</u>: Rate constants for *cis*-DCE and VC degradation (Objective 3.1) and concentrations of RDase peptides and genes in each microcosm at the time corresponding the beginning of the incubation used to extract the rate constants (Objective 3.2).

<u>Success Criteria</u>: The association between abundance of individual RDase peptides and genes, and the rate constants was tested by first performing correlation analysis. RDase biomarkers and rate constants with correlation factors that were positive and significantly different from zero at the 95% confidence interval were considered acceptable, then were carried forward into a power law least squares regression analysis where the predictive relationship was established.

3.4 Develop a multivariate regression (or other suitable) model, which predicts the *cis*-DCE and VC rate constants using RDase biomarkers as input

A simple power regression model was developed to allow an end user to predict the *cis*-DCE and VC degradation rate constant using qPCR and qProt data as model input parameters.

<u>Data Required</u>: Rate constants from microcosms and corresponding abundance of RDase proteins and functional genes so that the quantitative relationship between these measures can be modeled and the predictive tool can be developed.

<u>Success Criteria</u>: Quantify the rate-predictive power of the regression model using RDase protein abundance only, RDase functional gene abundance only, and a combination of the two together to establish the relative contribution of each measure to the predictive power of the model. This performance objective was established as a *qualitative* objective for this laboratory microcosm phase of the demonstration.

3.5 Effectively transfer the new technology to end users

Results of the microcosm study are the first to demonstrate use of qProt for predictions of reductive dechlorination rates under environmentally relevant conditions.

<u>Data Required</u>: Rate constants from microcosms and corresponding abundance of RDase biomarkers and functional genes so that the quantitative relationship between these measures can be modeled and the predictive tool can be developed.

<u>Success Criteria</u>: Distribute microcosm study findings using effective technology transfer platforms. Submit at least one manuscript describing the results and benefits of the approach to a top-quality, peer-reviewed journal. Present results at multiple national remediation conferences.

4.0 SITE DESCRIPTION

Three cVOC-contaminated DoD sites were selected for potential inclusion in this project: Naval Base Kitsap (NBK) Keyport Area 1, Joint Base Lewis-McChord (JBLM) Landfill 2, and Vandenberg Air Force Base (VAFB) Site SA288. These sites were selected because (1) collection of aquifer material and groundwater was possible with minimal cost to the project through leveraging pre-planned site characterization activities, and (2) each site is potentially suitable for a future field demonstration. The following subsections provide an overview of each DoD field site – and basis for inclusion or exclusion from this laboratory project.

4.1 NBK KEYPORT AREA 1, BREMERTON, WASHINGTON

Site Location and History. Keyport Area 1 is a former solid waste landfill at NBK Keyport, located 45 miles north of Tacoma, Washington on the Kitsap Peninsula. It comprises approximately 9 acres in the western portion of the base, next to a wetlands area and the tidal flats that flow into Dogfish Bay (Figure 4-1). The Area 1 landfill was the primary disposal area for domestic and industrial wastes generated by the base from the 1930s until 1973, when the landfill was closed. NBK Keyport became a Superfund site in 1989. The remedial investigation and feasibility study [30] identified cVOCs as contaminants of concern in site soil, sediment, tissue, groundwater, and surface water. The Record of Decision [31] for the Area 1 landfill specified cVOC hotspot treatment using phytoremediation by poplar trees in concert with natural attenuation, as well as landfill liner upgrades, monitoring and other best management practices. The landfill liner upgrade was completed in 2003 and phytoremediation was implemented in 1999 by planting two poplar plantations (Figure 4-1).

Site Geology/Hydrogeology. There are two aquifers at the site. The sandy unconfined upper aquifer is present throughout the landfill area and is 4 to 15 feet thick, with depth to water between 4 to 10 feet below ground surface (bgs) [30]. Approximately 5 feet of landfill material lies above the groundwater surface in the unsaturated zone; up to 10 feet of landfill material lies within the saturated upper aquifer. Upper aquifer groundwater generally flows west and discharges into the marsh pond (Figure 4-1). The upper aquifer is underlain by an aquitard consisting of sandy silt to clean silt, which is 4 to 15 feet thick where present. The underlying intermediate aquifer is 5 to 25 feet thick, with groundwater flow direction generally toward the tide flats (Figure 4-1). The intermediate aquifer is underlain at 25 to 40 feet bgs by a thick nonglacial silt and clay aquitard known as the Clover Park Silt, which is approximately 100 feet thick and separates the contaminated aquifers from the deeper regional water-bearing units [30].

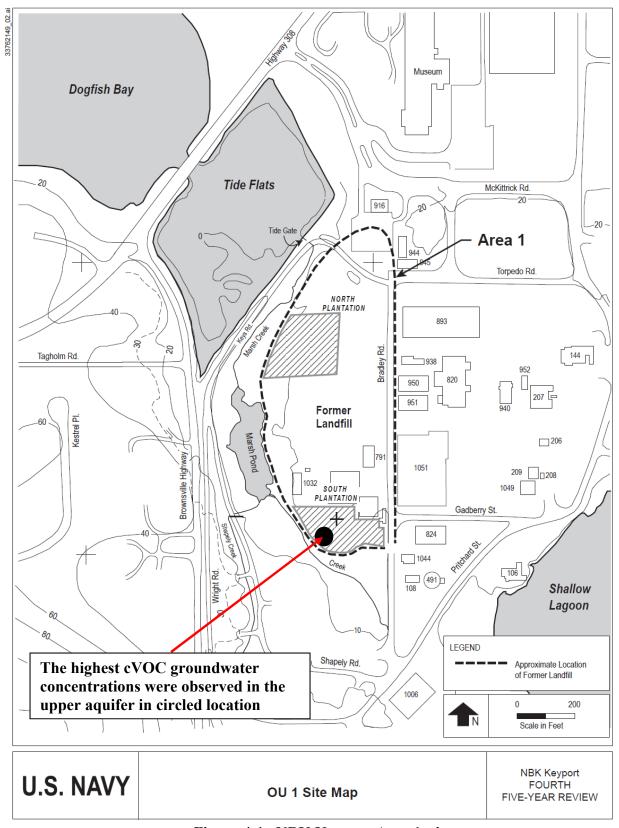


Figure 4-1. NBK Keyport Area 1, site map

Contaminant Distribution. In spite of a high degree of biodegradation and reductions in cVOC mass over time, groundwater concentrations of cVOCs beneath the south poplar plantation in the upper aquifer remain high and cVOC concentrations in surface water adjacent to the south plantation consistently exceed the surface water remediation goals. The maximum concentrations of cVOCs measured in upper aquifer monitoring wells in the south plantation were: TCE > 33,000 μ g/L, cis-DCE > 55,000 μ g/L, and VC > 6,000 μ g/L (Figure 4-1). Aquifer material and groundwater for this project will be collected from the upper aquifer, south plantation.

Project Inclusion Decision. Aquifer solids and groundwater were collected by NBK Keyport Area 1 contractors (Battelle Memorial Institute) in July and September 2017 and shipped on ice overnight to the Aptim laboratory in Lawrenceville, NJ. The quantity of aquifer material collected in July 2017 via direct-push drilling was limited but screening results were favorable so a larger quantity of aquifer material was collected in September 2017 via hollow stem auger. Unfortunately, the larger material sample exhibited a strong odor (suspected naphthalene, not confirmed). High/potentially inhibitory levels of contamination encountered in the material, coupled with suspected presence of inhibitory co-contaminants, resulted in a decision to exclude the NBK Keyport Area 1 material during the laboratory project phase. However, results of the recent expanded site characterization make NBK Keyport Area 1 a good candidate for a future field demonstration.

4.2 VAFB SITE SA 288, VANDENBERG, CALIFORNIA

Site Location and History. The site is located 4.1 miles east of the Pacific Ocean and 2.8 miles north of the Santa Ynez River within the Cantonment Area on the Burton Mesa portion of VAFB. The site consists of three buildings that were used for various industrial processes since the 1960s. Investigation activities initiated in 2008 involved installation of soil borings and temporary wells, which identified the former chemical storage shed and the former freon processing shed as potential cVOC source areas to groundwater (Figure 4-2). Additional soil borings and monitoring wells were installed and sampled in 2016, the results of which are summarized in the contaminant distribution section below. Additional site characterization is planned to support a "remedy in place" scheduled goal of second quarter 2018.

Site Geology/Hydrogeology. The site is underlain primarily by Quaternary Orcutt Sand [32] with bedrock occurring at approximately 45 ft below ground surface. Surface water that does not infiltrate into the subsurface at the unpaved areas of the site enters a storm drain system and ultimately discharges into the Santa Ynez River to the south. Groundwater can be detected near ground surface following significant rainfall events but is typically observed within a saturated sandy silt layer, which is 2 to 5 ft thick across the site. Depth to this saturated sandy silt layer varies across the site but is typically encountered around 10 ft below ground surface. Groundwater flows in a southeast direction from the presumed source area toward New Mexico Avenue (Figure 4-2).

Contaminant Distribution. Maximum cVOC concentrations in groundwater were encountered during the 2016 monitoring event in well SA288-MW-01, which is located approximately 150 ft downgradient of the former freon processing shed [33]. Groundwater concentrations of TCE and cis-DCE were 2,200 µg/L and VC was 76 µg/L (Figure 4-2). Aquifer material and groundwater use during this project will be collected from the saturated sandy silt perched aquifer.

Project Inclusion Decision. Aquifer solids and groundwater were collected by VAFB SA288 contractors (Geosyntec Consultants) and shipped on ice overnight to the Aptim laboratory in Lawrenceville, NJ. Unfortunately, the groundwater and aquifer solids collected from SA288 were naturally acidic (pH \sim 3.5), which is inhibitory for *Dhc* cells, and was therefore deemed not acceptable for inclusion in the laboratory portion of the project. However, other portions of the SA288 site or even other cVOC-contaminated aquifers present at VAFB could be candidates for a future field demonstration.

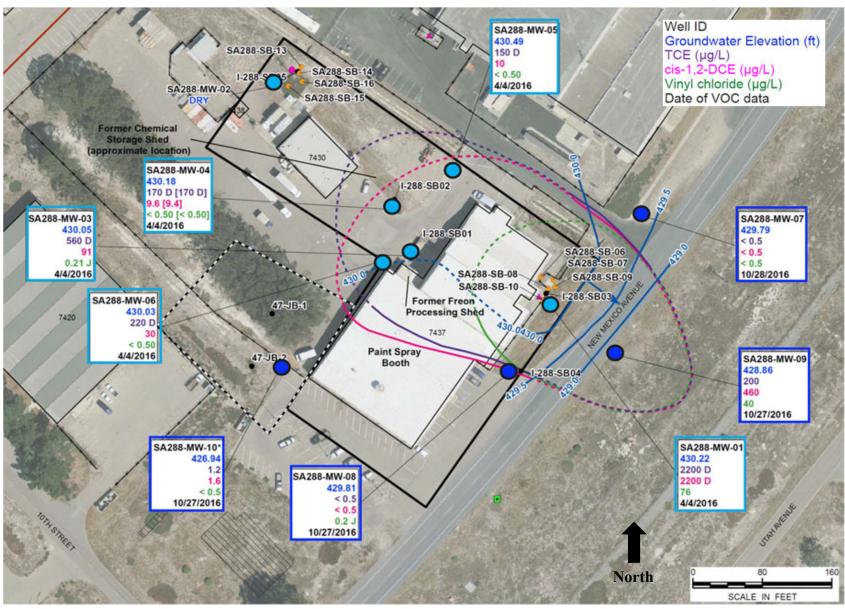


Figure 4-2. VAFB SA288 site map showing groundwater cVOC concentrations [33]

4.3 JBLM LANDFILL 2, TACOMA, WASHINGTON

Site Location and History. JBLM is a major military installation located approximately 15 miles southwest of Tacoma, Washington. Landfill 2 (LF2) was used to dispose of petroleum products and solvents generated by the Logistics Center between the 1940s to 1970s (Figure 4-3). Soils under the LF2 area are highly transmissive and the groundwater table is shallow; consequently, LF2 contributed to a very large TCE groundwater plume. The Logistics Center, which includes LF2, was listed as a Superfund site in 1989. The Record of Decision (ROD) [34] specified a pump and treat groundwater remedy for LF2. The ROD was subsequently modified to include multiple source area removal actions and enhancements to the pump and treat remedy.

Site Geology/Hydrogeology. LF2 is located within the unconfined Vashon Aquifer, which is comprised of interlayered outwash and glacial till to an approximate depth of 100 ft below ground surface. In the vicinity of the LF2 source area, the Vashon Aquifer is divided into the Upper Vashon and the Lower Vashon, which are separated by a discontinuous low permeability till layer. The Vashon Aquifer is separated from the underlying confined Sea Level Aquifer by a 10 to 20 feet thick non-glacial aquitard unit. A "window" in the aquitard unit downgradient of the LF2 source area resulted in formation of a large cVOC plume in the underlying Sea Level Aquifer.

Contaminant Distribution. Multiple source area removal actions (excavation, thermal treatment) and pump and treat remedy implementation since the 1990s have significantly reduced cVOC concentrations present in the LF2 source area wells (Figure 4-3, inset). However, only select groundwater wells are sampled during compliance monitoring events and those low concentrations (see contours, Figure 4-3) do not explain the $\sim 200~\mu g/L$ sustained TCE concentrations routinely encountered in extraction well PW-1. Groundwater TCE concentrations in the $\sim 1,000~\mu g/L$ range are expected in the source area vicinity. New investigation wells are being installed and a comprehensive groundwater monitoring event is being conducted to refine the LF2 conceptual site model and confirm remaining TCE concentrations in source area groundwater. These investigation activities in the LF2 source area will be leveraged to provide aquifer material and groundwater for use during this ESTCP project.

Project Inclusion Decision. Aquifer solids and groundwater were collected by U.S. Army Engineer Research Development Center (ERDC) and Seattle District U.S. Army Corps of Engineers (USACE) and shipped on ice overnight to the Aptim laboratory in Lawrenceville, NJ in May 2017. Aquifer solids and groundwater samples were stored at 4°C until use. Initial screening of the groundwater samples showed cVOC concentrations and pH were within acceptable ranges. Next, the LF2 groundwater was screened for potential reductive dechlorination inhibitory substances by conducting a simple microcosm study. SDC-9 cells (10⁸ cells/mL), *cis*-DCE (10 mg/L) and lactate (500 mg/L) were added to LF2 groundwater followed by measurements of *cis*-DCE, VC and ethane/ethene concentration and pH over time. Rapid reductive dechlorination was observed in the screening microcosm, which supported a "go" decision to include LF2 materials in the laboratory project. pH reduction observed during the screening microcosm prompted inclusion of calcium carbonate buffer during subsequent microcosm experiments.

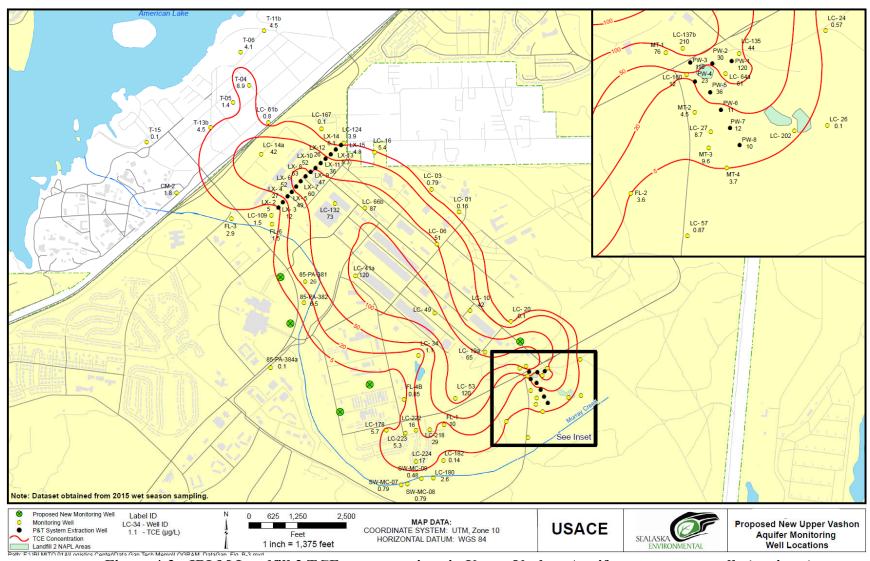


Figure 4-3. JBLM Landfill 2 TCE concentrations in Upper Vashon Aquifer source area wells (see inset)

5.0 TEST DESIGN

This section provides an overview of the experimental approach and field material collection (Sections 5.1 - 5.2), refinement and validation of the qProt assay specific to this project (Sections 5.3 - 5.5), as well as detailed experimental procedures, analytical methods and data analysis requirements for the project (Sections 5.6 - 5.8).

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

The goal of this project was to demonstrate the utility of measuring RDase biomarkers (RDase genes, peptides) via qPCR and qProt to estimate in situ cVOC degradation rates. First, the RDase protein targets were identified to finalize the qProt assay for use during this project (Section 5.3) then MDL and IDL studies were performed to establish the quantitative framework for the qProt assay (Section 5.4). Next, we performed a study using diluted SDC-9 culture to validate quantitation limits of the qProt assay (Section 5.5). Finally, a series of microcosm studies were performed using DoD site aquifer materials where cis-DCE and VC degradation rates were quantified as a function of RDase biomarker gene and protein abundances (Section 5.6). Microcosms were prepared by amending JBLM LF2 aquifer material with cis-DCE, lactate as a growth substrate, and calcium carbonate buffer. Varied quantities of the dehalogenating consortium SDC-9, which contains *Dhc* strains carrying RDase genes including *vcrA* and *tceA*, were added to the microcosms as illustrated in Figure 5-1. Samples were collected from the microcosms over time and analyzed for cVOCs so that degradation rates of cis-DCE and VC could be calculated. Samples also were collected from the microcosms at multiple points for analysis of selected RDase biomarkers. As described in Section 6.0 below, results of this laboratory project illustrated that RDase biomarkers can be reliably quantified over ranges of *Dhc* cell abundances relevant to cVOC site management – from low abundance/low activity relevant to MNA to high abundance/high activity relevant to enhanced bioremediation. Furthermore, the positive and significant correlations established between the biomarker abundances and reductive dechlorination rate coefficients in this laboratory study lay the foundation for a follow-on field study where the quantitative link between RDase biomarkers and in situ rates can be validated.

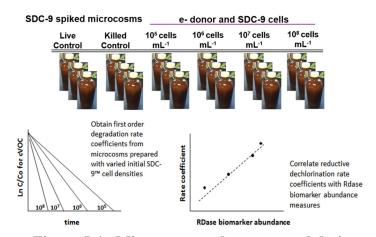


Figure 5-1. Microcosm study conceptual design

5.2 BASELINE CHARACTERIZATION ACTIVITIES

Aguifer material was collected from the saturated zone of the JBLM LF2 cVOC-contaminated aguifer the week of May 29, 2017. The aguifer materials were sieved in the field to remove gravel, collected in large Zip-Loc-type freezer bags, labeled, and then stored on ice pending overnight shipment to the Aptim laboratory (Aptim). Wet solids were shipped on ice in Zip-Loc type bags labeled "JBLM-L236 8-50' BGS". The solids (wet sand and some small pebbles) were homogenized under a nitrogen atmosphere at Aptim and placed in sterile 4-L glass jars. The jars were stored at 15 °C. The wet soil was allowed to settle for a week, and the water was decanted to lower the moisture content of the solids. This was repeated several times over 10 weeks until the soil moisture content was 15% (wt/wt). Groundwater was collected from the extraction well PW-1 sampling port the week of May 29, 2017, and then again the week of March 5, 2018, into 18-L stainless steel kegs that had been bleached (2,500 mg/L chlorine), rinsed with Nano-Pure water, and autoclaved (15 psi, 121 °C, 45 minutes). Groundwater samples were placed on ice and shipped overnight to Aptim. Site groundwater was analyzed for cVOCs using methods described below prior to use in microcosms. The cVOCs present in the groundwater were removed by purging with N₂ prior to groundwater use in microcosm preparation to prevent any potential impacts of the native cVOCs on the growth and activity of dechlorinators in the SDC-9 consortium. In order to ensure reductive dechlorination was not affected by inhibitors associated with the aquifer materials, a single microcosm was prepared and sampled as described in Section 5.3 below for screening purposes. This microcosm was bioaugmented to achieve a *Dhc* cell density of 10⁸ cells/mL with lactate as the electron donor, then screened for cis-DCE and VC degradation rates and pH changes only. If reductive dechlorination activity fell within the expected range (based upon historical data with the SDC-9 consortium), the aquifer material was considered acceptable and carried forward in the microcosm study. As described in Section 4.0, only aguifer material from JBLM LF2 was deemed acceptable to carry forward.

5.3 DEVELOPMENT OF RDASE PEPTIDE TARGETS

SDC-9 biomass was harvested during growth in a 4,000-L bioreactor maintained at Aptim at three time points and subjected to proteomic analysis. Targeted proteomic analysis was conducted using the same instrumentation and chromatographic method as used for shotgun proteomics except that the mass spectrometer was operated in parallel reaction monitoring (PRM) mode. A full scan spectrum was acquired (100 ms accumulation time) followed by product ion spectra of each target peptide (75 ms accumulation time), for a total cycle time of 1.9 seconds. The product ion scans were not time scheduled. Proteins were identified from MS/MS fragmentation data by searching the MS/MS data of the top n peaks against a custom FASTA library protein sequences acquired from the metagenome of consortium SDC-9. Searches were performed with the Paragon algorithm in Applied Biosystems ProteinPilot 4.5, with the following parameters: ID with 95% confidence, fixed modifications (carbamidomethyl), variable modifications (methionine oxidation).

ProtScore values for an identified protein were calculated by summing the ProtScore of each identified peptide after log transformation:

$$ProtScore = -log(1-Cn)$$
 equation 1

where n peptides with a confidence of Cn each contributes to the ProtScore of the identified protein. For example, a protein that has four peptides with 99% confidence match has a 99.999999% chance (1 - 0.014) of being a true identification. In this case, each peptide contributes 2 units to the ProtScore for every peptide identified with a 99% confidence ID. High-confidence, non-tryptic peptides were subjected to analysis using a suite of open-source software to provide explanation for the observed cleavage site as follows:

- PRED-TAT (http://www.compgen.org/tools/PRED-TAT4), which is used to predict signal peptide domains; and
- PROSPER (https://prosper.erc.monash.edu.au/5), PeptideCutter (http://web.expasy.org/peptide_cutter/5), and the MEROPS peptidase database (https://www.ebi.ac.uk/merops/search.shtml6), all of which are used to predict protease specificity for a given protein sequence.

5.4 ESTABLISHING MDL AND IDL FOR TARGET PEPTIDES

The SDC-9 culture-specific RDase peptides were identified for quantification. These specific RDases were then used in a MRM targeted proteomic assay to establish quantitative biomarker rate correlations, which are needed to generate degradation rate estimates for chlorinated ethenes. MDL/IDL study methods are summarized below; details are included in Appendix A.

For determination of the MDL, the 12.5 pmol/µL stock solution of isotopically labeled (IS) peptides was diluted in 50 mM ammonium bicarbonate to prepare the following concentrations (final in 25 μL): 250, 83, 27, 9, 3, 1, 0.34, and 0.11 fmol/μL. Each sample was digested with trypsin overnight and desalted using C18 spin columns. To confirm instrument functionality and detectability of each IS peptide, infusion and injection steps were performed. Each IS peptide was prepared as 12.5 pmol/µL in dimethyl sulfoxide (DMSO)/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. Concentrated solutions for each peptide were provided to the analyst for subsequent dilution and infusion directly into the mass spectrometer (Waters Xevo TQ-XS) for confirmation of precursor (parent) ion, charge state, product ions (daughters), and optimization of collision energies (CE) (Table 2, Appendix A). This optimization step is performed to confirm that a peptide of a given sequence is detectable in the mass spectrometer and to optimize signal intensity for product ions. Each peptide was diluted to 0.5 pmol/µL or 1.25 pmol/µL in HPLC-grade water +0.1% formic acid and was directly infused into the mass spectrometer at a flow rate of 10 μ L/min. For each peptide, a mass spectrum of the precursor ion was obtained. For each precursor ion, a mass spectrum was obtained for the product ions after fragmentation with CE of \geq 20 V. Using Waters Intellistart software, the CE for each peptide was optimized to maximize a signal from product ions. This was performed by infusing a single peptide into the mass spectrometer while Intellistart software varied cone voltage and CE to maximize a signal for each product ion. Skyline software was also used to output optimal CE for each peptide using equation 2 with parameters (slope, intercept) that are specific to Waters Xevo mass spectrometers (Table 5-1).

CE = slope *(precursor charge state) + intercept equation 2

Table 5-1. Parameters of Waters Xevo CE Equation

Precursor Charge State	Slope	Intercept
+2	0.037	-1.066
+3	0.036	-1.328

After optimization of CE per each IS peptide further development of multiple reaction monitoring assay was performed, including optimization of dwell time, CE, and solvent program. During this phase, peptides with relatively poor response were dropped from the MRM method file. The Skyline-optimized CEs were used in initial MRM method development. Comparison to Intellistart-optimized CEs was performed later in MRM development, however improvements in signal intensity were insignificant.

For MRM method development, peptides were prepared as a mixture at 1.25 pmol/µL in HPLC-grade water + 0.1% formic acid from a 12.5 pmol/µL mixture in DMSO/Milli-Q water. The solvent program and modified versions thereof were used (see Appendix A, Table 3). The chromatographic system used was the Waters M-Class equipped with a trap column (Acquity UPLC M-Class Trap Symmetry® C18; 5 µm particle size, 100Å pore size; 0.3 mm x 50 mm) and an analytical column (Acquity UPLC M-Class HSS T3 C18; 1.8 µm particle size, 0.3 mm x 50 mm). Based on the observed maximum peak heights of each peptide at 1.25 pmol/µL prepared in HPLC-grade water + 0.1% formic acid (MS Parameters from September 5, 2017: 123 transitions; 30 ms dwell time; 3.7 s cycle time), some peptides were removed from the transition list based on poor response (peak height or peak area) relative to other peptides. Only those peptides with the largest responses were retained on the transition list.

Using the modified transition list and a 1.25 pmol/µL standard prepared in HPLC-grade water + 0.1% formic acid, three dwell times (20 ms, 50 ms, and 70 ms) were examined to assess the sensitivity of the signal to variation in dwell time. Based on the quality of the output data (peak height, peak shape, and points across a peak), the 50 ms dwell time was pursued for MDL experiments. The dwell time parameter was adjusted to 30 ms after further method development was prompted by failure of the first MDL set.

To establish IDL, IS peptides were prepared as a mixture at 12.5 pmol/ μ L in DMSO/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. A mixed, concentrated solution (12.5 pmol/ μ L) was provided fresh to the analyst during each day of analysis. The analyst diluted the sample to 250 fmol/ μ L in in HPLC-grade water + 0.1% formic acid and serially diluted this solution three-fold to prepare the following concentrations: 83, 27, 9, 3, 1, 0.34, and 0.11 fmol/ μ L. The lowest measurable concentration for each peptide, defined as S/N \geq 3 (as measured by MassLynx) for the primary and secondary ion, represents the IDL for each peptide.

To determine the MDL of peptide targets, IS peptides were prepared as a mixture at 12.5 pmol/ μ L in DMSO/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. A mixed, concentrated solution (12.5 pmol/ μ L) was provided fresh to the analyst during each day of analysis. The analyst diluted the sample to 1.25 fmol/ μ L in HPLC-grade water + 0.1% formic acid to use as a control during the analysis sequence. The 12.5 pmol/ μ L stock solution was diluted in ammonium bicarbonate to prepare the following concentrations (final in 25 μ L): 250, 83, 27, 9, 3, 1, 0.3, and

0.1 fmol/ μ L. Each sample was digested with trypsin overnight and desalted using C18 spin columns. The lowest measurable concentration for each peptide, defined as S/N \geq 3 (as measured by MassLynx) for the primary and secondary ion, represents the MDL for each peptide.

A detailed report characterizes each step of system and IS peptide optimization (Appendix A). Data pertaining to system resolution check, calibration, and chromatograms of peptide detections are grouped per sample set.

5.5 VALIDATION OF QPROT ASSAY QUANTITATION LIMITS

After the development of the MRM assay and after the IDL and MDL values had been established for each RDase peptide, a study was performed with the SDC-9 consortium to identify the lowest *Dhc* cell titer that generated detectable and quantifiable concentrations of the RDase peptides selected for quantification. Validation study methods are summarized below; details are included in Appendix B.

To correlate the number of RDase proteins to *Dhc* cell abundances, qPCR was performed with the same samples. Briefly, to determine initial *Dhc* cell density, culture suspension (1 mL) of freshly grown SDC-9 consortium was filtered through 0.22 μm Durapore membrane filters (25 mm, Millipore, Billerica, MA) in triplicate to collect biomass, and then DNA was extracted by using a DNeasy PowerLyzer PowerSoil Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions except for application of bead-beating method for enhanced cell lysis (OMNI Bead Rupter Homogenizer, OMNI International, GA) at 5 m/s for 3 min. Total DNA concentrations were determined using the Qubit dsDNA BR Assay (Invitrogen, Carlsbad, CA). TaqMan qPCR analysis of DNA was performed to determine the *Dhc* cell abundances using a *Dhc* 16S rRNA gene-targeted primer-probe set and qPCR conditions given in Section 5.6.3.

Based on initial Dhc cell density/mL determined by TaqMan qPCR assay, the SDC-9 culture was diluted to Dhc cell densities of 10^5 , 10^6 and 10^7 cells/mL using reduced mineral salt medium [35] inside an anoxic chamber. Sample preparations were performed in triplicate in sterile 50-mL Falcon plastic tubes. Samples (15 mL) were taken from each dilution and filtered through a 0.2 μ m filter (0.22 μ m, Millipore, Billerica, MA) at low flow speed for qPCR and qProt analyses. Each filter was placed in a sterile 50-mL Falcon tube and stored at -80°C immediately.

For qProt analysis, the filters were sent to Battelle on dry ice with an overnight carrier. Proteins were extracted with the Protein Extraction Kit (MoBio) and protein concentration was calculated using published methods [36]. An aliquot corresponding to 100 μg of protein was mixed with 100 mM ammonium bicarbonate, 10 μg bovine serum albumin (BSA) and isotopic peptide mix, reduced with dithiothreitol (10 mM), and incubated for 30 minutes at 57°C. Proteins were then alkylated with iodoacetamide (50 mM) for 30 min at room temperature in the dark. Excess iodoacetamide was quenched with dithiothreitol (16 mM final concentration). Peptides were digested with trypsin added in a 1:50 trypsin/protein ratio for 10 hours at 37°C. Samples were then acidified with an equal volume of 3% trifluoroacetic acid (TFA), dried via SpeedVac, then suspended in 270 μL of 0.1% TFA. Samples were loaded on a C18 XTerra column (1 × 100 mm, 5 μm pore size, 100 Å; Waters Corporation, Milford, MA, USA), desalted using 0.1% TFA, and peptides were eluted with 70% acetonitrile. Samples were dried via SpeedVac, then suspended in

a 2% indexed Retention Time (iRT) solution (Waters) prior to injection onto a Xevo TQ-XS Triple Quadrupole Mass Spectrometer.

5.6 LABORATORY MICROCOSM TESTING

The microcosms utilized for this project consisted of 500 mL (groundwater only) or 1000 mL (groundwater + soil) amber, narrow mouth glass bottles. Each bottle was fitted with a Teflon®-lined screw-cap. Microcosm tests were performed using materials from the JBLM LF2 field site (Section 4). One of the microcosm tests was performed using groundwater only with no aquifer solids. Two of the microcosm tests were performed using a mixture of groundwater and aquifer solids. In order to most effectively simulate a groundwater aquifer, a high ratio of aquifer solids/groundwater was used in the solids-containing microcosms. All microcosms were placed on a bottle roller to ensure adequate mixing during incubation. Subsamples were collected as described in Section 5.6.3 and were subject to chemical, geochemical and molecular parameter analysis described in Section 5.7.

5.6.1 Growth of the SDC-9 Inoculum

The SDC-9 culture was inoculated in microcosms at varying densities to quantify and correlate rates of cVOC degradation with quantities of key RDase biomarkers. The SDC-9 inoculum was grown in reduced basal salts medium [37] in a 4,000-L fermenter using lactate as a source of carbon and electrons, PCE as a sole electron acceptor, and yeast extract as a source of nutrients. Further details concerning the fermentation and growth of SDC-9 are provided elsewhere [38]. For the current study, a volume of the culture was removed from the fermenter (or from a keg of culture previously grown and stored at 4°C for < 1 month), centrifuged, and suspended in medium to a *Dhc* density of $\sim 1 \times 10^{10}$ cells/mL based upon optical density (OD), $\alpha = 600$ nm [38]. The culture was then diluted for addition to microcosms as described below. Initial studies were conducted to estimate biodegradation rates of *cis*-DCE by SDC-9 prior to microcosm preparation.

5.6.2 Microcosm Preparation and Treatments

Microcosm #1, Groundwater Only Treatments. Six treatments (1-6) were tested in triplicate (a,b,c) microcosms. Microcosm construction and sampling was performed in a Coy anoxic chamber with a pure N_2 headspace. No H_2 gas was used in the chamber, which was thoroughly purged with N_2 prior to use to minimize any residual O_2 . Microcosms were constructed in 500 mL amber Boston Round analytical bottles with Teflon®-lined septa caps. Microcosm bottles were bleached, rinsed with deionized water (DI) and autoclaved prior to use. Due to the relatively low alkalinity and pH of the site water, 1.0 g calcium carbonate (CaCO₃) was added to each microcosm as a slow release buffer to maintain a neutral pH during incubation. Site water (475 mL) was added to each of the bottles. Bottles then received the following amendments to bring the final aqueous concentration in each to ~ 490 mL:

- 1. Lactic acid sodium salt (LASS) to 500 mg/L final concentration of lactate (4.1 mL of 6% LASS).
- 2. NaBr to a final Br⁻ concentration of 10 mg/L: (0.49 mL of 10,000 mg/L Br⁻ stock).
- 3. To individual bottles, washed SDC-9 culture was added in medium to achieve nominal *Dhc* titers listed below:

```
1a,b,c Add 4.9 mL of 10^{10} Dhc /mL (final = 10^{8}/cells Dhc per mL)

2a,b,c Add 4.9 mL of 10^{9} Dhc /mL (final = 10^{7}/cells Dhc per mL)

3a,b,c Add 4.9 mL of 10^{8} Dhc /mL (final = 10^{6}/cells Dhc per mL)

4a,b,c Add 4.9 mL of 10^{7} Dhc /mL (final = 10^{5}/cells Dhc per mL)

5a,b,c Add 4.9 mL of site water

6a,b,c Add 4.9 mL of site water and 0.333 g HgCl<sub>2</sub>
```

- 4. A 5 mg/L final concentration of *cis*-DCE was added to each bottle (2.45 mL of 1,000 mg/L *cis*-DCE in DI water).
- 5. Small amounts of additional site water were added to achieve a final volume of 490 mL in each incubation vessel.

After all amendments were added, the microcosms were "topped off" with groundwater so that < 1 mL headspace was present in each bottle. The bottles were then tightly sealed with Teflon[®]-lined caps and removed from the anoxic chamber. Each bottle was then placed at 15 °C on a bottle roller operating at ~ 0.5 rotations per minute for incubation for an appropriate period of time depending on the treatment prior to sampling again for analysis of *cis*-DCE. The sampling procedure and analytes measured are provided in Section 5.6.3 and 5.7, respectively.

Microcosm #2, Groundwater Only and Groundwater + Solids Treatments. Ten treatments were established in the JBLM 2 microcosms, which were prepared in 1000 mL clean, sterile Boston Round Bottles. All microcosms were amended with 500 mg/L LASS, 10 mg/L cis-DCE, and 10 mg/L Br⁻ (NaBr) prepared as previously described. Microcosms received SDC-9 at four expected Dhc cell titers; 10^7 Dhc/mL (1a, b, c and 1d, e, f), 10^6 Dhc/mL (2a, b, c and 2d, e, f), 10^5 Dhc/mL (3a, b, c) and 10⁴ Dhc/mL (4a, b, c) as previously described except that dilutions were prepared in site groundwater rather than medium. Live (5a, b; 5 c, d) and killed (6 a, b; 6c, d) controls were also prepared. For replicates a,b,c in treatments 1-4 and a,b in treatments 5-6, only groundwater was added to the bottles. The remaining bottles (replicates d,e,f in treatments 1 and 2 and replicates c, d in treatments 5 and 6) received 353 g of aquifer solids at 15% moisture content (300 g dry weight). After all amendments had been added, the microcosms were completely filled with site groundwater, sealed and placed on a bottle roller (1 rpm) at 15 °C. After 10 days of incubation, 2 g of CaCO₃ (solid) was added to all microcosms due to an observed decline in pH in some bottles with SDC-9 added. Treatment 1, with the highest *Dhc* concentration, was set up a second time with an initial 2 g of CaCO₃ added to ensure that the declining pH did not affect degradation kinetics. A photograph of the JBLM 2 microcosm bottles with and without sediments is provided in Figure 5-2.

5.6.3 Microcosm Sampling Procedure

Water samples were collected from microcosms in order to measure contaminant degradation and RDase biomarkers as described below.

Microcosm #1 Sampling. All sampling was performed in a Coy anoxic chamber with a N₂ headspace. Liquid samples for chemical analysis were removed from the microcosms with gastight syringes to appropriate sample containers. Samples volumes consisted of the following: 2 mL for cVOCs (EPA Method 8260); 4 mL for methane, ethane and ethene (EPA 3810/RSK-175); 1 mL for anions (EPA Method 300.0) and volatile fatty acids (EPA 300m); and 5 mL for pH determination. The methods of analysis are provided in Section 5.7 and Appendix C.

Duplicate 15-mL aqueous samples, one for qPCR and the other for proteomic analysis, were removed using a glass pipette and transferred to sterile screw-cap 50-mL conical tubes. Cells were collected by centrifugation for 40 min at 11,000 rpm using a refrigerated Sorvall Lynx 6000 Centrifuge and a F21-8x50y rotor (Thermo Scientific). Immediately after centrifugation, the supernatant was aspirated from the cell pellets and the samples frozen at -80 °C. Microcosms were refilled with site water removed during sampling, punctured septa were replaced, and bottles were returned to rollers operating at 1 rpm and 15 °C.

Samples from the microcosm treatments were collected at different intervals based upon the initial concentration of *Dhc* added. See summary of cVOC sampling times bulleted below. Samples for qPCR and proteomic analysis were not collected and/or analyzed at each of these time points, but generally at the beginning, middle, and end of the incubating periods. Specific times for sample collection from each microcosm bottle are provided in Appendix E.

- Set 1: 0, 1, 2, 4, 6, 8, and 24 hours
- Set 2: 0, 8, 24, 48, 72, 96, 120 hours
- Set 3: 0, 6, 9, 13, 16, 20, 23, 27, 36, 55, and 83 days
- Set 4: 0, 6, 13, 20, 27, 36, 55, and 83 days
- Set 5 and Set 6: 0, 6, 9, 13, 16, 20, 23, 27, 55, and 83 days





Figure 5-2. Photograph of microcosms with and without sediments on the bottle roller (left) and settling for sample collection (right).

Microcosm #2 Sampling. Microcosm sampling was conducted as described for Microcosm #1, except that bottles with solids were removed from the rollers for 30 minutes to allow solids to settle prior to liquid sampling. See summary of cVOC sampling times bulleted below. Samples for qPCR and proteomic analysis were not collected and/or analyzed at each of these time points, but generally at the beginning, middle, and end of the incubating periods.

- Set 1: 0, 3, 7, 10, 14, 17, 21, 24, and 28 days
- Set 1 (duplicate): 0, 0.3, 1, 1.3, 2, 2.3, 3, 3.3, and 7 days
- Set 2: 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 days
- Set 3 and Set 4: 0, 7, 14, 21, 28, 35, and 42 days
- Set 5 and Set 6: 0, 3, 7, 9, 14, 17, 21, 24, 28, 31, 35, 38, and 42 days

5.7 SAMPLING & ANALYSIS METHODS

This section provides a summary of all samples collected during the laboratory project (Table 5-2), as well as a summary of the analysis methods used (Table 5-3). Method SOPs and detailed QC procedures are included in Appendices C and D, respectively.

Table 5-2. Number of microcosm test samples by analysis

Component	Matrix	Number of Samples	Analyte	Locationa
Screen aquifer material from 3	Groundwater	3	cVOCs, pH	JBLM LF2, VAFB SA288, NBK Keyport Area 1
candidate cVOC- contaminated	Groundwater/Aquifer Material Slurries	3	cVOCs, pH	JBLM LF2, VAFB SA288, NBK Keyport Area 1
aquifer sites	Dhc activity inhibitor screening microcosm	3	cVOCs, pH	JBLM LF2 only
JBLM	Groundwater	24	peptides	JBLM LF2
Microcosm#1,	"	24	genes	"
Groundwater only	"	159	anions	"
	"	159	cVOCs	"
	"	159	VFAs	"
	66	159	dissolved gasses	"
JBLM	Groundwater	18	peptides	JBLM LF2
Microcosm#2,	"	18	genes	"
Groundwater only	"	182	anions	"
treatments	"	182	cVOCs	"
	"	182	VFAs	"
	"	182	dissolved gasses	"
JBLM	Groundwater ^b	18	peptides	JBLM LF2
Microcosm#2,	"	18	genes	"
Groundwater+	46	145	anions	"
aquifer solids	46	145	cVOCs	"
treatments	46	145	VFAs	"
P' 11 1	"	145	dissolved gasses	"

a. Field materials were collected from candidate sites described in Section 4.0.

Table 5-3. Summary of standard analytical and RDase biomarker methods

Sample Type	Analyte	Method	Container	Preservative	Hold Time
	peptides	Proteomics (Section 5.4.2, Appendix B)	Sterile 15 mL plastic Falcon tube	-80°C	N/A
	renec 1		Sterile 15 mL plastic Falcon tube	-80°C	N/A
Microcosm	Anions	EPA 300.0	1 mL	4°C	7 days
Samples	cVOCs	EPA Method 8260B	2 mL	HC1	28 days
	VFAs	EPA 300 m	< 100 μL (aliquot from anion sample)	4°C	7 days
	Dissolved gasses	EPA Method 3810, RSK-175	5 mL w/1 mL headspace	HCl	28 days

N/A reflects direct injection analysis for cVOCs and dissolved gasses upon sample collection

5.7.1 Analytical Methods: Standard Geochemical cVOC Analyses

Microcosm samples (groundwater/sediment mixture) were analyzed using the following standard EPA procedures or modifications of these procedures for the analytes of interest at Aptim. Detailed

b. Groundwater collected after allowing solids to settle per Section 5.6.3.

method procedures are included in Appendix C. Inorganic anions were determined according to EPA Method 300.0, which uses ion chromatography. cVOCs were determined by EPA Method 8260B using gas chromatography and mass spectrometry. cVOCs were introduced into the gas chromatograph by the purge-and-trap method 5030B. Dissolved gases including methane, ethane and ethene were analyzed according to EPA Method 3810, RSK-175 [39]. For this method, a 4-mL volume of water from the microcosm was added to a 5 mL serum vial, and the vial was then sealed and shaken to equilibrate the headspace with the aqueous phase. The headspace was then analyzed for dissolved gases by GC using direct injection. The Henry's law coefficient for each gas was then used to calculate the aqueous concentration. Volatile fatty acids (VFAs) including acetate, lactate, formate, and propionate were measured using ion chromatography via modification of EPA method 300 (EPA 300m). pH was measured using a pH meter and microprobe.

5.7.2 Analytical Methods: Proteomics

Samples collected during the microcosm study were frozen at -80 °C then shipped on dry ice overnight to Battelle Memorial Institute for proteomic analyses. Proteins were extracted from lyophilized groundwater/sediment slurry samples, reduced, alkylated, trypsin digested, and subjected to LC-MS/MS using a Nano 415 LC system in line with an ABI Sciex Triple TOF 5600 high resolution MS instrument (Sciex, Concord, Canada) (Figure 5-3). During processing, the entire sample was subjected to protein extraction since protein and peptide concentration determination is a prerequisite for optimal protein digestion and optimal sample loading amount in bottom-up proteomics. The protein and peptide concentrations were calculated with a tryptophan assay [36]. For qProt, samples were spiked with selected isotopically labeled peptides at the digestion step for quantification of native peptide equivalents.

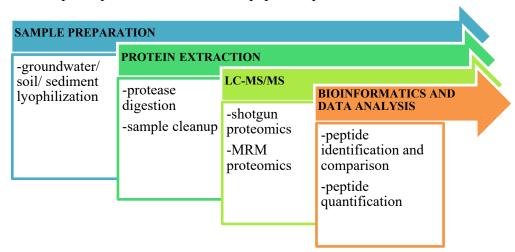


Figure 5-3. Steps involved in proteomic analysis of microcosm test samples

Sample MS and MS/MS data were acquired using an Eksigent Nano 415 liquid chromatograph system (Sciex, Concord, Canada) directly connected to a quadrupole time-of-flight (QqTOF) TripleTOF 5600 mass spectrometer (Sciex, Concord, Canada). The instrumentation is controlled using Analyst TF 1.6 and Eksigent software. A total of 25 µL of sample was injected onto a 0.3 mm x 150 mm Eksigent C18-CL-120 analytical column (3 µm particle size, 120 Å pore size,) using a trap-and-elute method. Peptide separation was achieved using a linear gradient of acetonitrile containing 0.1% (v/v) formic acid of different lengths depending on the acquisition

mode. Solvents used included 0.1% formic acid (v/v) (solvent A) and 0.1% formic acid (v/v) in acetonitrile (solvent B). Peptides were trapped on the loading column using 100% solvent A at a flow rate 5 μ L/min for 5 min. Trapped peptides were then separated at a predetermined flow rate using the following conditions: (1) 5% solvent B in A (from 0-5 min), (2) 5-35% solvent B in A (from 5-65 min), (3) 35-90% solvent B in A (from 65-66 minutes), and (4) 90% solvent B in A (from 66-70 minutes), with a total runtime of 90 min, including mobile phase equilibration.

Continuing mass calibration of the TOF MS and TOF MS/MS was performed throughout the analysis sequence by analyzing a digested β -galactosidase standard (Sciex, Concord, Canada). Mass spectrometric analysis was performed using data dependent acquisition (referred to as information dependent acquisitions, or IDA). Full scan spectra were acquired for specific m/z with a 250-millisecond acquisition time. For collision induced dissociation tandem mass spectrometry (CID MS/MS) in IDA mode, the mass window for precursor ion selection of the quadrupole mass analyzer was set to unit resolution (\pm 0.5 m/z). For MS/MS analysis, precursor ions were fragmented in a collision cell using nitrogen as the collision gas. For IDA analysis, the instrument was set to trigger product ion scans (from 100 to 1500 m/z) only after specific criteria were met by the precursor ions. These criteria were defined during the IDL and MDL analyses. The Rolling CE algorithm was used to determine the appropriate collision energy for each precursor mass.

For quantification, labeled RDase conserved peptides selected based on multiple sequence alignment of known RDase protein sequences and an internal bovine serum albumin control were spiked into sample extracts immediately prior to the protease digestion step. Native peptide concentrations were determined by comparing peak ratios of native and isotopically labeled peptides. Proteins were identified from LC-MS/MS spectra by searching against a database of protein sequences constructed from the metagenome sequences of the SDC-9 microbial community. In addition, sequences of protein contaminants typical for proteomic experiments (e.g., keratin and trypsin) were added to the database. The proteomic LC-MS/MS data were queried against this database and searched against the library of known selected enzymes involved in cVOC degradation. Only peptides with the "Protscore" for a particular protein higher than 1.3 were considered true positives. Statistical analyses of proteomic data were performed using Protein Pilot (confidence score and false discovery rate).

5.7.3 Analytical Methods: Quantitative PCR

Reductive dechlorination biomarker genes were enumerated in aqueous samples with qPCR following established procedures [7, 14]. Frozen cell pellets collected from the microcosms (details in Section 5.6.3) incubated in the Aptim laboratory were shipped overnight in a cooler with dry ice to the University of Tennessee. The samples were stored frozen at -80°C until analysis.

DNA extraction. The cell suspensions were thawed, and DNA was isolated from cell pellets with DNeasy PowerLyzer PowerSoil Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions except for using a bead-beating method (OMNI Bead Rupter Homogenizer, 5 m/s for 3 min) (OMNI International, GA) for enhanced cell lysis. DNA was eluted into nuclease-free water and DNA concentration and quality were determined with a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and a Qubit fluorometer (Invitrogen) using

double stranded DNA (dsDNA) Broad-Range assay kit according to the manufacturer's manual. DNA was stored at -80°C until analysis.

qPCR. Primers and probes to enumerate total bacterial 16S rRNA, *Dhc* 16S rRNA, *tceA* and *vcrA* genes have been reported [3, 7, 10, 40]. In addition, new primer and probe combinations have been designed for SDC9 24 *pceA* and *fdhA* (Table 5-4).

Table 5-4. Summary of specific qPCR assays run for microcosm samples

Assay ID	Organism(s)	Target Gene
Bac_16S	Total Bacteria	16S rRNA
Dhc_16S	Dhc-specific	16S rRNA
SDC9_24_pceA	Dehalobacter restrictus/ Desulfitobacterium hafniense	PCE reductive dehalogenase
SDC9_59_tceA	Dhc	TCE reductive dehalogenase
SDC9_212_vcrA	Dhc	VC reductive dehalogenase
fdhA (omeA)	Dhc-specific	Molybdoenzyme involved in electron transfer to the RDase

Specific primers and TaqMan probe sequences targeting SDC9_24 pceA and fdhA were designed using Geneious R11.0.2 (http://www.geneious.com, [41]) and primers were synthesized by IDT (Integrated DNA Technologies). In order to ensure specific hybridization at a uniform temperature, probes with Minor Groove Binder (MGB) modification were synthesized by Thermo Fisher Scientific. Design parameters for the target assays included primer and probe annealing temperature close to 60°C, primer and probe lengths ranged between 14-30 and 16-25 base pairs (bp), respectively and parameters were set to ensure that it was thermodynamically unlikely to form hairpin structures, self-dimers and heterodimers for primers and TaqMan probes. The specificity of the primers and probes was also verified using primer-BLAST analysis [42].

- (i) For regular qPCR, every 20- μ L reaction had 10 μ L of 2×TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA), 2 μ L of diluted (1:10 and 1:100) DNA template, and forward and reverse primers and probe at final concentrations of 300 nM each. Reactions were initially held for 2 min at 50°C and 10 min at 95°C following 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 1 min. The qPCR assay results were analyzed using the ViiA7 Software (Applied Biosystems, Carlsbad, CA). All qPCR assays were performed in triplicate.
- (ii) For high-throughput qPCR in 384-well microtiter plates, all qPCR reactions were performed with the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Carlsbad, CA), a flexible platform enabling the instrument to accommodate one 384-well plate. Every 10-μl reaction contained 5 μL of 2×TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA), 2 μL of diluted (1:10 and 1:100) DNA template, and forward and reverse primers and probe at final concentrations of 300 nM each. Reactions were initially held for 2 min at 50°C and 10 min at 95°C following 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 1 min. The qPCR assay results were analyzed using the QuantStudio 12K Flex Real-Time PCR System Software (Applied BiosystemsTM, Carlsbad, CA).

Plasmid DNA containing each of the cloned target gene was used as templates for standard curves. Standard curves were included with every qPCR plate using 10-fold serial dilutions of plasmid DNA over a 7 orders of magnitude range beginning at a 1 ng μ L⁻¹ concentration (~8 log gene copies) and decreasing to 10^{-7} ng μ L⁻¹. All standard curves had a total of eight calibration points and were run in triplicate. To calculate the number of gene copies in a known amount of DNA and gene copies per sample, previously published equations were applied [7].

From this original dilution series, Level of Detection (LOD) and Level of Quantitation (LOQ) were determined experimentally as 1-10 copies/ μ L and 10-50 copies/ μ L, respectively based on targeted assay. Examples of qPCR standard curves are given in Appendix C.

To further understand RDase expression and regulation as well as potentially correlate degradation rate to gene expression, transcript (mRNA) measurements were planned. Using transcript measurements, some correlation between dechlorination activity and RDase gene expression has been demonstrated, but the correlation is highly inconsistent and currently unpredictable [5]. This inconsistency is due to the fact that RDase gene expression is regulated by various environmental factors (e.g., growth phase, contaminant concentrations, etc.) through poorly understood accessory proteins. Further, reproducible and quantitative RNA extraction remains challenging due to the inherent susceptibility of RNA to degradation (i.e., unintended loss of biomarker) [43]. Thus, while informative, assays that use ribonucleic acid provide no reliable correlation to degradation rate and no direct information about the catalysts (i.e., the enzymes) that actually perform the biodegradation reaction, and measurements of transcripts to determine gene activity were not applied to samples in this study.

5.8 DATA ANALYSIS

Estimating Reductive Dechlorination Rates. First-order rate coefficients for biological reductive dechlorination of cis-DCE and VC (k_{cDCE} and k_{VC}) in the microcosms were estimated by fitting a numerical approximation of the first order reaction equations for sequential degradation of cis-DCE to VC and then to ethene to the change in molar concentrations of cis-DCE, VC, and ethene (target compounds) over time in the microcosms. Microsoft Excel Solver was used to minimize the sum of squares error between the measured and model-estimated values to obtain the best fit. Measured values were corrected for dilution by applying a dilution factor equal to the total moles of target compounds present in the microcosm when the sample was collected divided by the total moles of target compounds present at time zero. Dilution correction using bromide concentrations yielded similar results. The number of moles in individual target compounds in microcosms that contained aquifer solids were corrected for sorption to the solids by assuming linear partitioning and using equation 3 below,

$$M_{tot,sorption \ corrected} = C_w V_w + C_w M_s (0.63 K_{ow} f_{oc})$$
 equation 3

where C_w is the dilution-corrected molar concentration in water, V_w is the volume of water present in the microcosm, M_s is the mass of solids in the microcosm, K_{ow} is the VOC-specific octanol-water partitioning coefficient, and f_{oc} is the fraction of organic carbon present in the solids, which was assumed to be 0.001. The constant 0.63 converts units of the product of K_{ow} and f_{oc} into L/kg. K_{ow} values used for cis-DCE, VC, and ethane were 72.4, 28.8, and 13.5, respectively [44]. Rate coefficients for each test replicate microcosm were determined separately using this approach; rate

coefficients for live and killed controls were not determined. The 95% confidence interval associated with each fitted rate constant was determined using the approach described in Smith et al. 1998 [45].

Correlating Reductive Dechlorination Rates and Biomarker Abundances. Log-transformed rate coefficients and biomarker abundances were subject to the Shapiro-Wilk normality test. The Spearman Rank Order Correlation analysis followed by power law least squares regression analysis was used to quantify the relationship between biomarker abundances and rate coefficients. Rate coefficients represent an integrated measure of activity in each microcosm over time, whereas biomarker abundances provide a single measure at various time points during the incubation. Correlation analysis was performed twice: once using the global data, which included biomarker abundances collected at early, mid and late time points from each microcosm with the corresponding integrated rate coefficients measured for that microcosm, and again using only midpoint biomarker abundances and the corresponding rate coefficients.

Assessing Predictive Power of Biomarker Abundances. The microcosm experiments were divided into a "training set" and an "evaluation set." Rate coefficients and biomarker abundances from the "training set" microcosms were subject to power law least squares regression analysis of rate constants on abundance. Only data from microcosms where non-zero rate coefficients were obtained were included in the analysis. Measured biomarker abundances in the "evaluation set" of microcosms were entered as "x" variable in the regression equations to obtain a predicted rate coefficient. The predicted rate coefficient was then compared to the measured rate coefficient for that "evaluation set" microcosm. Two types of training sets were established. The first training set featured randomly selected microcosms that contained either 10⁶, 10⁷ or 10⁸ Dhc cells/mL. The second training set featured the global average of biomarker abundances and rate coefficients for all microcosms that contained either 10⁶, 10⁷ or 10⁸ Dhc cells/mL. The predictive power of the biomarkers were evaluated by comparing (1) the measured vs. predicted rate constants for each individual biomarker, (2) the measured vs. the average of the predicted rate constants for the various gene biomarkers, (3) the measured vs. the average of the predicted rate constants for the various peptide biomarkers, and (4) the measured vs. the average of the predicted rate constants for all of the biomarkers.

6.0 PERFORMANCE ASSESSMENT

6.1 DETERMINATION OF RDASE TARGETS WITH SHOTGUN PROTEOMICS

Shotgun proteomic analyses of SDC-9 extracts identified 35 RDase peptides. The tryptic peptides included the most abundant peptides of TceA, PceA and VcrA, which were detected with 99-100% peptide coverage. Five non-tryptic peptides (i.e., truncated from one end [either N-terminal or C-terminal] of the tryptic peptide), were observed with confidence levels exceeding 90% (three corresponding to PceA and two corresponding to TceA). A single peptide corresponding to VcrA was observed with low confidence (<50%). Sixteen peptides corresponding to PceA and fourteen peptides corresponding to TceA were identified with maximum confidence (100% sequence identity). The final list of SDC-9 unique RDase peptide targets and their transitions downselected for the MRM work are listed in Table 6-1.

Table 6-1. PRM transitions of selected RDase SDC-9 endogenous peptides

Protein	Peptide Sequence	Precursor m/z	RTd	Product m/z ^c	Ion	CVa	R ²	IDLb	Accession Number	
		705.4		1225.7	y11	4.3			G + D20700 2	
	IATQIPLLQDAAR	705.4 [M+2H] ²⁺	45.3	996.6	y9	3.5	0.98	5	CAD28790.2 WP 025206074.1	
		[101+211]		883.5	y8	5.1	•		W1_023200074.1	
		(24.2		1025.5	у9	2.2			CAD28790.2	
PceA	LESGYVQNMVK	634.3 [M+2H] ²⁺	33.2	938.5	y8	0.8	0.98	5	CDX02974.1	
		[141+211]		718.4	у6	3.2	•		WP_025206074.1	
		506.3		925.5	y8	7.3			CAD28790.2	
	VYTDLELAPDKPR	$[M+3H]^{3+}$	33.2	683.4	у6	5.1	0.97	1	CDX02974.1	
		[101+311]		612.3	у5	5.9			CDX02974.1	
		701.3		1188.6	y11	4.9	-			
	VNNEPWWVTTR	$[M+2H]^{2+}$	43.6	945.5	у7	1.7	0.97	5	WP_062900263.1	
				848.4	y6	4.1				
	YFGASSVGAIK	550.3 [M+2H] ²⁺	34.5	936.5	y10	2.9	-		WP_062900263.1	
TceA				789.4	у9	3.4	0.98	0.5		
				661.4	у7	1.3				
	YSGWNNQGAYFL	933.8		978.4	b9	4.5		100	WP_062900263.1	
	PEDYLSPTYTGR	$[M+3H]^{3+}$	54.5	1172.6	y10	1.6	NA			
	1251251111611			1057.5	у9	3.4				
				1104.4	у9	2.6	•		See peptide BLAST	
	VVTDLPIAPTPPID	806.7	56.7	989.4	y8	3.2	NA	100	results in SI; no match	
	AGMFEFCK	$[M+3H]^{3+}$	30.7	918.4	y7	3.2			with sequences from metagenomic hits	
				1067.5	y8	3.1	-		See peptide BLAST	
VcrA	SLNNFPWYVK	634.3	48.1	839.4	у6	6.8	0.98	50	results in SI; no match	
VUA		[M+2H] ²⁺	70.1	692.4	y5	6.6	0.70	50	with sequences from metagenomic hits	
	GLGLAGAGIGAVA			1211.1	y23					
	ASAP-	1086.9 [M+3H] ³⁺	ND	1161.5	y22	ND			AEI59454.1	
	VFHDIDEFVSSEA NSTK			1126.0	y21	ND			11111/1111	

a. CV calculated from n=3 replicates from a 25, 50, 250 or 500 fmol/ μ L isotopically-labeled standard; b. units are in fmol/ μ L and values represent IDL for the isotopically-labled standards; c. bolded product m/z represent those used for quantitation; d. retention time, minutes

6.2 METHOD DETECTION AND INSTRUMENT DETECTION LIMIT RESULTS

RDase peptide hits identified from shotgun mass spectrometry experiments (Table 6-1) and additional FdhA peptides were selected for targeted quantification based on the following selection criteria: confidence score >90%, no missed cleavages, non-tryptic, no methionine oxidation, and no carbamidomethylation. Peptides were then searched against the National Center for Biotechnology Information (NCBI) protein database to assess specificity to RDase targets. Three peptides per protein were monitored, and the three most sensitive transitions per peptide were reported. Isotopically labeled peptide standards were used to verify transitions, quantify selected peptides and determine retention time on the liquid chromatography system for all peptides. Instrument detection limits were reported as the lowest isotopically labeled standard that satisfied a signal-to-noise ratio ≥ 3 across three separate analyses. The most sensitive transition per peptide was used for quantification, and the remaining transitions were used as qualifiers. More specifically, several transitions for a given peptide ion were measured to validate the identification of a peptide, otherwise known as qualifiers. Only subsets of the transitions, typically the transition(s) with the highest intensities, were used for quantification of the peptide, also known as the quantifier. For example, the y9 ion of YFGASSVGAIK was the most sensitive ion for that peptide and was used for quantification, while detection of y7 and y10 ions were requisite to increase confidence in protein quantifications.

To determine the limit of detection, triplicate measurements of standard peptides at eight different concentrations in 0.1% formic acid were performed. The signal to noise ratio per each peptide was measured, and the standard deviations of the response at each concentration were calculated. CEs were optimized for initial 28 isotopically-labeled peptides to maximize the resulting signal from product ions. Following the optimization step, dwell time and solvent program were optimized and peptides that demonstrated poor signal response were discarded from the list. In total, 10 peptides were discarded from the list after optimization steps were completed and IDLs and MDLs were developed for the remaining peptides. Most peptides were observed in experimental samples during the IDL and MDL analysis, however VcrA peptides were observed exclusively in MDL experiments and not in IDL experiments (not observed is denoted as <250 fmol/μL), suggesting that sample digest and cleanup enhance the peptide signals for VcrA peptides. Two VcrA peptides (DQPWYVK and VPDHAVPINFK) were detectable in all MDL experiments while they were not detected in IDL experiments. Performance variation between IDL and MDL experiments are likely due to matrix effects. Some peptides performed similarly between MDL replicates (e.g., DQPWYVK) while others did not. Within the MDL set, inconsistencies were observed for sensitive peptides (e.g., TSPSLISSATVGK, VSSIIEPR, YFGASSVGAIK). This variation represents the variation present in the preparatory methods and instrumental analysis; it is unlikely that instrumental variation resulted in decreased sensitivity as control samples did not reveal loss of chromatographic quality or loss in mass spectrometer signal during the MDL runs.

Overall, the experiments performed allowed for identification of the most sensitive RDase and FdhA peptides for targeted quantification. MDL experiments resulted in detection of up to three of the most sensitive peptides per protein with up to three of the most intensive transition ions per peptide (Table 6-2).

Table 6-2. MDL for peptides analyzed for SDC-9 culture

						Established
Protein	ID	Peptide ¹	MDL 1	MDL 2	MDL 3	MDL
	FdhA2	SGSEIAFTGGLI K ¹	3	3	3	3
FdhA	FdhA5	ALGIVYLDSQA R	3	3	1	3
	FdhA8	NQAVSAPGEA K	3	3	3	3
	PceA4	IATQIPLLQDAA R	9	9	9	9
Dank	PceA5	LESGYVQNMV K	3	3	3	3
PceA	PceA7	DFWNNPEPI K	1	1	1	1
	PceA8	TSPSLISSATVG K	0.3	0.3	1	1
	TceA2	DVDDLLSAGK	0.3	3	3	3
TceA	TceA3	VSSIIEP R	0.3	0.3	1	1
1 ceA	TceA4	VNNEPWWVTT R	9	9	9	9
	TceA5	YFGASSVGAI K	0.3	0.3	1	1
	VcrA1	WGLYGPPHDSAPPDGSVP K	9	9	3	9
	VcrA2	YFGAGDVGALNLADP K	27	27	27	27
VcrA	VcrA3	VPDHAVPINF K	0.3	0.3	1	1
	VcrA4	GVYEGPPDAPFTSWGN R	83	27	27	83
	VcrA6	DQPWYV K	1	1	1	1

Units are fmol-peptide. A 1.0 mL sample was extracted to determine the MDLs.

The data generated during the optimization and calibration experiments were built into the MRM method (i.e. the "qProt assay") used for quantification of RDase peptides in the microcosm experiment samples obtained during this laboratory study.

6.2 VALIDATION OF MRM ASSAY QUANTITATION LIMITS RESULTS

Quantitation limits of the qProt MRM assay for FdhA, PceA, TceA and VcrA proteins were validated by analyzing triplicate samples of SDC-9 culture diluted to 10⁵, 10⁶, or 10⁷ Dhc cells/mL.

Only two out of three FdhA peptides, namely FdhA2 and FdhA5, were observed in the analyzed samples. The FdhA8 peptide was not detected. FdhA2 and FdhA5 peptides were detected in all SDC-9 cell dilutions (10^5 to 10^7 *Dhc* cells). However, only 10^7 *Dhc* cells concentrations rendered quantifiable concentrations above the lower limit of MDL for these two peptides. FdhA2 peptide was selected as a quantifier based on the detection of the fragment ions showing the lowest LOQ. Table 6-3 shows FdhA peptide concentrations per total *Dhc* cell number. Peptides PceA4, PceA7 and PceA8 showed lower sensitivity than the PceA5 peptide, which was detected in samples with 10⁵ Dhc cells/mL. The lowest abundance of Dhc cells that generated detectable and quantifiable concentrations of PceA4, PceA7 and PceA8 was 10⁶, while 10⁵ Dhc cells was needed to quantify the PceA5 peptide. The, PceA5 and PceA4 peptides were selected as quantifiers for the proceeding studies. The TceA2, TceA3 and TceA5 peptides had highest sensitivity and were detected and quantified in samples containing 10⁵ Dhc cells (Table 6-3). However, the TceA5 peptide had a relatively inconsistent retention time variation and the peptides TceA2 and TceA3 were selected as quantifiers for the proceeding studies. The TceA4 peptide was detected in samples with 10⁷ Dhc cells/mL. The VcrA peptides were least sensitive, with VcrA1, VcrA2, VcrA3 and VcrA6 peptides detected in the 10^6 and 10^7 Dhc cells and with small number of transition ions passing the accuracy

¹ Bolded letter denote heavy ¹³C and ¹⁵N labeled amino acid; the maximum of three MDL test replicates was established as the MDL

criteria. The VcrA4 peptide was detected but below the quantification limit in this study and most likely requires higher number of *Dhc* cells for quantification. Of all VcrA peptides, the VcrA3 was selected as a quantifier due to its highest sensitivity, detection of the highest number of transitions and its low MDL of 1 fmol/mL.

The analysis of the compiled data shows that the most sensitive peptides for quantification were FdhA2, PceA4 and PceA5, TceA2 and TceA3 and VcrA3 and required abundances of $2.2 \times 10^6 \, Dhc$ cells/mL or more in the 15 mL sample that was extracted (corresponding to $10^7 \, Dhc$ cells or more extracted) to be detected (Table 6-3). These peptides served as quantifiers in the next set of experiments. The remainder of qualifier peptides was used to confirm accuracy of the detection method and were analyzed in all subsequent experiments. Additionally, four to six transition ions were analyzed per peptide ion to confirm accuracy and sensitivity of the method and to confirm peptide sequence. In this study, up to six transitions for a given peptide ion were measured to validate the identification of a peptide.

The required lowest concentration of *Dhc* cells for detection of the remainder of peptides varied per protein, for example, to detect other TceA peptides a minimum of 10^5 *Dhc* cells are required, but to detect VcrA specific peptides the cell concentrations need to be an order of magnitude higher. Thus, the total recommended *Dhc* cells in a sample for targeted proteomics is 10^7 cells, regardless of sample volume.

Table 6-3. Results of qProt assay quantitation limit validation study

			<i>Dhc</i> ab	undance in	sample, <i>Dhc</i>	cells/mL ^b		D [46 47]	
		1.3x10 ⁵	2.2x10 ⁶	3.1x10 ⁷	1.3x10 ⁵	2.2x10 ⁶	3.1x10 ⁷	Previously reported [46, 47] protein concentrations in	
Protein	Peptide ID		le concentra lture, fmol/1			oncentration i /mL (protein/		culture, fmol/mL (protein/cell)	
	FdhA2a	<3.0x10 ⁰	<3.0x10 ⁰	8.5x10 ⁰	2.0.100	2.0.100	0.5.100	[46]KB1, D2 culture (TCE):	
FdhA	FdhA5	<3.0x10 ⁰	<3.0x10 ⁰	1.1x10 ¹	$<3.0 \text{ x}10^{0}$	<3.0 x10 ⁰	$\begin{array}{c} 8.5 \times 10^{0} \\ (3.8 \times 10^{3}) \end{array}$	$9.0x10^{1} - 1.0x10^{2} (2.3x10^{3} - 3.5x10^{3})$	
	PceA4 a	<9.0x10 ⁰	<9.0x10 ⁰	2.1x10 ¹					
PceA	PceA5 a	$6.3x10^2$	$1.9x10^2$	6.8x10 ¹	6.3×10^2	1.9x10 ²	4.4x10 ¹		
PceA	PceA7	<1.0x10 ⁰	2.2x10 ¹	1.9x10 ¹	0.3810				
	PceA8	<1.0x10 ⁰	1.7x10 ¹	1.2x10 ¹					
	TceA2 a	<3.0x10 ⁰	1.9x10 ¹	1.3x10 ¹			1.7x10 ¹		
TceA	TceA3 a	<1.0x10 ⁰	3.1×10^{1}	2.1x10 ¹	<1.0x10 ⁰	2.5x10 ¹			
IceA	TceA4	<9.0x10 ⁰	<9.0x10 ⁰	2.3x10 ¹		$(1.1x10^4)$	$(7.7x10^3)$	$(^{[47]}2.3x10^3)$	
	TceA5	<1.0x10 ⁰	2.4x10 ¹	1.7x10 ¹					
	VcrA1	<9.0x10 ⁰	$1.7x10^2$	1.8x10 ¹					
	VcrA2	<2.7x10 ¹	7.6x10 ¹	4.9x10 ¹	_		_		
VcrA	VcrA3 a	$<1.0x10^{0}$	5.7x10 ¹	9.3x10 ¹	$<1.0x10^{0}$	$5.7x10^{1}$ (2.6x10 ⁴)	$9.3x10^{0}$ $(4.2x10^{3})$	Difficult to quantify due to low peptide sensitivity	
	VcrA4	<8.3x10 ¹	<8.3x10 ¹	<8.3x10 ¹		(2.0/10)	(4.2810)	10.1. populae sensitivity	
	VcrA6	$<1.0x10^{0}$	5.8x10 ¹	1.1x10 ¹					

^aQuantifier peptides used to estimate protein abundance, in cases where multiple quantifier proteins exist those abundances are averaged to obtain protein abundance, a 1:1 peptide to protein ratio is assumed; ^b15mL of each cell density were extracted, corresponding to 2x10⁶ to 5x10⁸ *Dhc* cells extracted; ^cDetected proteins were expressed in both fmol/mL and protein/cell concentration units

6.3 MICROCOSM STUDY RESULTS

Reductive dechlorination of *cis*-DCE and VC and subsequent production of ethene were observed in the microcosm experiments as a general function of the SDC-9 inoculum concentration; Figure 6-1 provides an example of relevant microcosm data. Losses in uninoculated-live and killed-control microcosms were comparatively small and similar in magnitude, presumably due to volatilization during sample collection (see panel C in Figure 6-1 for data from an uninoculated-live microcosm). The data indicate that indigenous dechlorinating organisms did not contribute significantly to the observed rates of *cis*-DCE or VC degradation. In microcosms inoculated with SDC-9, lactate was generally fermented to acetate and propionate (Figure 6-1, panel B), which then declined slowly over time. Bromide was used as a conservative tracer to document losses of VOCs and fatty acids due to dilution as all water in the bottles was replaced with VOC- and VFA-free site water after sample collection (Figure 6-1, panel D).

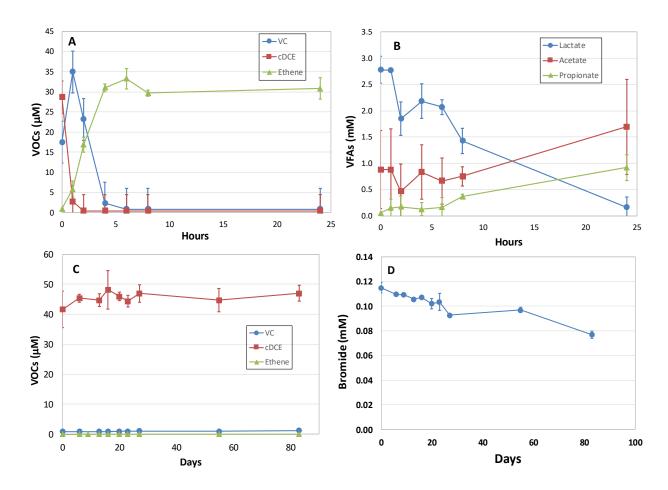


Figure 6-1. Concentrations of VOCs, VFAs and bromide in select JBLM #1 microcosms. Panel A and panel B show VOC and VFA concentrations, respectively, in microcosms receiving the highest SDC-9 inoculum ($\sim 10^8$ cells/ml). Panel C shows VOC concentrations in the live microcosms that were not inoculated with SDC-9, and Panel D shows bromide concentrations in these same microcosms (uninoculated) over time as a measure of dilution during sampling.

Example measured and model-fitted time-series *cis*-DCE, VC and ethene concentrations from selected microcosm sets are shown in Figure 6-2. Initial acceptance criteria for fitted rate constants required that the 95% confidence interval on the fitted rate constant be \leq the rate coefficient value itself. However, because best fit k_{cisDCE} , k_{vc} and global model R^2 are not independent, (i.e. reflect tradeoff between goodness of fit to parent and daughter product time-series concentrations subject to mass balance constraints), a more appropriate acceptance criterion was established to require (1) a global R^2 value of \geq 0.75, and (2) an average ratio of the 95% confidence interval on the k_{cisDCE} and k_{vc} rate constants of \geq 125%. Of the 40 microcosm tests performed (excluding live and killed controls), 26 and 15 tests respectively yielded acceptable quality k_{cisDCE} and k_{vc} rate coefficient data for further evaluation during this study (Table 6-4). A summary of all microcosm analytical data, as well as gene and protein abundance average and standard deviation values (triplicate analyses of single samples) obtained for each microcosm set at each time point sampled, is included in Appendix E.

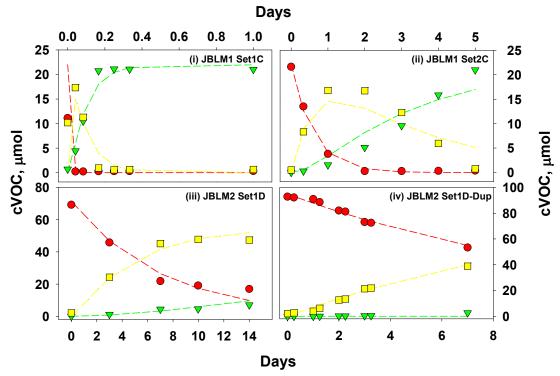


Figure 6-2. Measured and model-fitted cVOC concentrations in microcosms Measured (symbols) and fitted (dashed line) time series cVOC and ethene mass values measured in selected microcosms inoculated with 10⁹ (i), 10⁷(ii), 10⁶ (iii), 10⁷ (iv) *Dhc* cells/mL. Symbols for chlorinated ethenes are red circles (*cis*-DCE), yellow squares (VC), and ethene (green triangles).

Table 6-4. Summary of fitted k_{cisDCE} and k_{vc} by microcosm test

Table 6-4. Summary of fitted k_{cisDCE} and k_{vc} by microcosm test								
Microcosm Test Replicate	^a kcisDCE, day ⁻¹	^a kvc, day⁻¹	R ² model	Average Ratio of 95% Confidence Interval to Rate Constant for k _{cisDCE} , and k _{VC}				
- Itophicute	110102 02, 41115	JBLM1	11104101	Constant for Misbel, with My				
JBLM1 Set1A	62.6 ± 73.1	10.2 ± 3.9	0.85	77%				
JBLM1 Set1B	57.0 ± 53.7	10.2 ± 3.6	0.86	65%				
aJBLM1 Set1C	73.6 ± 146.0	11.3 ± 5.9	0.77	125%				
JBLM1 Set2A	1.48 ± 0.50	0.31 ± 0.073	0.88	29%				
JBLM1 Set2B	1.49 ± 0.48	0.34 ± 0.078	0.89	28%				
JBLM1 Set2C	1.68 ± 0.53	0.34 ± 0.070	0.91	26%				
JBLM1 Set3A	0.024 ± 0.0026	0.0015 ± 0.0018	0.90	65%				
JBLM1 Set3B	$0.016 \pm 1.20 \times 10^{-8}$	$0.00058 \pm 6.6 \times 10^{-8}$	0.88	0.0%				
JBLM1 Set3C	$0.026 \pm 2.20 \times 10^{-7}$	$0.0037 \pm 6.6 \text{x} 10^{-7}$	0.89	0.0%				
JBLM1 Set4A								
JBLM1 Set4B	0.0013 ± 0.00013	b	0.91					
JBLM1 Set4C	0.0014 ± 0.0014	0.000001 ± 0.026	0.91					
VB2	0.001.	JBLM2	0.51					
JBLM2 Set1A	1.05 ± 0.94	0.11 ± 0.018	0.94	53%				
JBLM2 Set1B	1.05 ± 1.14	0.13 ± 0.028	0.92	65%				
JBLM2 Set1C	1.13 ± 1.28	0.17 ± 0.041	0.83	68%				
JBLM2 Set1A Dup	0.28 ± 0.038	0.013 ± 0.031	0.99	128%				
JBLM2 Set1B Dup	0.30 ± 0.031	0.000027 ± 0.023	0.99	43591%				
JBLM2 Set1C Dup	0.22 ± 0.017	0.0067 ± 0.021	1.00	161%				
JBLM2 Set2A	0.0028 ± 0.00040	0.0031 ± 0.0086	0.49	145%				
JBLM2 Set2B	0.0018 ± 0.00024	0.0026 ± 0.0083	0.53	168%				
JBLM2 Set2C	0.00244 ± 0.00039	0.0042 ± 0.0099	0.34	125%				
JBLM2 Set3A	0.0010 ± 0.00026	0.0028 ± 0.013	0.05	255%				
JBLM2 Set3B	0.00088 ± 0.00022	0.0033 ± 0.013	0.13	210%				
JBLM2_Set3C	0.00096 ± 0.00025	0.0017 ± 0.013	0.06	401%				
JBLM2 Set4A	0.0011 ± 0.00030	0.0037 ± 0.014	0.03	197%				
JBLM2_Set4B	0.00082 ± 0.00021	0.0037 ± 0.013	0.12	189%				
JBLM2_Set4C		-						
JBLM2_Set5A		Live con	trole					
JBLM2_Set5B		Live con						
JBLM2_Set6A		Killed con	ntrols					
JBLM2_Set6B								
c*JBLM2_Set1D	0.14 ± 0.016	0.019 ± 0.0081	0.97	27%				
*JBLM2_Set1E	0.088 ± 0.013	0.018 ± 0.015	0.92	51%				
*JBLM2_Set1F	0.096 ± 0.015	0.021 ± 0.015	0.92	44%				
d*JBLM2_Set1D_Dup	0.078 ± 0.0046	0.00001 ± 0.016	0.97	81907%				
*JBLM2_Set1E_Dup	0.059 ± 0.0038	0.018 ± 0.020	0.96	57%				
*JBLM2_Set1F_Dup	0.057 ± 0.0035	0.0027 ± 0.018	0.96	343%				
*JBLM2_Set2D	0.001 ± 0.00026	0.00009 ± 0.015	-0.26	8447%				
*JBLM2_Set2E	0.001 ± 0.00029	0.00001 ± 0.017	-0.15	83025%				
*JBLM2_Set2F	0.00001 ± 0.00048	0.0001 ± 2.88	-0.22	1439925%				
*JBLM2_Set5C		Live con	trols					
*JBLM2_Set5D								
*JBLM2_Set6C		Killed con	ntrols					
*JBLM2_Set6D		1111100 001						

^{&#}x27;—"indicates no rate was computed; ^aBest fit rate constants ± the 95% confidence interval on the rate constant; grey-highlighted values did not meet acceptance criteria and were excluded from further analysis; "*" indicates microcosm included groundwater and aquifer solids

6.4 BIOMARKERS AS PREDICTORS OF REDUCTIVE DECHLORINATION RATES

Gene and protein abundances collected from early, middle and late time points from each microcosm were positively and significantly correlated with the k_{cisDCE} and k_{vc} rate coefficients extracted from those microcosms (Table 6-5). The correlation coefficients between biomarker abundances and rate coefficients were generally highest when only the middle time point biomarker abundances were included in the correlation analysis. For example, the correlation coefficient for VcrA protein and the k_{cisDCE} rate coefficient increased from 0.374 to 0.725 when only the midpoint protein abundances were considered (Table 6-5). This may reflect the general metabolic status of the dechlorinating organisms in the batch system (i.e., organisms had time to assimilate to the groundwater environment after inoculation and were actively biodegrading cis-DCE). This status may most effectively simulate that found in a flow-through aquifer system, where electron acceptor (e.g., cis-DCE) and nutrients are resupplied by groundwater flow. Accordingly, the rate coefficient vs. biomarker abundances regression analyses were performed using the midpoint biomarker abundances only. Note that while the pceA gene is carried by reductive dechlorinators present in the SDC-9 consortium, and both pceA gene and PceA protein abundances were found to be positively correlated with reductive dechlorination rates, these RDase biomarkers are not present in *Dhc* cells and are not reflective of complete dechlorination to ethene. Therefore, pceA gene and PceA protein abundances were not carried forward during rate and biomarker abundance regression analysis.

Table 6-5. Rate coefficients and biomarker correlations

Biomar	Biomarker abundance correlations with rate coefficients (biomarker abundance, all microcosm time points)									
	FdhA	PceA	TceA	VcrA	DHC_16S gene	tceA	vcrA	fdhA	pceA	
Log kcis	0.737	0.571	0.575	0.374	0.844	0.859	0.856	0.801	0.804	
p value	2x10 ⁻⁷	6x10 ⁻⁶	1x10 ⁻⁶	6x10 ⁻³	2x10 ⁻⁷					
n	57	55	62	54	64	64	64	64	62	
$\text{Log } k_{VC}$	0.774	0.797	0.652	0.678	0.932	0.934	0.93	0.905	0.91	
p value	2x10 ⁻⁷	2x10 ⁻⁷	3x10 ⁻⁵	3x10 ⁻⁵	2x10 ⁻⁷					
n	35	33	34	30	36	36	36	36	36	
Biomarl	ker abunda	nce correla	tions with rat	te coefficients	(biomarker abund	lance, mic	crocosm n	id-points	only)	
	FdhA	PceA	TceA	VcrA	DHC_16S gene	tceA	vcrA	fdhA	pceA	
Log kcis	0.852	0.793	0.755	0.725	0.863	0.905	0.918	0.881	0.854	
p value	2x10 ⁻⁷	2x10 ⁻⁷	2x10 ⁻⁷	7x10 ⁻⁴	2x10 ⁻⁷					
n	21	21	23	17	23	23	23	23	23	
Log kvc	0.925	0.836	0.765	0.916	0.934	0.953	0.962	0.966	0.943	
p value	2x10 ⁻⁷	2x10 ⁻⁷	4x10 ⁻⁴	2x10 ⁻⁷						
n	15	15	15	11	15	15	15	15	15	

Results of the power law least squares regression analysis of *cis*-DCE and VC rate coefficients versus gene and protein abundances are presented in Figure 6-3. Following the regression trends of target proteins down to the highest of the method detection limit for targeted proteins in this study of 3 fmol (e.g. TceA2, Table 6-1), which is equivalent to a typical 1-L groundwater sample containing $2x10^6$ proteins/mL, would translate to k_{cisDCE} and k_{vc} rate constants both in the range of 0.0001 day⁻¹ (~0.04 yr⁻¹), which is suitably low to be relevant to MNA sites¹. Thus, the proteomics

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 $^{^1}$ For example, an apparent first order degradation rate coefficient of 0.04 yr $^{-1}$ means 500 $\mu g/L$ VC would decrease to the 2 $\mu g/L$ VC maximum contaminant level in 138 years.

assay is sensitive enough to quantify proteins over a wide range of *Dhc* abundances and activities relevant to both biostimulated or bioaugmented sites where biomarker abundances and rates of reductive dechlorination are high, as well as to MNA sites where biomarker abundances and reductive dechlorination rates are quite low.

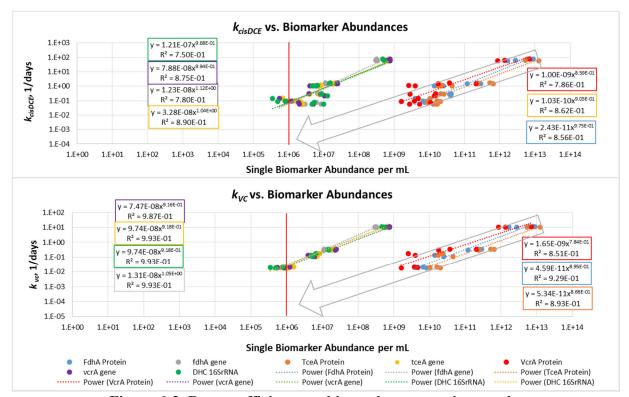


Figure 6-3. Rate coefficients vs. biomarker regression resultsMicrocosms that yielded acceptable rate coefficients (Table 6-4) and their corresponding midpoint RDase gene and biomarker abundances were used to complete regression results shown above.

The predictive power of gene and protein biomarkers was tested using regression equations featured in Figure 6-3 and biomarker abundances from microcosm tests that met the data quality acceptance screening criteria (Table 6-4) but that were not included in the regression analysis. The test was performed in two ways (1) using randomly selected biomarker abundances corresponding to a range of *Dhc* cell abundances, and (2) using global averages of all biomarker abundances that corresponded to *Dhc* cell abundances at 10⁶, 10⁷, 10⁸ cells/mL. The randomly-selected biomarker abundances corresponded to the time zero sampling from microcosm sets JBLM1_Set1B, JBLM1_Set2C, and JBLM2_Set1F. As shown in Figure 6-4, protein-based rate predictions (white bars with black dots) were within an order of magnitude of measured rate coefficients (green boxes) for all tests. Rates predicted using a combination of genes and proteins (green bars, Figure 6-4) were generally better than those predicted using proteins alone.

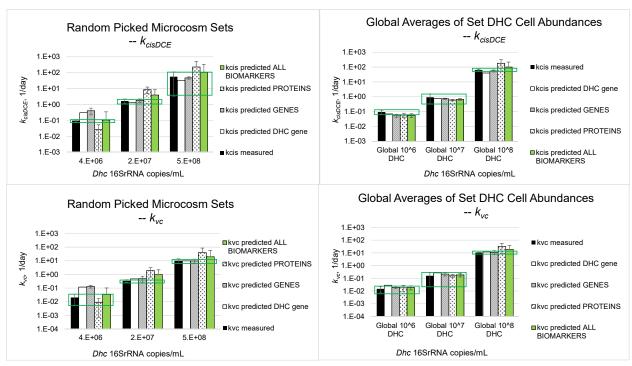


Figure 6-4. Biomarker-based rate predictions vs. measured rate coefficientsGreen boxes reflect the error range associated with the measured rate coefficients during the study. Rates predicted using a combination of RDase genes and proteins (green bars) generally yielded an improved rate prediction compared to RDase proteins alone.

6.5 CONSIDERING APPLICABILITY OF THE LABORATORY STUDY RESULTS TO FIELD SITES

The simple laboratory microcosm systems were appropriate for confirming a quantitative relationship between biomarker abundances and reductive dechlorination rates mediated by *Dhc* cells from the SDC-9 consortia. However, conditions in the laboratory microcosms do not perfectly emulate conditions in aquifers at cVOC-contaminated field sites, which is where the qProt tool must be useful to provide maximal benefit. Here we consider how the quantitative link established between the biomarker abundances in the laboratory might be different than the link established in the field.

First, the majority of the bioaugmented microcosm test completed under this project featured only groundwater; relatively few microcosms featured groundwater with aquifers solids. Results of the rate coefficient vs. protein biomarker abundance regressions are reproduced in Figure 6-5 below, here with the groundwater-only (blue symbols) and groundwater with soil (orange symbols) differentiated for each biomarker. Microcosms that featured groundwater with soil generally clustered at the low end of the biomarker abundances and activity rates. Although the plus soil treatments tended to have lower biomarker abundances and activities, the results were generally consistent with the entirety of the data set, suggesting no significant difference in the presence of aquifer solids. It should be noted that all microcosm tests with soil were performed relatively quickly, under continuously mixed conditions, and were not designed to assess or account for *Dhc* cell attachment that may occur overtime; attachment was assumed to be negligible during these

tests. The contribution of attached vs. planktonic *Dhc* cells was beyond the scope of this laboratory study but will be addressed in the pending follow-on field demonstration.

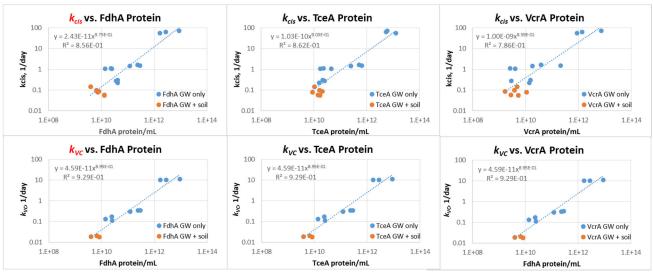


Figure 6-5. Rate coefficients vs. biomarker abundances with treatments distinguished. Blue and orange symbols represent biomarker and rate coefficient results from groundwater-only microcosms and groundwater with aquifer solids microcosms, respectively.

Second, in a natural aquifer system, the abundance of bacteria will adjust to a level where growth sustained by the supply of the limiting electron acceptor (e.g., cis-DCE) is balanced by maintenance and cell death (e.g., predation). Indeed, viable microbes present in a natural system may consume dead cells as a nutrient source [48] and thereby "turn over" the dead cells. This cell "turn over" in natural systems will, in theory, minimize the chance of detecting "carcass proteins" (i.e., in this case RDases associated with dead cells). While the presence of such RDases in nonviable cells would be difficult to quantify, we did evaluate whether the protein levels detected were reasonable based upon the density of *Dhc* cells measured. If protein to *Dhc* cell abundances were greater than physiologically expected, or if ratios were much larger than have been previously published for RDase proteins in *Dhc*, the presence of RDases not associated with viable cells could be a confounding factor in RDase biomarker vs. rate coefficient regressions established in this study. Observed ratios were generally between 10³ and 10⁵ proteins/cell (Figure 6-6), which is in the range of previously published values of 7.6x10³ and 2.60x10⁴ for TceA reported for KB-1 and D2 cultures, respectively [46]. Further, the theoretical maximum number of proteins that could "fit" in the periplasmic space of a *Dhc* cell was estimated using the computational approach of Milo 2013 [52] to be 10^5 proteins. If we assume 10% of the proteins in a *Dhc* cell are RDases, the maximum RDases per Dhc cell would be 104. Therefore, the observed range of RDase proteins per *Dhc* cell during this study do not exceed the realm of physiological possibility.

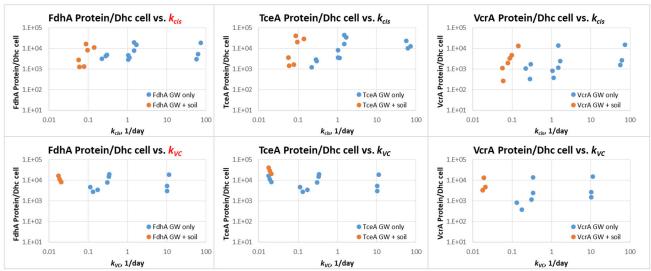


Figure 6-6. Protein/*Dhc* cell ratios vs. rate coefficients for microcosm tests Blue and orange symbols represent biomarker and rate coefficient results from groundwater-only microcosms and groundwater with aquifer solids microcosms, respectively.

Finally, because first order-based kinetics often describe reductive dechlorination at field scales reasonably well, our first order-based regressions (Figures 6-3 and 6-5) will be directly applicable for supporting qProt assay interpretation at many field sites. In a natural system – in this case a cVOC-contaminated aquifer undergoing monitored natural attenuation – Dhc abundance will adjust to a level where growth sustained by the supply of chlorinated ethenes is balanced by self-consumption to sustain metabolism, and predation. In this case the abundance of Dhc and concentration of chlorinated ethenes remain relatively constant at any one position along the flow path. In such cases cVOC degradation kinetics can be described by a pseudo first-order kinetic model; i.e. at any one location along the flow path, the overall rate of degradation of the substrate in the groundwater ($\mu g/L$) per day) divided by the concentration of the substrate ($\mu g/L$) is a fixed ratio. If an end user of the qProt assay wishes to use an alternative kinetic model to interpret test-or field site-specific results, published kinetic constants in the literature could be used to do so.

7.0 COST ASSESSMENT

7.1 COST MODEL

Standard analytical costs to obtain RDase activity rate estimates during the microcosm studies, as well as costs to obtain RDase biomarker abundance, were tracked as part of this laboratory project (Table 7-1). Costs to perform the microcosm study, analyze the data, and prepare a memorandum were tracked and recorded as well. Capital and operation and maintenance (O&M) costs were *not* tracked or reported. A field demonstration is necessary to a complete cost assessment with sufficient detail that a future "end user" of proteomics technology could compare costs between proteomics and existing MBTs and develop a reasonable cost estimate for conducting proteomic analysis at a cVOC-contaminated site. It should be noted that the microcosm testing conducted during this phase of the ESTCP Project would not be required during a field application of proteomic technology, so the associated costs are not relevant.

Table 7-1. Cost model for proteomics

Cost Element	Details	Tracked Demonstration Data	Discounted Costs		
Capital Costs					
System Design	Labor				
	Labor				
Well Installation and Development	Materials	These data were	not tracked as part of		
_	Subcontracts		oject but would be		
	Labor	tracked during su	ibsequent field		
System Installation	Equipment & Materials	demonstration			
	Subcontracts				
Travel					
		Subtotal			
Operation and Maintenance (O&I	M) Costs				
-	Labor				
Groundwater Sampling	Materials				
A 1 1 1 1	In-house Labor	These data were	not tracked as part of		
- Analytical	Laboratory	the laboratory pro	oject but would be		
G	Labor	tracked during subsequent field demonstration			
System O&M	Materials				
Reporting & Data Management	Labor				
Travel					
	•	Subtotal			
Other Technology-Specific Costs					
Site Selection	Labor & Travel				
	Labor				
Site Characterization	Materials				
	Subcontractor	The cost to set up	and run all the		
	Labor	microcosm tests,	including all the cVOC		
Treatability Testing	Materials	analysis and prep	paring the concentration		
, ,	Subcontractor		was \$215K. Cost per		
Meetings & Reporting	Labor & Travel		e biomarker analysis is		
Technology Transfer	Labor & Travel	described separat	ely in Section 7.3		
Demonstration Plan/Work Plan	Labor	below.			
Final Report	Labor				
Cost and Performance Report	Labor				
1	•	Subtotal			
		TOTAL COSTS			
ES	TIMATED TREATMENT				
	IMATED TREATMENT	` ,			
	XIMATE TREATMENT				
	IMATE TREATMENT C	` ,			
		(======================================			

7.2 COST DRIVERS

Implementation of advanced MBTs during the assessment phase, remedy implementation and monitoring of the project are impacted by the factors as described above. Although there are currently no regulatory requirements that specifically mandate advanced MBTs be used to assess a site, the data provided by the MBTs are meant to supplement and possibly replace other forms of data that provide lines of evidence that MNA is occurring and to estimate a degradation rate. Hence, the total sampling and analytical cost is driven by number of sample locations at a site and total number of samples collected (i.e., a greater number of samples equates to a higher cost). It should be noted however that the individual cost per sample for analyses with advanced MBTs 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 larger quantity of analyses.

7.3 COST ANALYSIS

The microcosm studies conducted for this project to correlate rates of cVOC biodegradation with RDase abundance were extensive and would not be required for field implementation. The cost for the microcosm work including analytical (cVOCs, VFAs, dissolved gases, anions, pH) for all studies, collection and shipping of samples for qPCR and proteomic analysis, as well as preparation of the treatability study workplan and keeping a project database was \$215K. This included \$209K in labor and \$6K in materials, supplies, equipment, and shipping costs. No subcontracts were issued as all work including analytical was conducted at Aptim's laboratory in Lawrenceville, NJ. Collection of site materials for the microcosm studies is not included in these costs.

With the exception of metagenomics and metaproteomics, the techniques used to assess the contaminant degradation and continued potential for natural attenuation are common and costs to apply these techniques are well documented in the literature [49-51]. As discussed in Section 7.2, costs are highly dependent on the number of samples collected, frequency of sampling, and number/types of analytes, which are primarily dictated by the nature/diversity of the contaminants of concern (COCs), size of the site, proximity of receptors, and regulatory requirements. Hence, it is not the intent of this demonstration report to generate a life-cycle cost estimate for a hypothetical site at which these techniques are applied to evaluate remedial performance and subsequent natural attenuation of the remaining COCs to achieve site remedial action objectives (RAOs).

Table 7-2 provides a general cost comparison of conventional MBTs (e.g., qPCR) to the advanced MBTs, including proteomics. As indicated in the last column of the table, many of these techniques 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 purpose, the cost of shotgun and quantitative metaproteomic analyses based on cost data collected during this demonstration were \$1,200 and \$800 per sample, respectively, assuming analysis of a batch of 12 samples. The cost of the metaproteomic analyses included use of an existing metaproteomic platform but assumed development of a workflow specific for cVOCs.

Table 7-2. Cost comparison of conventional MBTs (e.g., qPCR) to the advanced MBTs.

Molecular Tool	Identity/ Potential Activity/ Expressed Activity ^a	Quantitative, Qualitative (QA/QL)	Cost Range (\$) b	Availability ^c
	Conven	tional MBTs		
Compound specific isotope analysis	E	QA	100 to 2,500	C/R
Quantitative polymerase chain reaction	I/P/E	QA	275 to 425	WC
Microarrays	I/P/E	QL	1,250 to 5,000	C/R
Stable isotope probing	I/P/E	QA/QL	1,500 and up	C/R
Enzyme activity probes	E	QA	250 to 2,500	C/R
	Advanced	(omic) MBTs ^d		
Metagenomics (16S Sequencing)	I	QL	150 to 500	WC/R
Shotgun Metaproteomics	Е	QL	800 to 1,500	C/R
MRM Metaproteomics	E	QA	500 and up	C/R

Adapted from ITRC (2011). ^a I - identity of microorganisms (i.e., genus or species), P - potential activity (i.e., genetically capable of completing the activity), E - expressed activity (i.e., actually completing the activity at a given time). ^b Estimated price per sample. Low end represents compound specific restricted analysis. ^c WC - widely commercially available, C- minimally commercially available, R - available through university or other research laboratory. ^dThe cost of advanced omic MBTs represents cost from two commercial laboratories and Battelle metagenomic and proteomic lab. These costs are based on current costs from 2017 and higher number of batches (20 samples). These costs elements are reduced since the methods are maturing and proteomic analyses becomes more routinely used.

8.0 IMPLEMENTATION ISSUES

This section focuses on proteomic analysis and the potential of this technology to facilitate assessment of MNA. 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 access to specialized laboratories; and (3) technology cost compared to other more conventional monitoring options. These implementation issues are addressed in the following sections.

8.1 REGULATORY ACCEPTANCE

Proteomics is a new tool in environmental assessment and one which requires validation. The relationship between specific proteins and protein levels and degradation rates of various cVOCs is now being established, with the work reported herein providing key data in this regard. At present, proteomics can be used to provide a direct line of evidence that biodegradation is actively occurring based on the detection of proteins that are produced during the degradation process. However, in the future, it is conceivable that proteomics could provide a direct measure of degradation rates based on the concentrations of proteins that are measured in a sample, which could eliminate or reduce the need to measure concentrations of cVOCs. It is therefore expected that regulatory acceptance of this technology will in part be based on the application and end use of the resulting data.

As with any new technology, detailed demonstration and validation are required to ensure accuracy and precision of results for both techniques before widespread regulatory acceptance can be obtained. Standardized methods and procedures for sample collection and shipping, analytical methods, QA/QC and data evaluation must be further developed and validated to help ensure regulatory acceptance. In addition, technology transfer through SERDP/ESTCP, peer reviewed journal articles, webinars, conferences, and other meetings will play an important role to facilitate understanding and acceptance of these powerful tools.

8.2 LIMITED AVAILABILITY

Proteomic analysis of enzymes involved in the reductive dehalogenation of cVOCs in field samples is a relatively new endeavor. The results provided in this report provide a strong basis for moving forward with this MBT for site assessment purposes. However, as with any new technology, availability and data quality are important concerns. Due to the young state of the practice, QA/QC guidelines for environmental applications of proteomic analysis are not yet available. In addition, only few analytical environmental laboratories offer advanced MBTs, and qProt is not yet commercially available. As with other important MBTs (e.g., qPCR) it is anticipated that these issues will be resolved over time as the method becomes more widely accepted and commercially applied.

8.3 COST COMPARED TO OTHER MONITORING TOOLS

The costs for qProt analysis are high compared to conventional technologies but are expected to decrease substantially as the technology continues to advance. Although costs per sample currently range from several hundred dollars to about \$1,000 for these types of analyses, MBTs help to answer a variety of management questions and facilitate decision making that can result in a reduction of the life-cycle cost of a remedy. For instance, MBTs such as metagenomics and metaproteomics, may be used instead of laborious microcosm studies to definitively state if microorganisms of interest are performing required activities and are actively degrading specific contaminants. These new MBTs characterize the contaminant-degrading *in situ* microbiome with unprecedented resolution. Information provided by these new MBTs, together with data from conventional MBTs, provides a comprehensive assessment and enables site management decisions to be made with greater confidence. Not only will this likely result in a direct cost savings to the project since microcosm studies can be more costly than the MBT analyses, but it also reduces the time required for assessment because microcosm studies generally take 60 to 90 days to perform.

During remediation efforts, MBT data help to design the remedy, to optimize remedial strategies, and to troubleshoot unsuccessful treatment approaches. Results can be used to determine when to reapply amendments to optimize growth and distribution of the target organisms, which can help to minimize the time required for the active portion of the remedy. Conceivably, in the near future, proteomics may provide the necessary means to directly calculate degradation rates, which then can be augmented during the active portion of the remedy to facilitate removal of cVOCs, thereby reducing application time and life-cycle cost.

Proteomics can facilitate long-term monitoring efforts by confirming that active degradation is occurring across the site, and eventually may aid to estimate the rate of degradation to decide if site-specific cleanup goals can be achieved within a desired timeframe. This could result in less frequent monitoring events and or a reduced number of analytes, which may reduce the life-cycle long-term monitoring cost and may support more rapid site closure. As metagenomics and proteomics are increasingly used in environmental applications, and as more laboratories begin to offer these analyses, competition increases, and techniques are refined, which will bring down the costs.

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APPENDIX A

QUANTITATIVE PROTEOMICS METHOD DETECTION LIMIT AND INSTRUMENTATION DETECTION LIMIT STUDY RESULTS

Assessment of Instrument Detection Limit and Method Detection Limit for Reductive Dehalogenase Isotopic Peptides

REPORT

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List of Acronyms

CEs collision energies

DHC Dehalococcoides

IDL instrument detection limit

IS internal standards

LOD limit of detection
LOQ limit of quantification

LLOQ Lower level of quantification

MRM multiple reaction monitoring-based

m/z mass-to-charge ratios

PCE tetrachloroethene

RDase reductive dehalogenase

S/N Signal-to-noise

TCE trichloroethene

VC vinyl chloride

Introduction

In preparation for microcosm experiments planned for this project, the Battelle proteomics team, in collaboration with the University of Tennessee, has identified SDC-9 culture-specific reductive dehalogenase (RDase) peptides for quantification. These specific RDases are used in a multiple reaction monitoring-based (MRM) targeted proteomic assay to establish quantitative biomarker rate correlations, which are needed to generate in situ degradation rate estimates of chlorinated ethenes.

During the development phase, the MRM assay was thoroughly evaluated for limit of detection (LOD) and limit of quantification (LOQ). To maximize precision, stable isotope labeled internal standards (IS) are frequently used to account for errors and losses that can occur during sample handling and variability in peptide ionization in the analysis of peptides. Because of the rigors of establishing these assays and successfully performing them in complex matrices, they tend to be implemented on only a selected number of analytes in parallel. With only a small number of analytes measured, it is common to expend considerable time optimizing tune parameters and collision energies (CEs) of each analyte individually to attain the highest sensitivity possible.

Prior to the use of IS peptides for MRM proteomics, general MS instrument parameters that work well with the broad diversity of peptides to be targeted needed to be determined (**Figure 1**). Peptide standards are directly infused to optimize these parameters empirically. CE is an instrument parameter that is frequently optimized to maximize fragment ion intensities. Multiple instrument manufacturers offer automated routines for CE optimization by peptide infusion as part of the instrument tuning software.

Once the system is optimized (resolution and calibration) and optimal CEs are determined, instrument detection limits and method detection limits are established for each IS peptide. Most analytical instruments produce a signal even when a blank (matrix without analyte) is analyzed. This signal is referred to as the instrument background level. Noise is a measure of the magnitude of the background signal. It is generally measured by calculating the standard deviation of a number of consecutive point measurements of the background signal or by measuring the magnitude of a defined region of background. Signal-to-noise (S/N) is obtained by calculating the ratio between the magnitude of the signal and the magnitude of the noise. Thus, the instrument detection limit (IDL), also known as LOD, is the analyte concentration required to produce a signal that is distinguishable from the noise level.

For most applications, required sample preparation methods may result in alteration of clean analyte prepared in solvent. It may be necessary to remove unwanted matrix components, digest, extract and concentrate the analyte, or even derivatize the analyte for improved chromatography or detection. The analyte may also be further diluted or concentrated prior to analysis on an instrument. Additional steps in a sample preparation method add additional opportunities for error (losses). Determination of the detection limit when sample preparation/manipulation steps are incorporated into the preparatory and analysis scheme results in an identified method detection limit (MDL), also known as LOQ. An MDL or LOQ accounts for additional losses that occur during the course of sample manipulation/preparation. Theoretically, the IDL is lower than the MDL for a given target analyte.

In this report, each step of system and IS peptide optimization is characterized in the sections below. Data pertaining to system resolution check, calibration, and chromatograms of peptide detections are grouped per sample set in **Appendices C** through **H**.

• This optimization step is performed to confirm that a peptide of a given sequence is detectable in the mass spectrometer and to optimize signal intensity for product ions. • Every peptide is infused individually into the mass spectrometer at relatively high concentrations (0.5 pmol/ul or 1.25 pmol/ul). Infusion • This step is performed as a confirmation of instrument functionality. •IS mixture of all peptides is injected on a column at 1.25 pmol/ul. • Detections and nondetections of all expected peptides are observed and reported. Injection • Optimization of signal intensity for product ions at optimum CE for each peptide. • Final MRM method is established using CE parameters. Collision energy optimization • All IS peptides are injected at a range of concentrations in triplicate (250 fmol/ul, diluted serially to 0.1 fmol/ul). • Each set of dilutions is prepared and injected on a different day to account for instrument variability. IDL =• All IS peptides are prepared in an injection buffer (HPLC-grade water + 0.1% formic acid). Instrument detection limit •Lower level detection limit is established per peptide. • All IS peptides are injected at a range of concentrations in triplicate (250 fmol/ul to 0.1 fmol/ul). • All peptides are prepared exactly as experimental samples. This means that peptides are injected into the matrix (SDC-9 culture, palletted down, used only culture liquids to account for background proteome). • Each sample goes thorugh the extraction and cleanup procedure. MDL = Method • Samples are then run with the optimized MRM method. detection limit • Lower level of quantification (LLOQ) and level of detection (LOD) are established per each peptide.

Figure 1. Optimization of Instrument Parameters and Isotopic Standards Characteristics

RDase Peptide Selection, Protein Extraction and Quantification

RDase Peptide Selection

Dehalococcoides (DHC) comprise a genus-level group of bacteria within the phylum *Chloroflexi*, notable for their ability to respire halogenated compounds including recalcitrant groundwater contaminants. Their obligate use of halogenated organic compounds as an energy source has allowed successful development of DHC-containing enrichment cultures for bioaugmentation of chlorinated ethene-contaminated sites, such as SDC-9 consortium. Each DHC strain contains a unique complement of genes that are homologs of known RDases, the genes required for respiration of halogenated organic compounds. The SDC-9 consortium is a well-defined enrichment culture with a variety of robust tetrachloroethene (PCE) — vinyl chloride (VC) dechlorinators. A metagenome sequencing project for the SDC-9 consortium was completed by Battelle Memorial Institute and the University of Tennessee to determine specific RDase genes that could serve as targets in proteomic analyses.

Overall, 14 genes encoding RDases were identified, 10 of which best matched previously identified RDases from members of the genus *Dehalococcoides*. Of these 14 sequences, one *vcrA* and one *tceA* gene were identified, as well as two separate *pceA* genes. Of these 14 RDases, 12 were associated with small, protein coding RDase B genes that are predicted to have three membrane-spanning helices. Peptide sequences of RDases and several sequences of FdhA protein that encodes for formate dehydrogenase were selected for targeted proteomic analysis. The detailed list of peptide sequences is presented in **Table 1**.

Protein Extraction and Quantification

For determination of the MDL, the 12.5 pmol/ μ L stock solution was diluted in ammonium bicarbonate to prepare the following concentrations (final in 25 μ L): 250, 83, 27, 9, 3, 1, 0.34, and 0.11 fmol/ μ L. Each sample was digested with trypsin overnight and desalted using C18 spin columns. Protein extraction protocol, including protein detection with tryptophan assay and sample cleanup, has been developed for work with environmental samples and is located in **Appendix A** of this report. Copies of laboratory pages of sample extraction for MDL are also in **Appendix A**.

Table 1. List of SDC-9 Peptides Selected for Quantification

Protein	Peptide	Precursor (m/z)
	SELEVISSLLSR	671.9
	SGSEIAFTGGLI K	644.4
	SWDWALGEIAN K	699.4
	AAGASDWEE K	536.2
FdhA	ALGIVYLDSQA R	658.4
	VSSLQQLESPEELR	812.9
	LSWTYSTNPSAADVAK	859.9
	NQAVSAPGEA K	540.3
	TDTNTDYSYVNAI K	806.9
	VETWNHDVA R	412.9
	FDEWFGYSGPVNPEE R	969.9
	LLPWDLPK	495.3
DesA	IATQIPLLQDAA R	710.4
PceA	LESGYVQNMVK	638.3
	VYTDLELAPDKP R	509.6
	DFWNNPEPI K	634.3
	TSPSLISSATVG K	628.4
	FLGADLVGIAPYDE R	823.4
	DVDDLLSAGK	520.8
TceA	VSSIIEPR	455.8
	VNNEPWWVTT R	706.4
	YFGASSVGAI K	554.3
	WGLYGPPHDSAPPDGSVP K	662.3
	YFGAGDVGALNLADP K	808.4
VcrA	VPDHAVPINF K	415.6
VCIA	GVYEGPPDAPFTSWGN R	930.4
	TGAAIHW K	446.2
	DQPWYV K	472.2
	LVNELTEFAK	582.3
BSA*	AEFVEVTK	461.7
DOA	EYEATLEECCAK	751.8
	QTALVELLK tides were added to the method as in	507.8

*BSA peptides were added to the method as internal standard for peptide recovery based on discovery data run previously

Infusion and Injection of Isotopic Peptides

To confirm instrument functionality and detectability of each IS, infusion and injection steps were performed. Each isotopically labeled (IS) peptide was prepared as 12.5 pmol/ μ L in dimethyl sulfoxide (DMSO)/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. Concentrated solutions for each peptide were provided to the analyst for subsequent dilution and infusion directly into the mass spectrometer (Waters Xevo TQ-XS) for confirmation of precursor (parent) ion, charge state, product ions (daughters),

and optimization of CE (**Table 2**). This optimization step is performed to confirm that a peptide of a given sequence is detectable in the mass spectrometer and to optimize signal intensity for product ions. Each peptide was diluted to 0.5 pmol/ μ L or 1.25 pmol/ μ L in HPLC-grade water + 0.1% formic acid and was directly infused into the mass spectrometer at a flow rate of 10 μ L/min. For each peptide, a mass spectrum of the precursor ion was obtained (**Appendix B**). For each precursor ion, a mass spectrum was obtained for the product ions after fragmentation with CE of \geq 20 V.

Using Waters Intellistant software, the CE for each peptide was optimized to maximize a signal from product ions. This was performed by infusing a single peptide into the mass spectrometer while Intellistant software varied cone voltage and CE to maximize a signal for each product ion. Skyline software was also used to output optimal CE for each peptide using the following equation with parameters (slope, intercept) that are specific to Waters Xevo mass spectrometers (**Table 2**).

CE = slope *(precursor charge state) + intercept

Table 2. Parameters of Waters Xevo CE Equation

Precursor Charge State	Slope	Intercept
+2	0.037	-1.066
+3	0.036	-1.328

Multiple Reaction Monitoring (MRM) Method Development

After optimization of CE per each IS peptide further development of multiple reaction monitoring assay was performed, including optimization of dwell time, CE, and solvent program. During this phase, peptides with relatively poor response were dropped from the MRM method file. The Skyline-optimized CEs were used in initial MRM method development. Comparison to Intellistant-optimized CEs was performed later in MRM development, however improvements in signal intensity were insignificant.

For MRM method development, peptides were prepared as a mixture at 1.25 pmol/ μ L in HPLC-grade water + 0.1% formic acid from a 12.5 pmol/ μ L mixture in DMSO/Milli-Q water. The solvent program and modified versions thereof listed in **Table 2** were used. The chromatographic system used was the Waters M-Class equipped with a trap column (Acquity UPLC M-Class Trap Symmetry® C18; 5 μ m particle size, 100Å pore size; 0.3 mm x 50 mm) and an analytical column (Acquity UPLC M-Class HSS T3 C18; 1.8 μ m particle size, 0.3 mm x 50 mm). **Table 3** displays the solvent program used where A = HPLC-grade water + 0.1% formic acid and B = HPLC-grade acetonitrile + 0.1% formic acid.

Table 3. Solvent Program for M-Class Chromatographic System

Time (min)	Flow Rate (µL/min)	% A	% B
-	10	95	5
5	10	95	5
65	10	35	65
66	10	10	90
70	10	10	90
80	10	95	5
85	10	95	5

Based on the observed maximum peak heights of each peptide at 1.25 pmol/ μ L prepared in HPLC-grade water + 0.1% formic acid (MS Parameters from September 5, 2017: 123 transitions; 30 ms dwell time; 3.7 s cycle time), some peptides were removed from the transition list based on poor response (peak height or peak area) relative to other peptides. Only those peptides with the largest responses were retained on the transition list as specified in **Table 4** and **Table 5**.

Using the modified transition list and a 1.25 pmol/µL standard prepared in HPLC-grade water + 0.1% formic acid, three dwell times (20 ms, 50 ms, and 70 ms) were examined to assess the sensitivity of the signal to variation in dwell time. Based on the quality of the output data (peak height, peak shape, and points across a peak), the 50 ms dwell time was pursued for MDL experiments. The dwell time parameter was adjusted to 30 ms after further method development was prompted by failure of the first MDL set.

Calibration and System Suitability

Prior to each analytical run for IDL and MDL samples, the instrument was calibrated using a commercially available tuning solution (NAIRB) and a resolution check was performed. Instrument calibration ensures that the proper mass-to-charge ratios (m/z) have been assigned. The instrument is tuned in both MS1 and MS2 modes. Source and lens parameters are adjusted to optimize peak intensity and shape and the resolution and ion energy parameters are set for unit mass resolution on MS1 and MS2. This is performed by infusing a calibrant solution of NAIRB into the mass spectrometer and allowing the software to calibrate across the specified mass range (100-2000 m/z). Although triple quadrupole instruments are known to hold their calibrations for weeks to months, calibrations are performed or verified prior to each analysis sequence for sample sets. For infusion and optimization experiments, the instrument may or may not be calibrated prior to use each day. After instrument calibration, the mass accuracy (residuals) should be \pm 0.2 Da.

System suitability was determined by injecting a commercially available retention time synthetic peptide mixture (PierceTM Peptide Retention Time Calibration Mixture, Thermo Fisher Scientific) and a solvent spike (1.25 pmol/uL peptide mixture in HPLC-grade water + 0.1% formic acid) followed by solvent blank(s) (HPLC-grade water) before each sample set. Experimental samples were bracketed by injections of the retention time peptide mixture and the solvent spike to ensure the instrument functioned as anticipated and to track any loss of sensitivity or signal if observed during the course of the run. Checks for sensitivity, peak width, retention time, and carryover were performed qualitatively by inspection of the chromatograms of retention time peptide injections, solvent spike injections, and solvent blanks. While instrument sensitivity can vary day to day, no significant losses in instrument performance were observed during the course of IDL and MDL runs.

Table 4. List of SDC-9 Peptides Used in Optimization Experiments

Protein	Peptide	Precursor (m/z)	Observed (Y/N)	Retained (Y/N)
	SELEVISSLLSR	671.9	N	N
	SGSEIAFTGGLI K	644.4	Y	Y
	SWDWALGEIAN K	699.4	N	N
	AAGASDWEE K	536.2	Y	Y*
FdhA	ALGIVYLDSQA R	658.4	Y	Y
	VSSLQQLESPEEL R	812.9	Y	N
	LSWTYSTNPSAADVAK	859.9	Y	N
	NQAVSAPGEA K	540.3	Y	Y
	TDTNTDYSYVNAIK	806.9	Y	N
	VETWNHDVA R	412.9	N	N
	FDEWFGYSGPVNPEE R	969.9	N	N
	LLPWDLP K	495.3	Y	N
DasA	IATQIPLLQDAA R	710.4	Y	Y
PceA	LESGYVQNMV K	638.3	Y	Y
	VYTDLELAPDKP R	509.6	N	N
	DFWNNPEPI K	634.3	Y	Y
	TSPSLISSATVG K	628.4	Y	Y
	FLGADLVGIAPYDE R	823.4	Y	Y
	DVDDLLSAG K	520.8	Y	Y
TceA	VSSIIEPR	455.8	Y	Y
	VNNEPWWVTTR	706.4	Y	Y
	YFGASSVGAI K	554.3	Y	Y
	WGLYGPPHDSAPPDGSVP K	662.3	N	Y
	YFGAGDVGALNLADP K	808.4	N	Y
VcrA	VPDHAVPINF K	415.6	N	Y
VCIA	GVYEGPPDAPFTSWGN R	930.4	N	Y
	TGAAIHW K	446.2	N	N
	DQPWYV K	472.2	Y	Y
	LVNELTEFAK	582.3	N	Y
\mathbf{BSA}^{Ψ}	AEFVEVTK	461.7	N	Y
DOA	EYEATLEECCAK	751.8	N	Y
D 11 11 4	QTALVELLK	507.8	N	Y

Bolded letters represent isotopically labeled amino acids

^{*}Peptide was added to the transition list at a later date

⁴BSA peptides were added to the method based on discovery data run previously

Table 5. Selected Peptides Used in IDL/MDL Experiments

ID	Peptide	Precursor m/z	Product m/z	Ion	Charge
			1056.6	y10	+1
		644.4	927.6	y9	+1
FdhA2	SGSEIAFTGGLI K	644.4 [M+2H] ²⁺	814.5	y8	+1
		[141 211]	743.5	у7	+1
			1133.6	b12	+1
			929.4	y8	+1
	AAGASDWEE K	536.2	872.4	y7	+1
FdhA4		$[M+2H]^{2+}$	801.4	y6	+1
		[141 211]	714.3	y5	+1
			599.3	y4	+1
			1131.6	y10	+1
		658.4	961.5	y8	+1
FdhA5	ALGIVYLDSQAR	$[M+2H]^{2+}$	862.4	y7	+1
		[111 - 211]	699.4	у6	+1
			730.4	b7	+1
		540.3 [M+2H] ²⁺	837.5	у9	+1
	NQAVSAPGEA K		766.4	y8	+1
FdhA8			667.4	y7	+1
			580.3	y6	+1
			571.3	b6	+1
	IATQIPLLQDAA R	710.4 [M+2H] ²⁺	1235.7	y11	+1
			1134.7	y10	+1
PceA4			1006.6	y9	+1
			893.5	y8	+1
			796.5	y7	+1
		638.3 [M+2H] ²⁺	1162.6	y10	+1
			1033.5	у9	+1
PceA5	LESGYVQNMV K		946.5	у8	+1
			889.5	у7	+1
			726.4	y6	+1
		(2.1.2	1005.5	y8	+1
		634.3	819.4	y7	+1
PceA7	DFWNNPEPIK	$[M+2H]^{2+}$	705.4	у6	+1
			591.4	y5	+1
			677.3	b5	+1
			1067.6	y11	+1
		629.2	970.6	y10	+1
PceA8	TSPSLISSATVGK	628.3 [M+2H1 ²⁺	883.5	y9	+1
		$[M+2H]^{2+}$	770.4	y8	+1
			657.4	y7	+1
TceA1	FLGADLVGIAPYDE R	823.4	1385.7	y13	+1

		$[M+2H]^{2+}$	1029.5	у9	+1
		[141 · 211]	930.5	y8	+1
			873.4	y7	+1
			886.5	b9	+1
			826.4	y8	+1
	DVDDV 4 6 4 6 4 4	520.8	711.4	y7	+1
TceA2	DVDDLLSAG K	$[M+2H]^{2+}$	596.4	y6	+1
			483.3	y5	+1
			811.5	y7	+1
		455.0	724.4	y6	+1
TceA3	VSSIIEPR	455.8 [M+2H] ²⁺	637.4	у5	+1
		[M+2H]	524.3	y4	+1
			411.2	y3	+1
			1198.6	y9	+1
		706.4	1084.5	y8	+1
TceA4	VNNEPWWVTTR	706.4 [M+2H] ²⁺	955.5	y7	+1
			858.4	y6	+1
			1025.5	b8	+1
			944.5	y10	+1
	YFGASSVGAI K	554.3 [M+2H] ²⁺	797.5	у9	+1
TceA5			740.4	y8	+1
			669.4	y7	+1
			582.4	y6	+1
			899.9	y18	+2
	WGLYGPPHDSAPPDGSVP	662.3	871.4	y17	+2
VcrA1	K	$[M+3H]^{3+}$	814.9	y16	+2
	N.	[141 / 311]	733.4	y15	+2
			704.9	y14	+2
			1305.7	y14	+1
		808.4	1177.6	y12	+1
VcrA2	YFGAGDVGALNLADP K	$[M+2H]^{2+}$	1005.6	y10	+1
		L J	906.5	y9	+1
			1364.6	b14	+1
			725.4	6	+1
		415.6	626.4	5	+1
VcrA3	VPDHAVPINF K	$[M+3H]^{3+}$	573.3	10	+2
		. ,	467.3	8	+2
			520.3	5	+1
			1411.7	y13	+1
		930.4	1354.6	y12	+1
VcrA4	GVYEGPPDAPFTSWGN R	$[M+2H]^{2+}$	1254.6	y11	+1
		[1,1 - 211]	1045.5	y9	+1
**	DONNAMA	450.0	974.5	y8	+1
VcrA6	DQPWYV K	472.2	700.4	y5	+1

	$[M+2H]^{2+}$	603.3	y4	+1
		417.3	y3	+1
		811.4	у6	+1

Instrument Detection Limit (IDL)

To establish IDL, IS peptides were prepared as a mixture at 12.5 pmol/ μ L in DMSO/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. A mixed, concentrated solution (12.5 pmol/ μ L) was provided fresh to the analyst during each day of analysis. The analyst diluted the sample to 250 fmol/ μ L in in HPLC-grade water + 0.1% formic acid and serially diluted this solution three-fold to prepare the following concentrations: 83, 27, 9, 3, 1, 0.34, and 0.11 fmol/ μ L. The lowest measurable concentration for each peptide, defined as S/N \geq 3 (as measured by MassLynx) for the primary and secondary ion, represents the IDL for each peptide. Three trials were performed with the same dilution scheme; results generated are displayed in **Table 6** below.

Table 6. IDL Replicate Experiment Results

Protein	Peptide	IDL 1 (fmol/µL)	IDL 2 (fmol/μL)	IDL 3 (fmol/µL)
FdhA	SGSEIAFTGGLI K	83 ⁸	83	27
	AAGASDWEE K	>250	>250	>250
	ALGIVYLDSQA R	83	250	83
	NQAVSAPGEA K	27 [£]	27	27
	IATQIPLLQDAA R	83 ⁸	250 ⁸	83 ⁸
D 4	LESGYVQNMVK	250	250	250 ²
PceA	DFWNNPEPI K	>250	>250	>250
	TSPSLISSATVG K	27	27 [¥]	27 [£]
	FLGADLVGIAPYDE R	>250	>250	250
	DVDDLLSAG K	250	250 ^A	83
TceA	VSSIIEPR	9	33	9
	VNNEPWWVTT R	>250	>250	>250
	YFGASSVGAI K	250	>250	250
	WGLYGPPHDSAPPDGSVP K	>250	>250	>250
VcrA	YFGAGDVGALNLADP K	>250	>250	>250
	VPDHAVPINF K	>250	>250	>250
	GVYEGPPDAPFTSWGN R	>250	>250	>250
	DQPWYV K	>250	>250	>250

IDL units are fmol/μL

>250 fmol/µL denotes peptide was "not observed"

[¥]Primary and secondary ion pass at 3 fmol/μL with failure at 9 fmol/μL

^A Primary ion meets criteria at 3 fmol/μL

[£] Primary ion meets criteria at 9 fmol/µL

² Primary ion meets criteria at 27 fmol/µL

Secondary ion fails to meet criteria at 9 fmol/µL

Method Detection Limit (MDL)

To determine the MDL of peptide targets, IS peptides were prepared as a mixture at 12.5 pmol/ μ L in DMSO/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. A mixed, concentrated solution (12.5 pmol/ μ L) was provided fresh to the analyst during each day of analysis. The analyst diluted the sample to 1.25 fmol/ μ L in HPLC-grade water + 0.1% formic acid to use as a control during the analysis sequence. The 12.5 pmol/ μ L stock solution was diluted in ammonium bicarbonate to prepare the following concentrations (final in 25 μ L): 250, 83, 27, 9, 3, 1, 0.3, and 0.1 fmol/ μ L. Each sample was digested with trypsin overnight and desalted using C18 spin columns. The lowest measurable concentration for each peptide, defined as S/N \geq 3 (as measured by MassLynx) for the primary and secondary ion, represents the MDL for each peptide. Three trials were performed with the same dilution scheme; results generated are displayed in **Table 7**. An example total ion chromatogram displaying RDase peptides detected in the IS mix during the MDL study is shown in **Figure 2**.

Table 7. MDL Replicate Experiment Results

Protein	Peptide	MDL 1	MDL 2	MDL 3
	•	(fmol/ µL)	(fmol/ µL)	(fmol/ µL)
FdhA	SGSEIAFTGGLIK	27	83	83
	AAGASDWEE K	>250	>250	>250
	ALGIVYLDSQA R	3	83	83
	NQAVSAPGEA K	>250	>250	>250
PceA	IATQIPLLQDAA R	27^{F}	250^{G}	250
	LESGYVQNMVK	27^{D}	250^{C}	>250
	DFWNNPEPI K	27 ^D	250	250 ^C
	TSPSLISSATVG K	9	83	27
TceA	FLGADLVGIAPYDE R	27	250 ^C	250 ^C
	DVDDLLSAG K	1	83	250^{B}
	VSSIIEPR	3	27	83
	VNNEPWWVTT R	83	>250	>250
	YFGASSVGAI K	9	83 ^A	250^{G}
VcrA	WGLYGPPHDSAPPDGSVP K	83	>250	>250
	YFGAGDVGALNLADP K	83	>250	>250
	VPDHAVPINF K	3	9	9
	GVYEGPPDAPFTSWGN R	250	>250	>250
	DQPWYV K	27	27	27

MDL units are fmol/µL

>250 fmol/µL denotes peptide was "not observed"

A Primary and secondary ion pass at 3 fmol/μL with failure at 9 and 27 fmol/μL

B Primary and secondary ion pass at 9 fmol/μL with failure at 27 and 83 fmol/μL

^C Primary ion meets criteria at 83 fmol/μL

D Primary ion meets criteria at 9 fmol/μL

 $[^]F$ Primary ion meets criteria at 3 fmol/ μL

^G Primary ion meets criteria at 27 fmol/μL

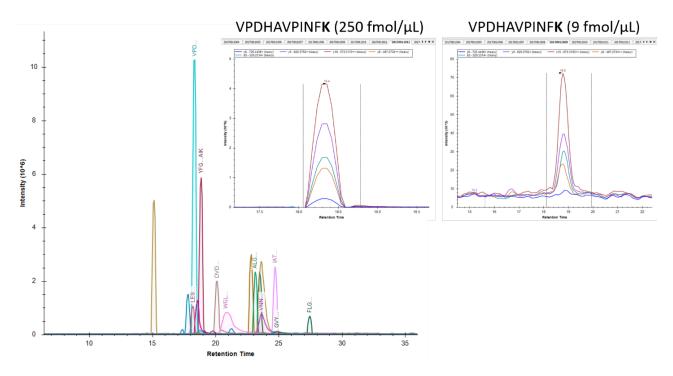


Figure 2. Representative Total Ion Chromatogram Displaying Detected RDase peptides; BSA peptides not shown as detection signals are significantly larger than for RDase peptides; the two panels to the right are more detailed chromatograms displaying the signal from each product ion for the VcrA peptide VPDHAVPINFK.

All BSA peptides were observed during MDL analysis. An example chromatogram of BSA peptides (10 µg of BSA was added prior to each MDL sample prior to digestion) is displayed in **Figure 3** (from the MDL dataset 9/18/2017).

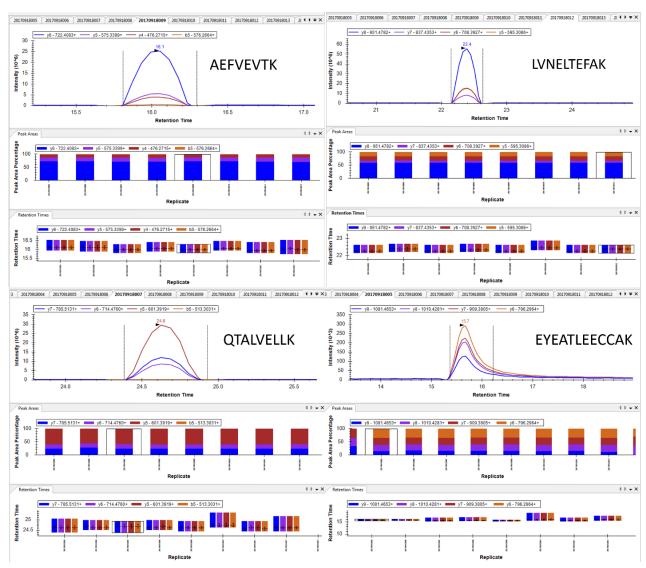


Figure 3. BSA Peptides Detected in MDL Samples

Two VcrA peptides (DQPWYVK and VPDHAVPINFK) were detectable in all MDL experiments while they were not detected in IDL experiments (**Figure 4**). The causes of performance variation between IDL and MDL experiments are likely due to matrix effects. Some peptides performed similarly between MDL replicates (e.g., DQPWYVK) while others did not. Within the MDL set, inconsistencies were observed for sensitive peptides (e.g., TSPSLISSATVGK, VSSIIEPR, YFGASSVGAIK). This variation represents the variation present in the preparatory methods and instrumental analysis; it is unlikely that instrumental variation resulted in decreased sensitivity as control samples of 1.25 pmol/uL mixed peptide prepared in HPLC-grade water + 0.1% formic acid and a Thermo Retention Time Peptide Mixture that bracketed samples did not reveal loss of chromatographic quality or loss in mass spectrometer signal during the course of the MDL runs.

For peptides that have poor secondary ion responses (e.g., YFGASSVGAIK), a more restrictive quality control scheme (that is, requiring two product ions to be present at $S/N \ge 3$) will result in higher detection limits. It is proposed that during sample analysis, the S/N of all analytes be calculated using MassLynx to satisfy the following acceptance criteria: those samples with $S/N \le 3$ would be categorized as not detected, and those that meet $S/N \ge 3$ would be accepted as true detections and would be reported with quantitative values.

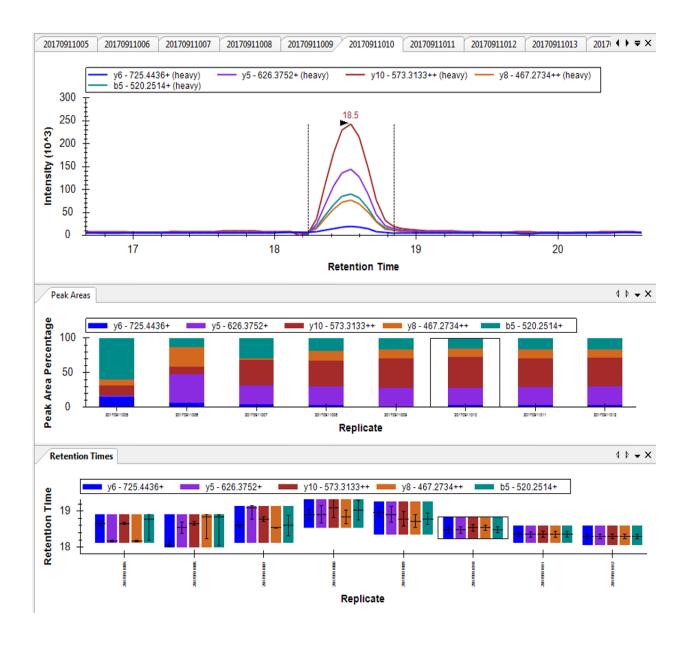


Figure 4. Representative detailed chromatogram, peak area contributions, and retention time variation for 0.11-250 fmol/μL VcrA peptide VPDHAVPINFK standard from MDL experiment 1 (9/11/2017).

Summary

In groundwater contaminated with chlorinated ethenes, the dominant and most productive biodegradation mechanism is typically reductive dechlorination. This is a process whereby the parent compound(s) PCE and/or TCE are sequentially dehalogenated to *cis*-1,2-dichloroethene (cis-DCE), vinyl chloride (VC) and finally ethene and/or ethane, which are considered environmentally benign. A number of different dehalogenating bacteria catalyze one or more steps throughout this process, with DHC being the only organismal group known to complete the entire pathway. In an effort to provide a robust and specific measurement that directly correlates to degradation rates, our team has developed a proteomics approach to quantify RDase proteins using a targeted MRM assay.

Prior to development of the MRM assay for targeted quantification, SDC-9 culture-specific RDase peptides were identified. Overall, a total of 28 peptide sequences, including those encoding for FdhA protein, were selected for quantification. During the initial steps of MRM assay development confirmation of instrument functionality and detection of peptide targets were performed. For that purpose, isotopically labeled peptide standards were injected into the mass spectrometer and their CE were optimized. Instrument and MDLs were subsequently established for each respective peptide.

In this study, CEs were optimized for initial 28 IS peptides to maximize the resulting signal from product ions. Following the optimization step, dwell time and solvent program were optimized and peptides that demonstrated poor signal response were discarded from the list. In total, 10 peptides were discarded from the list after optimization steps were completed and IDLs and MDLs were developed for the remaining peptides. Most peptides were observed in experimental samples during the IDL and MDL analysis, however VcrA peptides were observed exclusively in MDL experiments and not in IDL experiments (not observed is denoted as >250 fmol/µL), suggesting that sample digest and cleanup enhance the peptide signals for VcrA peptides. Two VcrA peptides (DQPWYVK and VPDHAVPINFK) were detectable in all MDL experiments while they were not detected in IDL experiments. Performance variation between IDL and MDL experiments are likely due to matrix effects. Some peptides performed similarly between MDL replicates (e.g., DQPWYVK) while others did not. Within the MDL set, inconsistencies were observed for sensitive peptides (e.g., TSPSLISSATVGK, VSSIIEPR, YFGASSVGAIK). This variation represents the variation present in the preparatory methods and instrumental analysis; it is unlikely that instrumental variation resulted in decreased sensitivity as control samples did not reveal loss of chromatographic quality or loss in mass spectrometer signal during the MDL runs.

Overall, the experiments performed during this study allowed for identification of the most sensitive RDase and FdhA peptides for targeted quantification. MDL experiments resulted in detection of up to three most sensitive peptides per protein with up to three most intensive transition ions per peptide. The data generated during the optimization and calibration experiments will be built into the MRM method used for quantification of native RDase peptides in microcosm experiment samples planned to be tested in the next phase of this project.

Re-Assessment of Instrument Detection Limit and Method Detection Limit for Reductive Dehalogenase Isotopic Peptides

REPORT

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Introduction

A suite of SDC-9 culture-specific reductive dehalogenase (RDase) peptides have been previously identified for quantification. These specific RDases are used in a multiple reaction monitoring-based (MRM) targeted proteomic assay to determine their quantities. During the initial development phase, the MRM assay was evaluated for empirical limits of detection (LOD) and limit of quantification (LOQ). While LOD and LOQ data were obtained during initial method development, the data was poorly reproducible among replicates (see Table 1 and 2 below) with some peptides not being observed even at the highest calibration level. Preparation and analysis of a second batch of samples was initiated to improve the reproducibility of the data. Changes to instrumental methods and updated tables are discussed below.

Table 1. Previously Determined Limits of Detection for RDase peptides

Protein	Peptide	IDL 1	IDL 2	IDL 3
FdhA	SGSEIAFTGGLI K	83 ⁸	83	27
	AAGASDWEE K	>250	>250	>250
	ALGIVYLDSQA R	83	250	83
	NQAVSAPGEA K	$27^{\mathfrak{t}}$	27	27
	IATQIPLLQDAA R	83 ⁸	250 ⁸	83 ⁸
PceA	LESGYVQNMV K	250	250	250 ⁸
rceA	DFWNNPEPI K	>250	>250	>250
	TSPSLISSATVGK	27	$27^{\text{\frac{4}{5}}}$	$27^{\mathfrak{t}}$
	FLGADLVGIAPYDE R	>250	>250	250
	DVDDLLSAG K	250	250⁴	83
TceA	VSSIIEP R	9	33	9
	VNNEPWWVTTR	>250	>250	>250
	YFGASSVGAI K	250	>250	250
	WGLYGPPHDSAPPDGSVP K	>250	>250	>250
VcrA	YFGAGDVGALNLADP K	>250	>250	>250
	VPDHAVPINF K	>250	>250	>250
	GVYEGPPDAPFTSWGN R	>250	>250	>250
	DQPWYV K	>250	>250	>250

Units are fmol/mL

Table 2. Previously Determined Limits of Quantification for RDase peptides

>250 fmol/ mL denotes peptide was "not observed"

⁴ Primary and secondary ion pass at 3 fmol/μL with failure at 9 fmol/mL

A Primary ion meets criteria at 3 fmol/mL

[£] Primary ion meets criteria at 9 fmol/mL

⁸ Primary ion meets criteria at 27 fmol/mL

Secondary ion fails to meet criteria at 9 fmol/mL

Protein	ID	Peptide	MDL 1	MDL 2	MDL 3	MDL 4	MDL 5
	FdhA2	SGSEIAFTGGLIK	27	83	83	83	250 ^{A,G}
FdhA	FdhA5	ALGIVYLDSQA R	3	83	83	83	27
	FdhA8	NQAVSAPGEAK	>250	>250	>250	>2250	>2250
	PceA4	IATQIPLLQDAA R	27^{F}	250^{G}	250	83^{G}	750^{G}
DesA	PceA5	LESGYVQNMV K	27^{D}	250 ^C	>250	250 [°]	750^{H}
PceA	PceA7	DFWNNPEPI K	27^{D}	250	250^{C}	83	250
	PceA8	TSPSLISSATVG K	9	83	27	27	27
	TceA1	FLGADLVGIAPYDE R	27	250^{C}	250^{C}	750^{H}	750^{H}
	TceA2	DVDDLLSAG K	1	83	250^{B}	83 ^D	83 ^G
TceA	TceA3	VSSIIEP R	3	27	83	9	9
	TceA4	VNNEPWWVTT R	83	>250	>250	750	1000^{I}
	TceA5	YFGASSVGAI K	9	83 ^A	250^{G}	83^{G}	83 ^F
	VcrA1	WGLYGPPHDSAPPDGSVP K	83	>250	>250	750	750
VcrA	VcrA2	YFGAGDVGALNLADP K	83	>250	>250	750^{H}	1000
	VcrA3	VPDHAVPINF K	3	9	9	27	27
	VcrA4	GVYEGPPDAPFTSWGN R	250	>250	>250	2250^{I}	2250
	VcrA6	DQPWYV K	27	27	27	250^{G}	750^{D}

Units are fmol/mL

>250 fmol/ µL denotes peptide was "not observed"

Calibration and System Suitability

Prior to sample analysis, the instrument was tuned in both MS1 and MS2 modes to maximize transmission of ions using a commercially available tuning solution (NAIRB). Using the instrument's automated tuning program, source and lens parameters were auto-adjusted to optimize peak intensity and shape, and the resolution and ion energy parameters were set for unit mass resolution on MS1 and MS2. A resolution check was also performed by the analyst to confirm the instrument met unit mass resolution. The instrument was also calibrated prior to each run using NAIRB. This was performed by infusing a calibrant solution of NAIRB into the mass spectrometer and allowing the software to auto-calibrate across the specified mass range (100-2000 m/z).

System suitability was determined by injecting a commercially available retention time synthetic peptide mixture (PierceTM Peptide Retention Time Calibration Mixture, Thermo Fisher Scientific) and a solvent spike (31 fmol/mL peptide mixture in HPLC-grade water + 0.1% formic acid) followed by solvent blank(s) (HPLC-grade water) before each sample set. Experimental samples were bracketed by injections of the retention time peptide mixture and the solvent spike to ensure the instrument functioned as anticipated and to track changes in sensitivity during the analytical run.

A Primary and secondary ion pass at 3 fmol/μL with failure at 9 and 27 fmol/mL

^B Primary and secondary ion pass at 9 fmol/μL with failure at 27 and 83 fmol/mL

^C Primary ion meets criteria at 83 fmol/mL

^D Primary ion meets criteria at 9 fmol/mL

F Primary ion meets criteria at 3 fmol/mL

^G Primary ion meets criteria at 27 fmol/mL

^HPrimary ion meets criteria at 250 fmol/mL

^IPrimary ion meets criteria at 750 fmol/mL

Multiple Reaction Monitoring (MRM) Method Updates

A new column was purchased for use on the program. It was observed that peptide FdhA8 displayed poor retention on the trap and analytical columns. The solvent program (Table 3) was modified by increased the percentage of starting acetonitrile (organic phase), which improved retention of peptide FdhA8.

Table 8. Solvent Program for M-Class Chromatographic System

Time (min)	Flow Rate (µL/min)	% A	% B*
-	10	95	1
5	10	95	1
65	10	35	65
66	10	10	90
70	10	10	90
80	10	95	1
85	10	95	1

^{*}Starting acetonitrile (organic phase) changed from 5% to 1%

The peptides monitored were updated to reflect most recent practices (Table 4).

Table 4. Mass Transitions Used for Updated IDL/MDL Experiments

ID	Peptide	Precursor m/z	Product m/z	Ion	Charge
FdhA2	SGSEIAFTGGLI K	644.4	814.5	y8	+1
FullAZ	SOSEIAI TOOLI K	[M+2H] ²⁺	743.5	y7	+1
FdhA5	ALGIVYLDSQA R	658.4	961.5	y8	+1
FullAS	ALGIV I LDSQA K	[M+2H] ²⁺	862.4	y7	+1
FdhA8	NQAVSAPGEA K	540.3	837.5	y9	+1
runao	NQAVSAI OLAK	[M+2H] ²⁺	667.4	y7	+1
PceA4	IATQIPLLQDAA R	710.4	1006.6	y9	+1
I CCA4	IATQII EEQDAA K	[M+2H] ²⁺	893.5	y8	+1
Dan A.F	LECCYMONMAN	638.3	1033.5	y9	+1
PceA5	LESGYVQNMV K [M+2H] ²⁺		726.4	у6	+1
PceA7	DFWNNPEPIK	634.3 [M+2H] ²⁺	819.4	y7	+1
rceA/			591.4	у5	+1
PceA8	TSPSLISSATVG K	628.3 [M+2H] ²⁺	770.4	y8	+1
1 00/10	151 5L155A1 VOK		657.4	y7	+1
TceA2	DVDDLLSAG K	520.8	826.4	y8	+1
100112	DVDDEESNOR	[M+2H] ²⁺	711.4	y7	+1
TceA3	VSSIIEPR	455.8	724.4	y6	+1
1 00/10	V SSHELL IX	[M+2H] ²⁺	524.3	y4	+1
TceA4	VNNEPWWVTTR	706.4	1198.6	у9	+1
1 00/14	VINIALI WWW TITE	[M+2H] ²⁺	955.5	y7	+1
TceA5	YFGASSVGAIK	554.3	797.5	у9	+1
10010	11 Griss v Griff	[M+2H] ²⁺	669.4	y7	+1
VcrA1	WGLYGPPHDSAPPDGSVP K	662.3	814.9	y16	+2
VUAI	W GET GITTIDOM T DOSVI K	$[M+3H]^{3+}$	733.4	y15	+2
VcrA2	YFGAGDVGALNLADP K	808.4	1177.6	y12	+1

		$[M+2H]^{2+}$	906.5	y9	+1
VcrA3	VPDHAVPINF K	415.6	573.3	y10	+2
	VEDHAVEINEK	$[M+3H]^{3+}$	626.4	y5	+1
VcrA4	GVYEGPPDAPFTSWGN R	930.4 [M+2H] ²⁺	1411.7	y13	+1
			974.5	y8	+1
VcrA6	DQPWYV K	472.2 [M+2H] ²⁺	700.4	y5	+1
			417.3	y3	+1

Bovine serum albumin (Table 5) was used to monitor digestion efficiency for MDL sample preparation. This was performed by spiking in $10 \mu g$ of BSA into ammonium bicarbonate alongside the IS peptides prior to digestion and C18 desalting.

Table 5. Mass Transitions Used to Monitor Digestion Efficiency

ID	Peptide	Precursor m/z	Product m/z	Ion	Charge
DCA1	LVNELTEFAK	582.3	951.5	y8	+1
BSA1	LVNELTEFAK	[M+2H] ²⁺	708.4	у6	+1
BSA2	AEFVEVTK R	461.7	722.4	у6	+1
DSAZ	ALT VEVIK K	$[M+2H]^{2+}$	575.3	у5	+1
DCA2	EYEATLEECCA K	751.8 [M+2H] ²⁺	909.4	у7	+1
BSA3			796.3	у6	+1
DC A 4	OTALVELLV	507.8	785.5	y7	+1
BSA4	QTALVELL K	$[M+2H]^{2+}$	604.4	у5	+1

Digestion Efficiency

Digestion efficiencies for each BSA peptide were calculated by taking the ratio between the peak areas for the peptide in the MDL standard (containing IS peptides and BSA) and the digestion control (reference containing BSA only) (Table 6).

Table 6. Mass Transitions Used to Monitor Digestion Efficiency

			Digest Efficiency (%)					
Protein ¹	ID	Peptide	MDL 1	MDL 2	MDL 3	Efficiency (%)	deviation (%)	(%)
	BSA1	LVNELTEFAK	111	112	ND	111.5	0.7	0.6
DCA1	BSA2	AEFVEVTK	112	116	119	115.7	3.5	3.0
BSA1	BSA3	EYEATLEECCAK	ND	161	157	159.0	2.8	1.8
	BSA4	QTALVELLK	113	108	ND	110.5	3.5	3.2

¹ Level of BSA spiked was 10 μg

Depending on the peptide, the digestion efficiency ranged from 111-159%. While BSA peptides 3 and 4 are diagnostic of efficiency, however these peptides should not be used as they are prone to cyclizing because of their N-terminal glutamine and glutamate amino acids. The replicates (n = 7 standards) were

ND Peptide was not detected in the reference digest sample; calculation of efficiency could not be calculated RSD (relative standard deviation)

precise with relative standard deviations $\leq 3\%$. While this data indicates that the digestion efficiency is well controlled, it is recommended that the level of BSA spiked be reduced by half to mitigate any detector saturation that could occur. While no saturation was observed in these sample sets, the intensities of BSA peptide in the samples (>10e6) are close to the level of detector saturation (10e7 - 10e8).

Instrument Detection Limit (IDL)

Isotopically labeled peptides were prepared as a mixture at 12.5 pmol/µL in DMSO/Milli-Q water (50/50), aliquoted, and frozen at -80°C until use. Mixed IS peptide solution (12.5 pmol/μL) was provided fresh to the analyst during each day of analysis. The analyst diluted the sample to 250 fmol/µL in in HPLC-grade water + 0.1% formic acid and serially diluted this solution 3-fold in water + 0.1% formic acid to prepare the following concentrations: 83, 27, 9, 3, 1, and 0.34 fmol/µL. The lowest measurable concentration for each peptide, defined as signal-to-noise $(S/N) \ge 3$ (as measured by MassLynx) for the primary and secondary ion, was assigned as the IDL for each peptide. Three trials were performed with the same dilution scheme; results generated are displayed in Table 7 below.

Table 7. IDL Replicate Experiment Results

Protein	ID	Peptide	IDL 1	IDL 2	IDL 3	Revised Average IDL		
	FdhA2	SGSEIAFTGGLI K	0.3	0.3	0.3	0.3		
FdhA	FdhA5	ALGIVYLDSQA R	0.3	0.3	0.3	0.3		
	FdhA8	NQAVSAPGEA K	9	1	1	9		
	PceA4	IATQIPLLQDAA R	3	1	0.3	3		
PceA	PceA5	LESGYVQNMV K	1	0.3	0.3	1		
rceA	PceA7	DFWNNPEPI K	3	1	0.3	3		
	PceA8	TSPSLISSATVGK	0.3	0.3	0.3	0.3		
	TceA2	DVDDLLSAG K	0.3	0.3	0.3	0.3		
TceA	TceA3	VSSIIEP R	0.3	0.3	0.3	0.3		
TCEA	TceA4	VNNEPWWVTTR	9	3	3	9		
	TceA5	YFGASSVGAI K	0.3	0.3	0.3	0.3		
	VcrA1	WGLYGPPHDSAPPDGSVP K	27	83	27	83		
	VcrA2	YFGAGDVGALNLADP K	27	9	3	27		
VcrA	VcrA3	VPDHAVPINF K	0.3	0.3	0.3	0.3		
	VcrA4	GVYEGPPDAPFTSWGN R	83	83	27	83		
	VcrA6	DQPWYV K	1	0.3	0.3	1		
Units are fr	Units are fmol/mL of sample							

The revised IDL was assigned as the largest IDL observed between the three replicate measurements. The revised IDL represents the lowest standard in diluent (water + 0.1% formic acid) that was observed on the instrument with signal to noise ≥ 3 .

Method Detection Limit (MDL)

To determine the MDL of peptide targets, the 12.5 pmol/µL stock solution of IS peptides was diluted in ammonium bicarbonate to prepare the following final concentrations: 250, 83, 27, 9, 3, 1, and 0.3 fmol/μL. Each sample was digested with trypsin overnight and desalted using C18 spin columns. This procedure mimics the matrix that is used during digestion and cleanup of field samples. The lowest measurable concentration for each peptide, defined as S/N ≥ 3 (as measured by MassLynx) for the primary and

¹ Bolded letters denote heavy ¹³C and ¹⁵N labeled amino acid

secondary ion, was assigned as the MDL for each peptide. Three trials were performed with the same dilution scheme; results generated are displayed in Table 8.

Table 8. MDL Replicate Experiment Results

Protein	ID	Peptide ¹	MDL 1	MDL 2	MDL 3	Revised Average MDL		
	FdhA2	SGSEIAFTGGLI K	3	3	3	3		
FdhA	FdhA5	ALGIVYLDSQA R	3	3	1	3		
	FdhA8	NQAVSAPGEA K	3	3	3	3		
	PceA4	IATQIPLLQDAA R	9	9	9	9		
D 4	PceA5	LESGYVQNMV K	3	3	3	3		
PceA	PceA7	DFWNNPEPI K	1	1	1	1		
	PceA8	TSPSLISSATVGK	0.3	0.3	1	1		
	TceA2	DVDDLLSAG K	0.3	3	3	3		
TD A	TceA3	VSSIIEPR	0.3	0.3	1	1		
TceA	TceA4	VNNEPWWVTT R	9	9	9	9		
	TceA5	YFGASSVGAI K	0.3	0.3	1	1		
	VcrA1	WGLYGPPHDSAPPDGSVP K	9	9	3	9		
	VcrA2	YFGAGDVGALNLADP K	27	27	27	27		
VcrA	VcrA3	VPDHAVPINF K	0.3	0.3	1	1		
	VcrA4	GVYEGPPDAPFTSWGN R	83	27	27	83		
	VcrA6	DQPWYV K	1	1	1	1		
Units are	Units are fmol/mL of extract							

¹ Bolded letters denote heavy ¹³C and ¹⁵N labeled amino acid

The revised MDL was assigned as the largest MDL observed between the three replicate measurements. The revised MDL represents the lowest standard in matrix (ammonium bicarbonate with subsequent C18 desalting) that was observed on the instrument with signal to noise ≥ 3 .

Comparison of Revised IDL and MDL Levels

The Battelle proteomics team expected the measured MDL to be larger than the measured IDL for two reasons: (1) matrix effects can result in suppression or enhancement of an analyte's response and, (2) sample handling and cleanup can result in signal losses. Note that for the majority of peptides, this expectation holds true: the measured MDL > measured IDL. For 3 peptides (FdhA8, PceA7, VcrA1, highlighted in red in Table 9), the relationship was reversed: measured MDL < measured IDL. This indicates that the analyte's response enhanced by addition of matrix (ammonium bicarbonate with C18 cleanup). For 4 peptides (TceA4, VcrA2, VcrA4, and VcrA6), the measured MDL = measured IDL. This suggests that the peptide response was not affected by the addition of matrix (ammonium bicarbonate with C18 cleanup).

While the measured IDL is useful for method development, the measured MDL is the more important in quantitative experiments as it represents the minimum quantifiable value for a given method. For subsequent quantitative experiments with microcosm samples, the MDL for each peptide will be used as the minimum quantifiable level. Samples with quantified peptide levels that are below the MDL are not reportable values but can be important for diagnostic purposes and for subsequent method development (Table 9).

Table 9. Comparison of Experimental Instrument and Method Detection Limits

Protein	ID	Peptide ¹	Experimental IDL	Experimental MDL		
	FdhA2	SGSEIAFTGGLIK	0.3	3		
FdhA	FdhA5	ALGIVYLDSQA R	0.3	3		
	FdhA8	NQAVSAPGEAK	9	3		
	PceA4	IATQIPLLQDAA R	3	9		
DasA	PceA5	LESGYVQNMV K	1	3		
PceA	PceA7	DFWNNPEPI K	3	1		
	PceA8	TSPSLISSATVG K	0.3	1		
	TceA2	DVDDLLSAG K	0.3	3		
Took	TceA3	VSSIIEP R	0.3	1		
TceA	TceA4	VNNEPWWVTTR	9	9		
	TceA5	YFGASSVGAI K	0.3	1		
	VcrA1	WGLYGPPHDSAPPDGSVP K	83	9		
	VcrA2	YFGAGDVGALNLADP K	27	27		
VcrA	VcrA3	VPDHAVPINF K	0.3	1		
	VcrA4	GVYEGPPDAPFTSWGN R	83	83		
	VcrA6	DQPWYV K	1	1		
Units are fmol/mL of extract						

¹ Bolded letters denote heavy ¹³C and ¹⁵N labeled amino acid

Comparison of Theoretical Detection Limits with IDL/MDL Results

A theoretical IDL for each peptide was also determined with existing IDL data (Table 10). Calculated (theoretical) IDL values are typically the IDL levels reported for publication. The theoretical IDLs were determined by measuring signal to noise for the secondary ions for each peptide at a concentration yielding signal to noise between 10 and 20. The proportionality between concentration and signal to noise was used to calculate the concentration of peptide that would theoretically yield a signal to noise = 3. This calculation assumes a linear relationship between concentration and signal to noise.

Table 10. Comparison of Detection Limits

Protein	ID	Peptide ¹	Theoretical IDL	Experimental IDL	Experimental MDL			
	FdhA2	SGSEIAFTGGLIK	0.08	0.3	3			
FdhA	FdhA5	ALGIVYLDSQA R	0.02	0.3	3			
	FdhA8	NQAVSAPGEAK	0.84	9	3			
	PceA4	IATQIPLLQDAA R	0.58	3	9			
DooA	PceA5	LESGYVQNMVK	0.59	1	3			
PceA	PceA7	DFWNNPEPI K	0.21	3	1			
	PceA8	TSPSLISSATVG K	0.09	0.3	1			
	TceA2	DVDDLLSAG K	0.04	0.3	3			
Took	TceA3	VSSIIEP R	0.02	0.3	1			
TceA	TceA4	VNNEPWWVTTR	2.17	9	9			
	TceA5	YFGASSVGAI K	0.03	0.3	1			
	VcrA1	WGLYGPPHDSAPPDGSVP K	19.15	83	9			
	VcrA2	YFGAGDVGALNLADP K	9.68	27	27			
VcrA	VcrA3	VPDHAVPINF K	0.33	0.3	1			
	VcrA4	GVYEGPPDAPFTSWGN R	28.89	83	83			
	VcrA6	DQPWYV K	0.25	1	1			
	Units are fmol/mL of extract 1 Bolded letters denote heavy ¹³ C and ¹⁵ N labeled amino acid							

¹ Bolded letters denote heavy ¹³C and ¹⁵N labeled amino acid

APPENDIX B VALIDATION STUDY OF QPROT ASSAY QUANTITATION LIMITS

Determination of RDase Peptide Concentrations in the Validation Study

REPORT

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List of Acronyms

CEs collision energies

DHC Dehalococcoides

IDL instrument detection limit

IS internal standards

LOD limit of detection
LOQ limit of quantification

MDL method detection limit

MRM multiple reaction monitoring-based

PCE tetrachloroethene

RDase reductive dehalogenase

VC vinyl chloride

Introduction

In preparation for microcosm experiments planned for this project, the Battelle proteomics team, in collaboration with the University of Tennessee, has identified SDC-9 culture-specific reductive dehalogenase (RDase) peptides for quantification. These specific RDases are used in a multiple reaction monitoring-based (MRM) targeted proteomic assay to establish quantitative biomarker rate correlations, which are needed to generate in situ degradation rate estimates of chlorinated ethenes.

After the development of the MRM assay and determination of instrument detection limit (IDL) and method detection limit (MDL) for each RDase, dilution study was performed. The dilution study severed to identify the lowest concentration of DHC cells that generate detectable and quantifiable concentrations of reductive dehalogenases (RDases) selected for quantification.

To maximize precision, stable isotope labeled internal standards (IS) were used to account for errors and losses that can occur during sample handling and variability in peptide ionization in the analysis of peptides. Because of the rigors of establishing these assays and successfully performing them in complex matrices, they tend to be implemented on only a selected number of analytes in parallel. With only a small number of analytes measured, it is common to expend considerable time optimizing tune parameters and collision energies (CEs) of each analyte individually to attain the highest sensitivity possible.

This report discusses set up of the dilution study and generated results. Data pertaining to system resolution check, calibration, and chromatograms of peptide detections are grouped per sample set in **Appendices C** and **D**.

Validation Study Setup, Protein Extraction and Quantification

Validation Study Set up

SDC-9 dechlorinating consortium was shipped to Battelle from Aptim in September 2017. The culture was aliquoted into 50 mL tubes and kept frozen in -80 °C until use. For the purposed of the dilution study, one 50 mL tube was shipped to the University of Tennessee for determination of DHC cell concentration and RDase genes (**Table 1**). Another tube was used for proteomic analysis. Starting SDC-9 culture of 2 x 10⁷ DHC cells was diluted in triplicate to concentrations: 10⁴, 10⁵, 10⁶ and 10⁷, and subject to protein extraction, tryptic digestion and quantification.

Table 1. Gene specific qPCR analysis of SDC-9 sample

Sample ID:	SDC-9 Culture
Analysis: qPCR	
Volume of sample filtered (mL)	30
Isolated DNA concentration (ng/μL)	67.5
Volume of purified DNA (μL)	50
qPCR Assays (gene copies/mL)	
General Bacteria 16S rRNA gene	3.18E+8
Dehalococcoides 168 rRNA gene	2.26E+7
Dehalococcoides vcrA gene (cDCE→Ethene)	2.70E+7
Dehalococcoides bvcA gene (cDCE→Ethene)	ND
Dehalococcoides tceA gene (TCE→VC)	2.23E+7

RDase Isotopic Peptides

Dehalococcoides (DHC) comprise a genus-level group of bacteria within the phylum *Chloroflexi*, notable for their ability to respire halogenated compounds including recalcitrant groundwater contaminants. Their obligate use of halogenated organic compounds as an energy source has allowed successful development of DHC-containing enrichment cultures for bioaugmentation of chlorinated ethene-contaminated sites, such as SDC-9 consortium. Each DHC strain contains a unique complement of genes that are homologs of known RDases, the genes required for respiration of halogenated organic compounds. The SDC-9 consortium is a well-defined enrichment culture with a variety of robust tetrachloroethene (PCE) — vinyl chloride (VC) dechlorinators. A metagenome sequencing project for the SDC-9 consortium was completed by Battelle Memorial Institute and the University of Tennessee to determine specific RDase genes that could serve as targets in proteomic analyses.

Overall, 14 genes encoding RDases were identified, 10 of which best matched previously identified RDases from members of the genus *Dehalococcoides*. Of these 14 sequences, one *vcrA* and one *tceA* gene were identified, as well as two separate *pceA* genes. Peptide sequences of RDases and several sequences of FdhA protein that encodes for formate dehydrogenase were selected for targeted proteomic analysis. The detailed list of peptide sequences is presented in **Table 2**.

Table 2. RDase peptides used in MRM assay

Protein	Peptide ID	Peptide	MDL 1 (fmol/ μL)	MDL 2 (fmol/ μL)	MDL 3 (fmol/ µL)
	FdhA2	SGSEIAFTGGLIK	27	83	83
FdhA	FdhA5	ALGIVYLDSQA R	3	83	83
	FdhA8	NQAVSAPGEA K	>250	>250	>250
	PceA4	IATQIPLLQDAA R	27^{F}	250^{G}	250
DesA	PceA5	LESGYVQNMVK	27 ^D	250 ^C	>250
PceA	PceA7	DFWNNPEPI K	27 ^D	250	250 ^C
	PceA8	TSPSLISSATVG K	9	83	27
	TceA2	DVDDLLSAG K	1	83	250^{B}
	TceA3	VSSIIEPR	3	27	83
TceA	TceA4	VNNEPWWVTT R	83	>250	>250
ICCA	TceA5	YFGASSVGAI K	9	83 ^A	250^{G}
	VcrA1	WGLYGPPHDSAPPDGSVPK	83	>250	>250
	VcrA2	YFGAGDVGALNLADP K	83	>250	>250
VcrA	VcrA3	VPDHAVPINF K	3	9	9
	VcrA4	GVYEGPPDAPFTSWGN R	250	>250	>250
	VcrA6	DQPWYV K	27	27	27
MDL units are fmol/μL >250 fmol/μL denotes peptide was "not observed" A Primary and secondary ion pass at 3 fmol/μL with failure at 9 and 27 fmol/μL B Primary and secondary ion pass at 9 fmol/μL with failure at 27 and 83 fmol/μL C Primary ion meets criteria at 83 fmol/μL D Primary ion meets criteria at 9 fmol/μL F Primary ion meets criteria at 3 fmol/μL G Primary ion meets criteria at 27 fmol/μL					

Protein Extraction and Quantification

For protein quantification, each sample was digested with trypsin and desalted using C18 spin columns. Protein extraction protocol, including protein detection with tryptophan assay and sample cleanup, has been developed for work with environmental samples and is located in **Appendix A** of this report.

Protein Concentrations in Dilution Study Samples

On average, 1 mg/mL protein was extracted in each sample. Peptide concentration data is tabulated in Excel file entitled "USACE ESTCP Dilution Study 11.15.17" attached to this report.

Only two out of three FdhA peptides, namely FdhA2 and FdhA 5, were observed in the dilution study. The FdhA8 peptide was not detected. FdhA 2 and FdhA 5 peptides were detected above method detection limit in **all** SDC-9 cell dilutions (10⁴ to 10⁷ DHC cells). However, their per cell concentrations were higher than reported in the literature. **Figure 1A** shows FdhA peptide concentrations per total cell number.

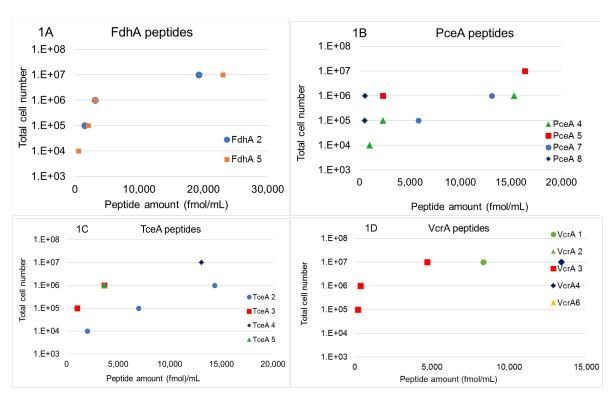


Figure 1. Concentration of (A) FdhA, (B) PceA, (C) TceA and (D) VcrA peptide in Dilution Study Samples

Figure 1B shows PceA peptide concentrations per total cell number. PceA5, PceA7 and PceA8 showed lower sensitivity than PceA4 peptide which was detected in 10⁴ starting DHC cells. The lowest concentration of DHC cells that generated detectable and quantifiable concentrations of PceA5 and PceA7 was 10⁵, while 10⁶ cells was needed to quantify PceA8 peptide. Thus, PceA4 peptide will be selected as a quantifier for the proceeding studies.

The TceA2 peptide, had highest sensitivity and was detected and quantified in samples containing 10⁴ DHC cells (**Figure 1C**). This peptide will be selected as a quantifier for the proceeding studies. TceA3 peptide was detected in 10⁵ DHC cell concentration and TceA4 and TceA5 were the least sensitive. These two peptides required 10⁶ and 10⁷ starting cell concentrations for quantification.

VcrA peptides were the least sensitive, with only VcrA3 peptide detected in 10⁵ and 10⁶ DHC cells (**Figure 1D**). The other two peptides, VcrA1 and VcrA4, were detected within their corresponding MDLs in 10⁷ DHC cell concentration.

The analysis of the compiled data shows that the lowest concentration of DHC cells for quantification of FdhA, PceA and TceA peptides is 10⁵. However, quantification of VcrA peptides is possible when DHC concentrations of 10⁶ or 10⁷ are used.

Summary

Dilution study was set up to establish the minimum concentration of DHC cells required to detect FdhA, PceA, TceA and VcrA peptides using a targeted MRM assay. Prior to sample analysis, SDC-9 culture-specific RDase peptides were down selected and IDL and MDL were established for each peptide.

In this study, sample of SDC-9 culture was diluted in triplicate to 10⁷, 10⁶, 10⁵, 10⁴ DHC cells and concentration of RDase and FdhA protein was analyzed using MRM proteomics.

Overall, the most sensitive peptides for quantification were FdhA5, PceA4, TceA2 and VcrA3 and required between 10⁴ and 10⁵ DHC cells to be detected. These peptides will serve as quantifiers in the next set of experiments. The required lowest concentration of DHC cells for detection of the reminder of peptides varied per protein, for example, to detect other TceA peptides a minimum of 10⁵ cells need to be provided, but to detect VcrA specific peptides the cell concentrations need to be couple orders of magnitude higher. Thus, the total recommended DHC concentration for targeted proteomics is 10⁶ cells, regardless of sample volume.

APPENDIX C METHOD STANDARD OPERATING PROCEDURES AND CALIBRATION OF ANALYTICAL EQUIPMENT

Primer and probe design

The amino acid sequences of RDases determined in SDC-9TM metagenomic analysis were compared to other published sequences in NCBI using BLASTP (http://www.ncbi.nlm.nih.gov/BLAST), and sequences were aligned with Geneious R11.0.2 (http://www.geneious.com, Kearse et al., 2012). The specificity of primers and probes targeting regions of the target genes met the criteria of the Geneious R11.0.2 and the specificity of the primers and probes was also verified using BLAST analysis.

To validate each assay, primers were tested first with SYBR Green chemistry using QuantStudio 12K Flex Real-Time PCR System (Life Technologies, Grand Island, NY). The 10 μ L qPCR mixture was composed of 5 μ L of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 300 nM of each primer, 2.0 μ L of template DNA and the remaining volume sterile nuclease-free water. The PCR cycle parameters applied were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. After amplification, a melting curve analysis was carried out to confirm that the signal obtained in SYBR Green qPCR originated from specific target PCR products, not from primer dimers or non-specific amplifications. SYBR-Green qPCR assay criteria were used to validate each assay (i.e., the efficiency of the reaction should be 90-110%; the slope of standard curve should be between -3.1 to -3.6, and R² should >0.99, respectively). Amplification efficiencies were calculated by the method of Pfaffl (2001).

Following validation and optimization of each assay with SYBR Green qPCR, the primers and probe specific to each target assay were used for TaqMan qPCR. Each 10 μ L mixture contained 5 μ L of TaqMan Universal PCR Master Mix No AmpErase UNG (Applied Biosystems, Foster City, CA), 300 nM of each primer, 300 nM of probe and 2.0 μ L of template DNA. TaqMan qPCR assays were run using QuantStudio 12K Flex Real-Time PCR System under the same PCR cycle conditions as described for SYBR Green qPCR. The target assays had amplification efficiencies between 90-110%, a standard curve slope between -3.3 to -3.6 and a standard curve with R²> 0.99. These values met the parameters suggested in literature (Holmes et al., 2006; Karlen et al., 2007; Ritalahti et al., 2009). The default instrument settings were used and LOD and LOQ values were determined as 1-10 and 10-50 copies per μ L for each assay.

Standard curve preparation: Plasmid DNA was served as templates for standard curve preparation. Template plasmid DNA (pDNA) was synthesized utilizing the pMK-RQ vector and incorporated into *E. coli* by Life Technologies (Grand Island, NY) or the target gene fragment was inserted into the pCRTM□2.1 Vector using the Invitrogen TA CloningTM□ kit (Life Technologies, Grand Island, NY) according to maufacturer's instructions. The *E. coli* transformant was grown in Luria Broth with ampicillin (100 mg/L) or kanamycin (50 mg/L) at 37°C overnight. pDNA was isolated using the Zymo Research ZyppyTM Plasmid Miniprep Kit (Zymo Research Corp., Irvine, CA) and quantified using a NanoDrop and the Qubit 2.0 Fluorometer.

Standard curves were included with every qPCR plate using 10-fold serial dilutions of plasmid DNA over a 7 orders of magnitude range beginning at a 1 ng μ L⁻¹ concentration (~8 log gene copies) and decreasing to 10⁻⁷ ng μ L⁻¹. All standard curves had a total of eight calibration points

and were run in triplicate. To calculate the number of gene copies in a known amount of DNA and gene copies per sample, the equations given in Ritalahti et al. (2006) were applied.

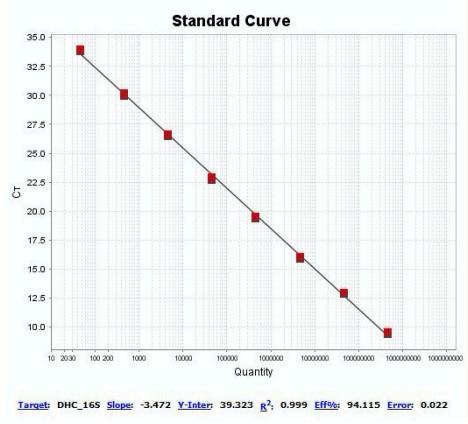


Figure 1. Example of standard curve for DHC_16S assay

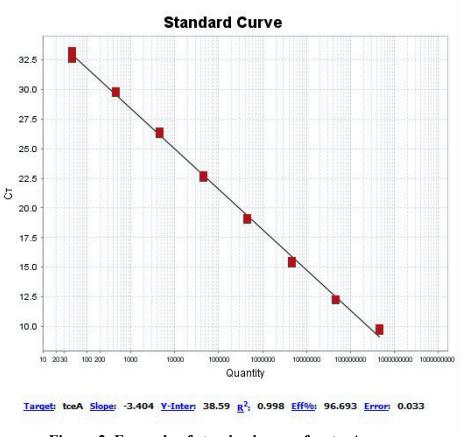


Figure 2. Example of standard curve for tceA assay

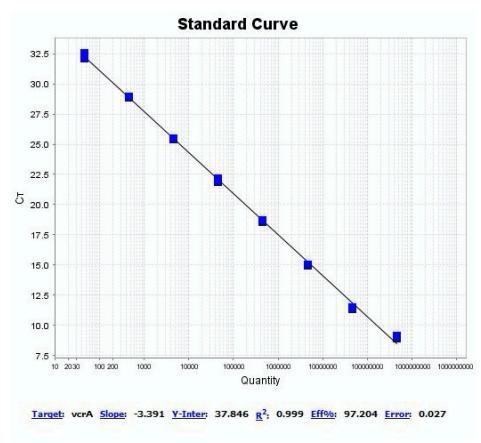


Figure 3. Example of standard curve for *vcrA* assay

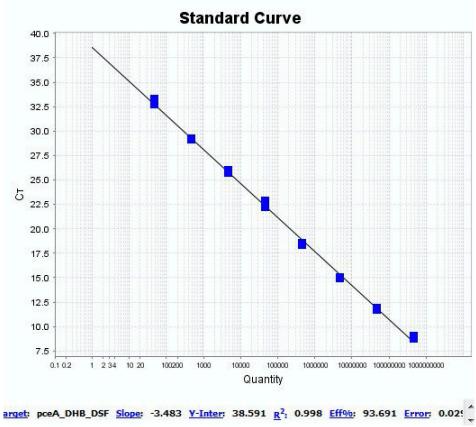
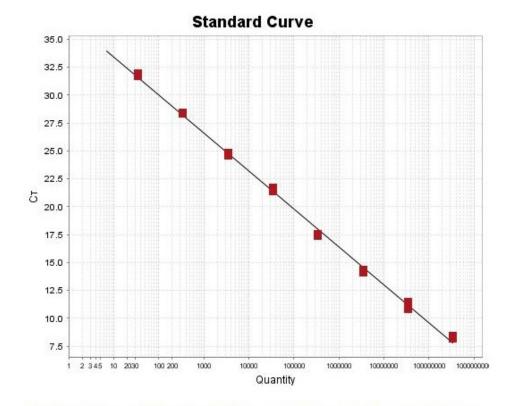


Figure 4. Example of standard curve for SDC9_24 pceA assay



<u>Target</u>: fdhA_DHC_All <u>Slope</u>: -3.403 <u>Y-Inter</u>: 36.858 <u>R</u>²; 0.998 <u>Eff%</u>: 96.721 <u>Error</u>: 0.03

Figure 5. Example of standard curve for fdhA assay

SHOTGUN AND TARGETED PROTEOMICS

The procedure described below has been developed and used by the Battelle Memorial Proteomics lab and is protected under the provisional patent.

Initial Discovery - Shotgun (bottom-up) Proteomics

- 1. Samples are analyzed by reverse-phase microflow HPLC-ESI-MS/MS using an Eksigent Nano 415 liquid chromatograph system (Sciex, Concord, CAN) which is directly connected to a quadrupole time-of-flight (QqTOF) TripleTOF 5600 mass spectrometer (Sciex, Concord, CAN).
- 2. The instrumentation is controlled using Analyst TF 1.6 and the Eksigent control software.
- 3. A total of 100 μL of sample is injected onto the analytical column (Eksigent 3C18-CL-120, 3 μm particle size, 120 Å pore size, 0.3 x 150 mm) using a trap-and-elute method. In order to achieve 100 μL on column, 10 full loop volumes (10 μL loop) are injected and trapped onto a Pepmap 300 cartridge (C18, 5 μm particle size, 0.3 x 5 mm, Thermo Scientific, Rockwood, TN). Each loop injection is washed with mobile phase A.
- 4. Once fully loaded, the samples are eluted from the trap and separated. All solvent concentration changes are linear with respect to time. Mobile phase solutions are purchased from Burdick and Jackson and are as follows: mobile phase A: 0.1% formic acid (v/v) in water (LC-MS grade); and mobile phase B: 0.1% formic acid (v/v) in acetonitrile (LC-MS grade).
- 5. Continuing mass calibration of the TOF MS and TOF MS/MS is performed throughout the analysis sequence by analyzing a digested beta-galactosidase standard (Sciex, Concord, CAN). Mass spectrometric analysis is performed using data dependent acquisition (referred to as information dependent acquisitions, or IDA). Full scan spectra are acquired from 400 to 1250 m/z with a 250 millisecond acquisition time.
- 6. For collision induced dissociation tandem mass spectrometry (CID MS/MS) in IDA mode, the mass window for precursor ion selection of the quadrupole mass analyzer is set to unit resolution (± 0.5 m/z). For MS/MS analysis, precursor ions were fragmented in a collision cell using nitrogen as the collision gas. For IDA analysis, the instrument is set to trigger product ion scans (from 100 1500 m/z) only after specific criteria are met by the precursor ions. The Rolling Collision Energy algorithm is used to determine the appropriate collision energy for each precursor mass.

Additional Discovery plus Select Targets - Shotgun (bottom-up) Proteomics

Based on the results of the initial shotgun analysis, specific targets are identified for further investigation. These targets are divided into two groups and samples are analyzed in two separate analyses. The instrument is set up with an inclusion list of these specific targets that would automatically trigger a product ion scan if the specified precursor was detected. The instrumentation, mobile phase, LC method, and injection volume were the same as the initial discovery analysis.

Labeled Peptide Analysis

- 1. Isotopically labeled peptides are purchased from Thermo Fisher Scientific based on the results of the targeted analysis.
- 2. Individual stocks of each peptide are prepared in 0.1% formic acid. From these stocks, multiple mixed solutions are prepared at 0.1, 1.0, 10, 100, and 1000 Xmol/μL in order to determine the approximate detection limit of each peptide and also the instrumental linearity.
- 3. Two test samples that were previously analyzed are split and spiked at a final on-column concentration of 200 fmol. Using these samples, 90 minute is udes for all further analysis. All instrumentation, mobile phase, and injection volumes were the same as the initial discovery analysis

Shotgun with Peptide Targets

Test samples are split and spiked with each labeled peptide. These samples are analyzed using the 90 minute gradient for specific CVOC targets plus any additional discovery data that may have been missed on the first initial analyses. The instrumentation, mobile phase, LC method, and injection volume were the same as the labeled peptide analysis.

MRMHR for MTBE Quantification

- 1. A mass spectrometric method will be built to perform product ion scans of the specific targets plus associated labeled peptides that were previously purchased. The analysis is performed using a 15 minute gradient, with a total runtime of 16 minutes, including mobile phase equilibration. The samples will be spiked with both labeled peptides for a final concentration of X mol on column. The instrumentation, mobile phase, and LC method are the same as the labeled peptide analysis.
- 2. Integrated reconstructed ion chromatograms of precursor-product ion transitions of both the labeled and un-labeled peptide targets are produced using MultiQuant software version 2.1 (Sciex, Concord, CAN). Multiple precursor-product ion transitions are plotted to add confidence to the tentative target detections.

3.	Using the response of each labeled peptide and its spiked concentration, a response factor is calculated. These calculated response factors are used for quantification of the unlabeled peptide targets.

CALLIBRATION OF AB SCIEX TripleTOF® 5600/5600+ INSTRUMENT

For tuning the system, use the following solutions that come with the installation kit:

For positive mode:

- For optimizing TOF MS MSMS high resolution or MSMS High Sensitivity, use the Tuning Solution.
- For Q1calibration, use the PPG POS solution.

In negative mode:

- For optimizing TOF MS MSMS High Resolution or MSMS High Sensitivity, use Taurocholic acid.
- For Q1calibration, use the PPG 3000 solution.

Required material

- Tuning solutions that are supplied in the Standards Chemical Kit shipped with the system. If needed, a new Kit can be ordered from AB SCIEX.
- Gas-tight syringes (1.0 ml is recommended)
- PEEK (red) sample tubing

Prerequisites

- Make sure that a printer is configured.
- Make sure that the spray is stable and that the proper tuning solution is being used.

Optimize the Instrument

The following procedure shows how to verify the performance of the instrument.

- 1. In the Navigation bar, under Tune and Calibrate, double-click Manual Tuning.
- 2. Run a TOF MS or Product ion scan type and confirm that there is a stable TIC and that the peaks of interest are present in the spectrum.
- 3. In the Navigation bar, under Tune and Calibrate, double-click Instrument

Optimization.

Note: AB SCIEX recommends that after using the Taurocholic acid, repeat the channel alignment using the PPG 3000 solution.

- 4. Select a tuning solution. Make sure that the tuning solution matches the reference table.
- 5. The Verify Performance Only check box is preselected. Click Next.

For this example, leave this option selected. If the report indicates that the instrument needs tuning, then run Instrument Optimization again and select one or more scan modes to optimize. Make sure that the ion source and syringe parameters are suitable.

7. Click GO.

The Verifying Performance screen appears. After the process has completed, the Results Summary appears showing the resolution and intensity for each scan mode.

Example of continuing calibration method with beta galactosidase is detailed in Table A1. Figures A1 and A2 show reference table editor for the opening calibration with tuning solution. And beta-galactosidase. The editor references the compounds and masses used during calibration.

Table H1. Continuing calibration method with beta-galactosidase.

HPLC	
Mass Spectrometer	Eksigent Nano 415
	AB Sciex 5600+ Triple ToF
Mass Spec Source	Electrospray, positive ion mode
Mass Spec Parameters	Experiment 1: Scan Type: ToF MS ToF Mass Range: 400 - 1500 Da Accumulation Time: 0.250 seconds
	Experiment 2: Scan Type: Product Ion Products of: 729.37 Da ToF Mass Range: 100-1500 Da Accumulation Time: 0.500 seconds
HPLC Column	Eksigent 3C18-CL-120, 3 μm, 120 A, 0.3 x 150 mm
Column Temperature	30° c
Mobile Phase	A= 0.1% formic acid inwater
Components	B= o.;% formic acid in methanol
Gradient Profile	All changes are linear with respect to time: 1. T %8 Flow rate,
Injection Volume	1μL (Trap and Elute, 5 minute wash with Mobile Phase A at 5μL/min)
Run Time	16 min

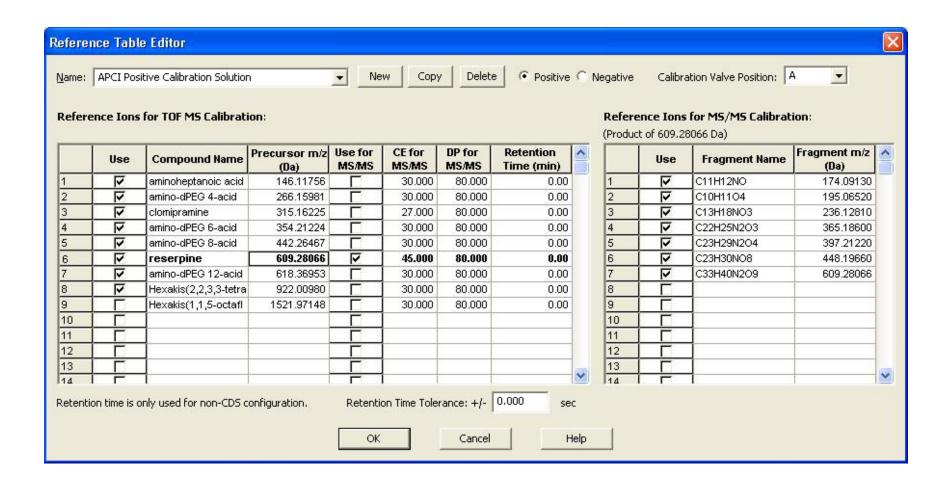


Figure H1. Reference table editor for the opening calibration with tuning solution. The editor references the compounds and masses used during calibration. Not all compounds are used and have a check mark if the mass is used for calibration.

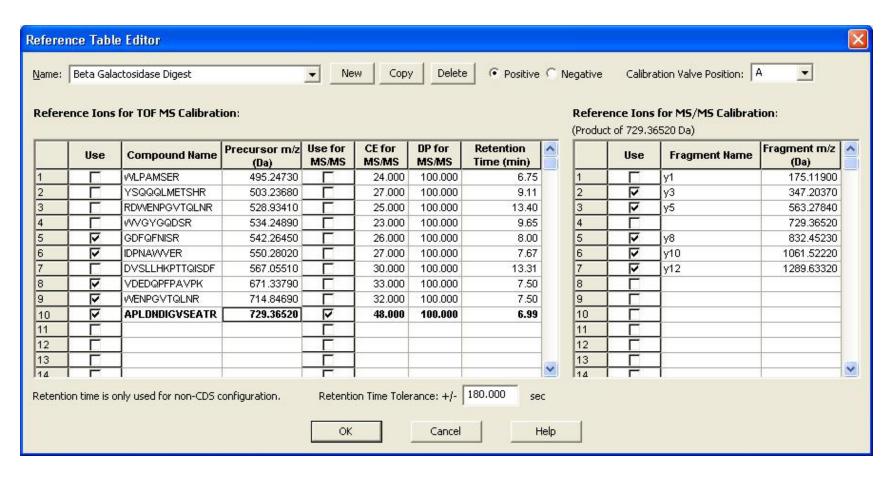


Figure H2. Reference table editor for the opening calibration with betagalactosidase. The editor references the compounds and masses used during calibration. Not all compounds are used and have a check mark if the mass is used for calibration.

APPENDIX D MICROCOSM ANALYTICAL AND BIOMARKER ABUNDANCE DATA

SET 1	Fo	rt Lewis JBN	ΛL	Start 7/18/2	.017 9:30 A	ιM																							
08 DHC/n	nL																												
								VOC's ug/I					Meth	hane/Ethene	ug/L				Anions mg/I						VFA mg/L				
DATE	TIME (hrs)	Bottle	pН	mean/SD	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide	Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	Q	mean
		1A	6.87			1060	D		3100	D		9.45			19.8			9.84			243	D		77.7	D		3.7	U	
7/18/17 9:30	0	1B	6.76	6.88	9721	938	D	1093	3090	D	2787	9.35		9.87	21.16		26.82	9.25		9.70	276	D	250.33	77.1	D	51.91	3.7	U	3.70
		1C	7.01	0.13		1280	D	173.32	2170	D	534.07	10.8		0.81	39.5		11.00	10.0		0.40	232.0	D	22.90	0.93	U	44.15	3.7	U	0.00
		1A	7.23			2230	D		301	D		32.7			133			9.05			252	D		0.93	U		3.7	U	
/18/17 10:30	1	1B	7.26	7.26	9721	2320	D	2183	465	D	269	30.5		37.27	130		164.00	8.58		8.66	250	D	249.67	65.6	D	51.91	24.9	D	10.77
		1C	7.28	0.03		2000	D	165.03	41	U	213.80	48.6		9.88	229		56.31	8.35		0.36	247	D	2.52	89.2	D	45.70	3.7	U	12.24
		1A	7.19			1450	D		41	U		156			430			7.86			160	D		20.3	D		3.7	U	
/18/17 11:30	2	1B	7.45	7.38	9721	1620	D	1447	41	U	41.00	158		167.00	456		472.00	7.56		7.65	198	D	166.33	0.93	U	27.38	3.7	U	12.43
		1C	7.49	0.16		1270	D	175.02	41	U	0.00	187		17.35	530		51.88	7.53		0.18	141	D	29.02	60.9	D	30.60	29.9	D	15.13
		1A	7.16			163	J		41	U		594			896			7.77			188	D		28.9	D		3.7	U	
/18/17 13:30	4	1B	7.4	7.36	9721	182	J	145	41	U	41.00	504		559.00	844		867.67	7.51		7.73	230	D	196.67	84.6	D	49.13	3.7	U	9.00
		1C	7.52	0.18		88.6	J	49.36	41	U	0.00	579		48.22	863		26.31	7.91		0.20	172	D	29.96	33.9	D	30.82	19.6	JD	9.18
		1A	7.18			53	U		41	U		1130			1000			8.16			175	D		25.3	D		27.7	D	
/18/17 15:30	6	1B	7.47	7.39	9721	53	U	53.00	41	U	41.00	940		1026.67	937		931.33	7.29		7.72	185	D	186.67	69.0	D	39.10	3.7	U	11.70
		1C	7.52	0.18		53	U	0.00	41	U	0.00	1010		96.09	857		71.67	7.71		0.44	200	D	12.58	23	D	25.92	3.7	U	13.86
		1A	7.38			53	U		41	U		1340			823			7.56			149	D		38.6	D		25.3	D	
/18/17 17:30	8	1B	7.46	7.43	9721	53	U	53.00	41	U	41.00	1290		1343.33	855		830.67	7.12		7.40	106	D	128.67	56.8	D	44.20	24.5	D	26.60
		1C	7.46	0.05		53	U	0.00	41	U	0.00	1400		55.08	814		21.55	7.53		0.25	131	D	21.59	37.2	D	10.93	30.0	D	2.97
		1A	6.90			53	U		41	U		5200			912			7.79			34.6	D		75.9	D		63.2	D	
7/19/17 9:30	24	1B	6.98	6.94	9721	53	U	53.00	41	U	41.00	5180		5153.33	901		863.00	7.88		7.80	2.23	U	14.62	62.9	D	99.93	51.1	D	67.00
		1C	6.95	0.04		53	U	0.00	41	U	0.00	5080		64.29	776		75.54	7.74		0.07	7.02	JD	17.47	161	D	53.28	86.7	D	18.10

SET 2	For	rt Lewis JBN	νī.	Start 7/18/2	017 9:45 A	M																							
10 ⁷ DHC/m																													
To Differn								VOC's ug/I					Meths	ne/Ethene	ug/L		_	_	Anions mg/l						VFA mg/L				$\overline{}$
DATE	TIME (hrs)	Bottle	рН	mean	ATL ID	VC	0	mean	cDCE	0	mean	Methane	0	mean	Ethene	0	mean	Bromide	0	mean	Lactic	0	mean	Acetic	0	mean	Proprionic	0	mean
		2A	7.51			22.7	j		4730	D		3.41			0.44	Ù		9.28	_		261	D		0.93	Ù		3.7	Ù	
7/18/17 9:45	0	2B	7.67	7.62	9721	52.1	J	46	4470	D	4473	3.15		3.29	0.44	U	0.44	9.35		9.32	197	D	210.00	0.93	U	0.93	3.7	U	3.70
		2C	7.67	0.09		62.6	J	21	4220	D	255	3.31		0.13	0.44	U	0.00	9.32		0.04	172	D	45.90	0.93		0.00	4		0.00
		2A	7.89			828	D		2450	D		28.9			15.2			8.25			160	D		0.93	U		3.7	U	
7/18/17 17:45	8	2B	8.04	7.99	9721	851	D	879	2540	D	2467	28.7		28.87	15.2		15.07	7.99		8.32	159	D	171.33	0.93	U	0.93	3.7	U	3.70
		2C	8.04	0.09		958	D	69	2410	D	67	29.0		0.15	14.8		0.23	8.73		0.38	195	D	20.50	0.93	U	0.00	3.7	U	0.00
		2A	7.50			1930	D		1030	D		115			76.7			7.97			197	D		0.93	U		3.7	U	
7/19/17 9:45	24	2B	7.61	7.56	9721	1720	D	1867	902	D	873	114		113.33	77.9		78.17	8.46		8.20	159	D	181.67	0.93	U	0.93	3.7	U	3.70
		2C	7.58	0.06		1950	D	127	686	D	174	111		2.08	79.9		1.62	8.17		0.25	189	D	20.03	0.93	U	0.00	3.7	U	0.00
		2A	7.33			2550	D		41	U		182			228			8.07			265	D		24.8	D		3.7	U	
7/20/17 9:45	48	2B	7.58	7.50	9724	2270	D	2163	41	U	41	186		178.67	242		233.00	8.07		8.11	465	D	341.67	22.4	D	28.30	11.9	JD	7.33
		2C	7.60	0.15		1670	D	450	41	U	0	168		9.45	229		7.81	8.18		0.06	295	D	107.86	37.7	D	8.23	6.38	JD	4.18
		2A	7.58			1510	D		41	U		285			431			7.95			271	D		26.9	D		7.21	JD	
7/21/17 9:45	72	2B	7.60	7.60	9724	1230	D	1320	41	U	41	274		278.33	426		428.00	8.12		8.02	278	D	282.33	26.6	D	30.73	9.83	JD	8.68
		2C	7.63	0.03		1220	D	165	41	U	0	276		5.86	427		2.65	7.99		0.09	298	D	14.01	38.7	D	6.90	8.99	JD	1.34
		2A	7.21			721	D		41	U		462			581			7.46			212	D		41.8	D		38.9	D	
7/22/17 9:45	96	2B	7.24	7.23	9724	573	D	597	41	U	41	549		503.00	678		617.00	7.97		7.84	229	D	209.67	66.2	D	60.67	48.9	D	49.50
		2C	7.23	0.02		496	D	114	41	U	0	498		43.71	592		53.11	8.1		0.34	188	D	20.60	74.0	D	16.80	60.7	D	10.91
		2A	6.93	_		81.5	JD	_	41	U		1010			779			7.62			2.23	U		79.6	D		166	D	1
7/23/17 9:45	120	2B	6.98	6.97	9724	53	U	63	41	U	41	927		959.33	708		716.33	7.99		8.02	2.23	U	2.23	110	D	98.87	145	D	154.00
		2C	7.01	0.04		53	U	16	41	U	0	941		44.43	662		58.94	8.45		0.42	2.23	U	0.00	107	D	16.75	151	D	10.82

SET 3	Fo	ort Lewis JBN	ML	START		7/18/17 10:55																							
06 DHC/m	L							VOC's ug/I	,				Met	hane/Ethe n	e ug/L				Anions mg	/L					VFA mg/L				
								VOC's ug/I	,				Met	hane/Ethe n	e ug/L				Anions mg	/L					VFA mg/L				
DATE	TIME (days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide	Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	Q	mean
		3A	7.62			53	U		4560	D		3.03			0.44	U		9.08			152	D		0.93	U		3.7	U	
7/18/17 10:55	0	3B	7.77	7.74	9721	53	U	53.00	4680	D	4427	3.45		2.97	0.44	U	0.44	8.98		9.17	180	D	180.67	0.93	U	0.93	3.7	U	3.70
		3C	7.84	0.11		53	U	0	4040	D	340	2.44		0.51	0.44	U	0.00	9.44		0.24	210	D	29.01	0.93	U	0.00	3.7	U	0.00
		3A	7.17			159	JD		4040	D		13.3			0.44	U		9.47			196	D		45.4	D		100	D	
7/24/17 9:00	5.9	3B	7.50	7.31	9742	67.6	JD	124.87	4020	D	3963.33	10.8		12.20	0.44	U	0.44	8.77		8.94	296	D	219.00	22.9	D	33.07	13.7	JD	57.80
		3C	7.25	0.17		148	D	49.89843	3830		115.9023	12.5		1.28	0.44	U	0.00	8.57		0.47	165	D	68.46	30.9	D	11.41	59.7	D	43.18
		3A	6.97			64.8	JD		2700	D		63.3			0.44	U		8.07			2.23	U		114	D		220	D	
7/27/17 9:00	8.9	3B	6.92	7.00	9726	61.2	JD	84.67	3050	D.	2956.67	63.6		64.93	0.44	U	0.44	8.69		8.48	2.23	U	2.23	121	D	119.67	226	D	225.00
		3C	7.10	0.09		128	JD.	37,57091	3120	D	225.0185	67.9		2.57	0.44	U	0.00	8.69		0.36	2.23	U	0.00	124	D	5.13	229	D	4.58
		3A	7.00			577	D		3180	D		145			2.42			9.46			2.23	U		187	D		170	D	
7/31/17 9:00	12.9	3B	7.06	7.04	9727	323	D	474.33	3430	D	3260.00	271		200.00	1.08	J	1.99	9.34		9.25	1.16	U	1.52	109	D	129.67	120	D	158.33
		3C	7.07	0.04		523	D	133.8108	3170	D	147.3092	184		64.51	2.47		0.79	8.96		0.26	1.16	U	0.62	93	D	50.29	185	D	34.03
		3A	6.97			695	D		3050	D		227			4.61			8.60			1.16	U		108	D		195	D	
8/3/17 9:00	15.9	3B	6.98	6.99	9728	433	D	595.67	3320	D	3073.33	300		262.00	1.73	J	3.39	8.33		8.35	1.16	U	1.16	101	D	101.60	186	D	188.67
		3C	7.02	0.03		659	D	142.0188	2850	D	235.8672	259		36.59	3.84		1.49	8.12		0.24	1.16	U	0.00	95.8	D	6.12	185	D	5.51
		3A	7.12			932	D		2810	D		273			9.9			8.50			1.12	U		76	D		138	D	
8/7/17 9:00	19.9	3B	7.10	7.13	9730	529	D	774.00	3120	D	2793.33	334		297.00	2.97	J	6.78	8.23		8.30	1.12	U	1.12	100	D	91.00	184	D	166.67
		3C	7.16	0.03		861	D	215.1255	2450	D	335.3108	284		32.51	7.46		3.52	8.18		0.17	1.12	U	0.00	97	D	13.08	178	D	25.01
		3A	7.05			977	D		2410	D		225			11.1			7.52			1.12	U		90.6	D		164	D	
8/10/17 9:00	22.9	3B	7.05	7.06	9734	586	D	849.00	2840	D	2490.00	268		234.33	3.30		7.90	7.97		7.76	1.12	U	1.12	102	D	93.60	187	D	176.00
		3C	7.07	0.01		984	D	227.7916	2220	D	317.6476	210		30.11	9.29		4.08	7.78		0.23	1.12	U	0.00	88.2	D	7.37	177	D	11.53
		3A	7.06			1110	D		2210	D		228			17.5			6.94			1.12	U		91.5	D		164	D	
8/14/17 9:40	26.9	3B	7.12	7.12	9735	740	D	1000.00	2750	D	2290.00	154		201.00	2.88	J	12.49	6.98		6.99	1.12	U	1.12	101	D	97.50	183	D	175.67
		3C	7.18	0.06		1150	D	226.0531	1910	D	425.6759	221		40.85	17.1		8.33	7.04		0.05	1.12	U	0.00	100	D	5.22	180	D	10.21
		3A	7.06			1820	D		1760	D		199			0.37	U		7.25			1.12	U		108	D		208	D	
8/23/17 9:00	35.9	3B	7.08	7.08	9738	1250	D	1566.67	2250	D	1733.33	210		199.33	0.37	U	0.37	7.42		7.44	1.12	U	1.12	115	D	113.33	221	D	219.00
		3C	7.09	0.02		1630	D	290.2298	1190	D	530.5029	189		10.50	0.37	U	0.00	7.66		0.21	1.12	U	0.00	117	D	4.73	228	D	10.15
		3A	7.09			1770	D		600	D		146			75.5			7.04			1.12	U		78	D		170	D	
9/11/17 9:00	54.9	3B	7.00	7.05	9739	1390	D	1576.67	1250	D	776.00	248		209.67	33.1		92.53	7.13		7.05	1.12	U	1.12	74.1	D	98.03	177	D	221.00
		3C	7.06	0.05		1570	D	190.0877	478	D	415.0036	235		55.52	169		69.53	6.99		0.07	1.12	U	0.00	142	D	38.13	316	D	82.35
		3A	7.48			1820	D		41	U		241			168			5.35			1.16	U		103	D		213	D	
10/9/17 10:00	83.0	3B	7.44	7.46	9752	1690	D	1663.33	340	D	140.67	188		195.33	100		160.00	5.25		5.34	1.16	U	1.16	96.7	D	102.23	208	D	214.33
		3C	7.46	0.02		1480	D	171.5615	41	U	172.6277	157		42.48	212		56.43	5.42		0.09	1.16	U	0.00	107	D	5.19	222	D	7.09

SET 4	Fc	ort Lewis JBN	ИL	START		7/18/17 11:50																							
10 ⁵ DHC/mL								VOC's ug/l					Metha	ne/Ethene	ug/L				Anions mg/	L					VFA mg/L				
								VOC's ug/l						ne/Ethene					Anions mg/						VFA mg/L				
DATE	TIME(days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide	Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	Q	mean
		4A	7.80			53	U		4380	D		5.43			2.89			9.13			298	D		39.4	D		3.7	U	
7/18/17 10:55	0	4B	7.81	7.80	9721	53	U	53.00	4470	D	4480	3.29	ľ	3.74	0.44	U	1.26	9.06		9.20	194	D	238.00	29.4	D	40.50	3.7	U	3.70
		4C	7.78	0.02		53	U	0.00	4590	D	105.36	2.51		1.51	0.44	U	1.41	9.40		0.18	222	D	53.81	52.7	D	11.69	3.7	U	0.00
		4A	7.70			53	U		4500	D		2.07			0.44	U		8.77			337	D		10.2	JD		3.7	U	
7/24/17 10:00	5.9	4B	7.94	7.88	9742	53	U	53.00	5110	D	4580.00	1.88		1.95	0.44	U	0.44	8.74		8.71	311	D	333.00	11.8	JD	13.97	3.7	U	3.70
		4C	7.99	0.16		53	U	0.00	4130	D	494.87	1.9		0.10	0.44	U	0.00	8.62		0.08	351	D	20.30	19.9	JD	5.20	3.7	U	0.00
		4A																											
7/31/17 10:00	12.9	4B	6.85	6.90	9727	52.8	U	52.80	4560	D	4300.00	3.51		3.07	0.44	U	0.44	8.61		9.03	1.16	U	1.70	150	D	142.50	147	D	179.50
		4C	6.94	0.06		52.8	U	0.00	4040	D	367.70	2.62		0.63	0.44	U	0.00	9.45		0.59	2.23	U	0.76	135	D	10.61	212	D	45.96
		4A																											
8/7/17 10:00	19.9	4B	6.97	6.99	9730	52.8	U	52.80	4420	D	4190.00	33.4		32.45	0.44	U	0.44	8.40		8.40	1.12	U	1.12	108	D	104.50	194	D	188.50
		4C	7.01	0.03		52.8	U	0.00	3960	D	325.27	31.5		1.34	0.44	U	0.00	8.39		0.01	1.12	U	0.00	101	D	4.95	183	D	7.78
		4A																											
8/14/17 10:40	27.0	4B	6.98	7.02	9735	52.8	U	52.80	4310	D	4065.00	57.5		63.75	0.44	U	0.44	7.54		7.54	1.12	U	1.12	110	D	119.50	196	D	208.00
		4C	7.06	0.06		52.8	U	0.00	3820	D	346.48	70.0		8.84	0.44	U	0.00	7.54		0.00	1.12	U	0.00	129	D	13.44	220	D	16.97
		4A																											
8/23/17 11:00	36.0	4B	7.03	7.04	9738	90.7	J	102.85	2930	D	3235.00	62.3		68.80	0.44	U	0.44	6.86		7.47	1.12	U	1.12	104	D	119.00	188	D	219.50
		4C	7.05	0.01		115	J	17.18	3540	D	431.34	75.3		9.19	0.44	U	0.00	8.07		0.86	1.12	U	0.00	134	D	21.21	251	D	44.55
		4A																											
9/11/17 10:00	54.9	4B	7.08	7.09	9739	177	J	164.50	3940	D	3645.00	86.5		95.75	0.44	U	0.44	7.52		7.50	1.12	U	1.12	169	D	131.00	369	D	292.50
		4C	7.09	0.01		152	J	17.68	3350	D	417.19	105		13.08	0.44	U	0.00	7.47		0.04	1.12	U	0.00	93	D	53.74	216	D	108.19
		4A																											
10/9/17 10:30	82.9	4B	7.26	7.33	9752	239	D	220.00	3030	D	2795.00	76.1		89.05	0.44	U	0.44	5.98		6.08	1.16	U	1.16	88.1	D	109.05	174	D	205.00
		4C	7.39	0.09		201	J	26.87	2560	D	332.34	102		18.31	0.44	U	0.00	6.18		0.14	1.16	Ű	0.00	130	D	29.63	236	D	43.84

SET 5	For	rt Lewis JBN	МL	START		7/18/17 12:30																							
Live Control								VOC's ug/I	,				Met	hane/Ethene	ug/L				Anions mg/I	L					VFA mg/L				
								VOC's ug/I					Met	hane/Ethene	ug/L				Anions mg/I	L					VFA mg/L				
DATE	TIME (days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide	Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	Q	mean
		5A	7.79			53	U		4010	D		3.12			0.44	U		8.97			254	D		76.6	D		3.7	U	
7/18/17 12:30	0	5B	7.88	7.86	9721	53	U	53.00	4630	D	4030	2.82		2.98	0.44	U	0.44	9.56		9.18	292	D	294.33	0.93	U	40.48	3.7	U	3.70
		5C	7.92	0.07		53	U	0	3450	D	590	2.99		0.150	0.44	U	0	9.02		0.33	337	D	41.55	43.9	D	37.95	3.7	U	0.00
		5A	7.60			53	U		4220	D		1.97			0.44	U		8.76			388	D		21.2	D		3.7	U	
7/24/17 11:00	5.9	5B	7.99	7.88	9742	53	U	53.00	4310	D	4197	0.95	U	1.67	0.44	U	0.44	8.74		8.76	336	D	340.67	17.4	D	17.83	3.7	U	3.70
		5C	8.06	0.25		53	U	0	4060	D	127	2.09		0.626	0.44	U	0	8.79		0.03	298	D	45.18	14.9	JD	3.17	3.7	U	0.00
		5A	7.65			75.5	U		2590	Ð		311			0.44	U		8.78			288	D		19.7	JD		3.7	U	
7/27/17 11:00	8.9	5B	8.05	7.93	9726	75.5	U	75.50	1540	D		1.93		2.23	0.44	U	0.44	8.72		8.72	341	D	305.67	15.7	JD	16.60	3.7	U	3.70
		5C	8.08	0.24		75.5	U	0	3390	D	928	1.65		0.775	0.44	U	0	8.67		0.06	288	D	30.60	14.4	JD	2.76	3.7	U	0.00
		5A	7.75			52.8	U		4070	D		1.73			0.44	U		8.45			358	D		0.47	U		1.85	U	
7/31/17 11:00	12.9	5B	7.88	7.88	9727	52.8	U	52.80	4120	D	3987	1.15		1.52	0.44	U	0.44	8.47		8.45	256	D	313.33	0.93	U	0.78	3.70	U	3.08
		5C	8.02	0.14		52.8	U	0.00	3770	D	189	1.68		0.321	0.44	U	0	8.42		0.03	326	D	52.17	0.93	U	0.27	3.70	U	1.07
		5A	7.41			52.8	U		5000	D		1.95			0.44	U		8.62			332	D		0.47	U		1.85	U	
8/3/17 11:00	15.9	5B	7.82	7.71	9728	52.8	U	52.80	4210	D	4360	2.07		2.04	0.44	U	0.44	8.59		8.57	349	D	339.33	0.47	U	0.47	1.85	U	1.85
		5C	7.91	0.27		52.8	U	0.00	3870	D	580	2.1		0.079	0.44	U	0	8.49		0.07	337	D	8.74	0.47	U	0.00	1.85	U	0.00
		5A	7.33			52.8	U		4030	D		1.79			0.44	U		8.55			322	D		0.47	U		1.85	U	
8/7/17 11:00	19.9	5B	7.68	7.65	9730	52.8	U	52.80	4020	D	3953	1.68		1.63	0.44	U	0.44	7.96		8.16	342	D	324.67	0.47	U	0.47	1.85	U	1.85
		5C	7.95	0.31		52.8	U	0.00	3810	D	124	1.42		0.19	0.44	U	0	7.96		0.340637	310	D	16.17	0.47	U	0.00	1.85	U	0.00
		5A	7.47			52.8	U		3890	D		1.98			0.44	U		8.14			320	D		0.47	U		1.85	U	
8/10/17 11:00	22.9	5B	7.88	7.75	9734	52.8	U	52.80	4020	D	3867	1.31		1.54	0.44	U	0.44	8.86		8.27	342	D	329.00	0.47	U	0.47	1.85	u	1.85
		5C	7.89	0.24		52.8	U	0.00	3690	D	166	1.34		0.378462	0.44	U	0	7.80		0.541233	325	D	11.53	0.47	U	0.00	1.85	U	0.00
		5A	7.05			52.8	U		3730	D		1.89			0.44	U		7.34			329	D		0.47	U		1.85	U	
8/14/17 13:00	27.0	5B	7.77	7.57	9735	52.8	U	52.80	3870	D	3673	1.66		1.87	0.44	U	0.44	7.47		7.41	301	D	326.67	0.47	U	0.47	1.85	U	1.85
		5C	7.88	0.45		52.8	U	0.00	3420	D	230	2.05		0.196044	0.44	U	0	7.43		0.066583	350	D	24.58	0.47	U	0.00	1.85	U	0.00
		5A	7.35			52.8	U		3510	D		3.01			0.44	U		7.82			418	D		0.47	U		1.85	U	
9/11/17 11:00	54.9	5B	7.73	7.60	9739	52.8	U	52.80	4020	D	3657	2.94		2.83	0.44	U	0.44	7.86		7.75	379	D	392.33	0.47	U	0.47	1.85	U	1.85
		5C	7.72	0.22		52.8	U	0.00	3440	D	317	2.53		0.259294	0.44	U	0	7.57		0.157162	380	D	22.23	0.47	U	0.00	1.85	U	0.00
		5A	7.77			52.8	U		3160	D		2.77			0.44	U		6.32			329	D		84.3	D		1.85	U	
10/9/17 12:00	83.0	5B	7.94	7.88	9752	52.8	U	52.80	3130	D	3047	1.69		2.14	0.44	U	0.44	5.90		6.15	485	D	391.33	0.47	U	28.41	1.85	U	1.85
		5C	7.94	0.10		52.8	U	8.7E-15	2850	D	171	1.95		0.563678	0.44	U	0	6.22		0.219393	360	D	82.59	0.47	U	48.40	1.85	U	0.00

SET 6	For	rt Lewis JBN	ИL	START		7/18/17 14:00																							
Killed Con	trol							VOC's ug/	L				Meth	nane/Ethe n	e ug/L				Anions mg/	L					VFA mg/L				
								VOC's ug/	L				Meth	nane/Ethe n	e ug/L				Anions mg/	L					VFA mg/L				
DATE	TIME (days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethe ne	Q	mean	Bromide	Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	Q	mean
		6A	6.60			53	U		3450	D		2.95			0.44	U		8.13			331	D		33.4	D		3.7	U	
7/18/17 14:00	0	6B	6.59	6.59	9721	53	U	53.00	2810	D	3293.33	3.23		3.28	0.44	U	0.44	6.61		7.85	317	D	334.00	63.1	D	38.13	3.7	U	3.70
		6C	6.58	0.01		53	U	0.00	3620	D	427.12	3.66		0.36	0.44	U	0.00	8.81		1.13	354	D	18.68	17.9	D	22.97	3.7	U	0.00
		6A	6.93			53	U		4570	D		1.74			0.44			7.84			298	D		14.9	JD		3.7	U	
7/24/17 12:00	5.9	6B	6.94	6.93	9742	53	U	53.00	4250	D	4433.33	1.69		1.66	0.44		0.44	8.22		8.09	359	D	343.00	0.93	U	5.59	3.7	U	3.70
		6C	6.93	0.01		53	U	0.00	4480	D	165.03	1.55		0.10	0.44		0.00	8.20		0.21	372	D	39.51	0.93	U	8.07	3.7	U	0.00
		6A	6.92			75.5			1700	В		1.73			0.44	U		8.43			384	D		0.93	U		3.7	U	
7/27/17 12:00	8.9	6B	6.94	6.94	9726	75.5	U	75.50	1520	D	2633.33	1.09		1.49	0.44	U	0.44	8.97		8.70	357	D	343.67	0.47	U	0.78	1.85	U	3.08
		6C	6.96	0.02		75.5	L.	0.00	2680	D	1090.75	1.66		0.35	0.44	U	0.00	8.70		0.27	290	D	48.40	0.93	U	0.27	3.7	U	1.07
		6A	6.98			52.8	U		4230	D		1.71			0.44	U		9.87			357	D		130	D		1.85	U	
7/31/17 12:00	12.9	6B	6.96	6.97	9727	52.8	U	52.80	4080	D	4113.33	1.34		1.49	0.44	U	0.44	9.17		9.26	321	D	346.67	27.9	D	56.90	3.7	U	3.08
		6C	6.98	0.01		52.8	U	0.00	4030	D	104.08	1.43		0.19	0.44	U	0.00	8.73		0.57	362	D	22.37	12.8	D	63.76	3.7	U	1.07
		6A	6.96			52.8	U		4290	D		2.21			0.44	U		8.92			317	D		103	D		1.85	U	
8/3/17 12:00	15.9	6B	6.98	6.97	9728	52.8	U	52.80	4170	D	4066.67	0.19	U	1.30	0.44	U	0.44	8.31		8.47	356	D	344.33	24.1	D	48.87	1.85	U	1.85
		6C	6.98	0.01		52.8	U	0.00	3740	D	289.19	1.51		1.03	0.44	U	0.00	8.18		0.40	360	D	23.76	19.5	D	46.94	1.85	U	0.00
		6A	6.99			52.8	U		4200	D		1.59			0.44	U		7.29			338	D		0.47	U		1.85	U	
8/7/17 12:00	19.9	6B	7.01	7.01	9730	52.8	U	52.80	4130	D	4010.00	1.64		1.49	0.44	U	0.44	7.64		7.55	342	D	330.00	0.47	U	0.47	1.85	U	1.85
		6C	7.02	0.02		52.8	U	0.00	3700	D	270.74	1.24		0.22	0.44	U	0.00	7.71		0.23	310	D	17.44	0.47	U	0.00	1.85	U	0.00
		6A	7.00			52.8	U		4160	D		1.68			0.44	U		7.22			341	D		0.47	U		1.85	U	
3/10/17 12:00	22.9	6B	7.05	7.04	9734	52.8	U	52.80	3950	D	3963.33	1.37		1.47	0.44	U	0.44	7.45		7.38	345	D	347.67	0.47	U	0.47	1.85	U	1.85
		6C	7.06	0.03		52.8	U	0.00	3780	D	190.35	1.36		0.18	0.44	U	0.00	7.46		0.14	357	D	8.33	0.47	U	0.00	1.85	U	0.00
		6A	6.88			52.8	U		3940	D		1.35			0.44	U		7.82			343	D		0.47	U		1.85	U	
3/14/17 13:45	27.0	6B	6.97	6.95	9735	52.8	U	52.80	3800	D	3920.00	1.70		1.49	0.44	U	0.44	6.97		7.29	356	D	355.33	0.47	U	0.47	1.85	U	1.85
		6C	7.00	0.06		52.8	U	0.00	4020	D	111.36	1.43		0.18	0.44	U	0.00	7.09		0.46	367	D	12.01	0.47	U	0.00	1.85	U	0.00
		6A	6.98			52.8	U		3640	D		2.37			0.44	U		6.05			380	D		0.47	U		1.85	U	
9/11/17 12:00	54.9	6B	6.99	6.99	9739	52.8	U	52.80	3620	D	3600.00	2.72		2.49	0.44	U	0.44	7.09		6.84	376	D	387.67	0.47	U	0.47	1.85	U	1.85
		6C	7.00	0.01		52.8	U	0.00	3540	D	52.92	2.39		0.20	0.44	U	0.00	7.38		0.70	407	D	16.86	0.47	U	0.00	1.85	U	0.00
		6A	7.23			52.8	U		2980	D		1.48			0.44	U		5.48			529	D		0.47	U		1.85	U	
10/9/17 11:30	82.9	6B	7.10	7.17	9752	52.8	U	52.80	3160	D	2936.67	1.62		1.58	0.44	U	0.44	5.97		5.81	538	D	510.00	0.47	U	0.47	1.85	U	1.85
		6C	7.19	0.07		52.8	U	0.00	2670	D	247.86	1.64		0.09	0.44	U	0.00	5.97		0.28	463	D	40.95	0.47	U	0.00	1.85	U	0.00

SET 1 (A,B,C=	water only)	F	ort Lewis JB!	ML	Start	3/12/18 8:00										-											
10 ⁷ DHC/mL																_					\perp				_		
DATE		D1		(CD)	ATT ID	N/C		VOC's ug/L	D.CE			24.0		thane/Ethene		-			ns mg/L		_			FA mg			0
DATE	TIME (hrs)	Bottle 1A	pH 7.41	mean/SD	ATL ID	VC 148	Q U	mean	eDCE 8820	Q	mean	Methane 2.41	Q	mean	Ethene 0.44	Q U	mean	Bromide 11.70	Q mean	Lactic 632	Q	mean	Acetic 0.9	Q U	mean		Q mean
3/12/18 8:00	0.0	1B	7.41	7.45	9789	148	U	148	11580	-	10047	3.07		2.64	0.44	U	0.44	11.70	12.07	593		584.67	393.0	U	131.60		U 3.70
3/12/18/8:00	0.0	1C	7.5	0.05	7107	148	U	0.00	9740		1405.32	2.44		0.37	0.44	U		12.80	0.64	529		52.00	0.9	U			U 0.00
		1D	7.30	0.05		148	U	0.00	7540		1403.32	2.19		0.57	0.44	Ü	0.00	12.10	0.04	606		32.00	0.9	U	220.30		U 0.00
3/12/18 8:00	0.0	1E	7.46	7.42	9789	148	U	148	12400	-	9953	2.00		1.97	0.44	U	0.44	11.00	11.70	500		549.00	0.9	U	0.90		U 3.70
		1F	7.50	0.11		148	U	0.00	9920		2430.17	1.72		0.24	0.44	U	0.00	12.00	0.61	541		53.45	0.9	U			U 0.00
		1A	7.08			4270			76	U		26.1			237	\top		10.10		400			66.0			56.1	
3/15/18 8:00	3.0	1B	7.09	7.09	9790	5441		4634	76	U	76	26.90		24.93	235		240.00	10.60	10.47	359		355.67	58.4		57.87	45.7	46.03
		1C	7.11	0.02		4192		699.68	76	U	0.00	21.8		2.74	248		7.00	10.70	0.32	308		46.09	49.2		8.41	36.3	9.90
•••••••••••••••••••••••••••••••••••••••		1D	7.58		*******************************	1669			4860			1.87			37.9			10.50		426			66.8			6.1	J
3/15/18 8:00	3.0	1E	7.61	7.62	9790	1689		1671	7160		5973	1.78		1.84	29.7		34.10	9.42	10.14	342		366.00	56.3		56.83	4.3	J 4.68
		1F	7.67	0.05		1656		16.62	5900		1151.75	1.87		0.05	34.7		4.13	10.50	0.62	330		52.31	47.4		9.71	3.7	U 1.24
		1A	6.54			1587			76	U		75.80			512			10.10		4.1			94.9			191	
3/19/18 8:00	7.0	1B	6.53	6.54	9791	2339		1583	76	U	76	84.40		95.73	663		656.67	10.20	10.13	21.4		11.75	120.0		106.63	229	210.33
		1C	6.54	0.01		823		758.01	76	U	0.00	127.00		27.42	795		141.61	10.10	0.06	9.8	J	8.83	105.0		12.63	211	19.01
		1D	7.08	_		3193			2380			2.87			141			10.30		2.2	U	_	152.0		_	256	
3/19/18 8:00	7.0	1E	7.25	7.22	9791	2938		2914	4090		3263	2.37		2.43	119		127.33	10.20	10.20	2.2	U	2.20	109.0		125.33	186	213.4
		1F	7.34	0.13		2612		291.22	3320		856.41	2.05		0.41	122		11.93	10.10	0.10	2.2	U	0.00	115.0		23.29	198	37.39
		1A	6.05			1044			76	U		97.8			530			9.69		2.2	U		97.6			202	
3/22/18 7:25	10.0	1B	6.04	6.06	9792	622		605	76	U	76	195		150.93	1180		890.00	10.00	9.85	2.2	U	2.20	97.4		93.53	226	210.6
		1C	6.09	0.03		148	U	448.25	76	U	0.00	160		49.23	960		330.61	9.86	0.16	2.2	U	0.00	85.6		6.87	204	13.32
		1D	6.81			2844			1750	-		2.26			123	٠,		10.00		2.2	U		125.0			219	
3/22/18 7:25	10.0	1E	6.87	6.85	9792	3000		2844	4000		3023	4.45		3.00	103	Н,	113.00	9.90	9.96	2.2	U	2.20	130.0		127.33	227	225.0
		1F	6.87	0.03		2689		155.50	3320		1153.96	2.29		1.26	113	-	10.00	9.98	0.05	2.2	U	0.00	127.0		2.52	229	5.29
	140	1A	7.60	7 7.00	0702	223	J	172	76	U	7/	278		202.22	1140	-	1200.00	9.65	9.80	2.2	U	2.20	136.0		120.00	274	262.2
3/26/18 8:00	14.0	1B 1C	7.66	7.65	9793	148 148	U	173 43,30	76 76	U	76 0.00	307 325		303.33 23.71	1540 1220	,	1300.00 211.66	9.88 9.88	0.13	2.2	U	2.20 0.00	121.0 133.0		130.00 7.94	245 268	262.3 15.31
		1D	7.70	0.03		3113		43.30	1720		0.00	3.51		23./1	210		211.00	9.88	0.13	2.2	U	0.00	135.0		7.94	238	13.31
3/26/18 8:00	14.0	1E	7.28	7.30	9793	3020		2916	3850	-	2863	2.73		3.07	134	٠,	165.67	9.67	9.64	2.2	I	2.20	108.0		125.33	195	224.3
3/20/18 8:00	14.0	1F	7.38	0.07	7193	2614		265,36	3020		1073.61	2.73		0.40	153		39.55	9.68	0.06	2.2	U		133.0		15.04	240	25,42
		1A	7.68	0.07		148	U	205.50	76	U	1073.01	246		0.40	987	+	37.33	9.48	0.00	2.2	U	0.00	116		13.04	248	23.42
3/29/18 8:00	17.0	1B	7.72	7.74	9794	148	U	148	76	U	76	273		271.67	1220	,	1066.00	9.64	9.60	2.2	U	2.20	125		119.33	264	250.33
3/29/18 8300	17.0	1C	7.81	0.07	7174	148	U	0.00	76		0.00	296		25.03	991	1	133.38	9.68	0.11	2.2	U	0.00	117		4.93	239	12.66
		1D	7.50	0.07		2087		0.00	1390		0.00	3.12		25.05	179		133.30	9.79		2.2	U	0.00	122			233	12.00
3/29/18 8:00	17.0	1E	7.38	7.45	9794	2010		1953	3190		2377	2.19		2,66	93.6		132.20	9,59	9,68	2.2	U	2.20	111		116.33	217	223.6
		1F	7.46	0.06		1761		170.39	2550		912.43	2.68		0.47	124		43,29	9,65	0.10	2.2	U	0.00	116		5.51	221.0	8.33
		1A	7.70			148	U		76	U		325			1100	\neg		9.09		2.2	U		91.7			200	
4/2/18 8:00	21.0	1B	7.74	7.73	9795	148	U	148	76	U	76	239		297.00	961		1009.00	9.05	9.08	2.2	U	2.20	108		99.57	180	187.3
		1C	7.76	0.03		148	U	0.00	76	U	0.00	327		50.24	966		78.85	9.1	0.03	2.2	U	0.00	99		8	182	11.02
		1D	7.33			2009			1240			3.51			171			8.98		2.2	U		108			196	
4/2/18 8:00	21.0	1E	7.32	7.34	9795	1609		1863	2760		2287	2.88		3.19	125		148.00	8.79	8.86	2.2	U	2.20	102		104.33	187	195.6
		1F	7.36	0.02		1970		220.55	2860		907.82	3.17		0.32	148		23.00	8.8	0.11	2.2	U	0.00	103		3.21	204.0	8.50
		1A	7.75			148	U		76	U		291			1050			8.32		2.2	U		84			185	
4/5/18 8:00	24.0	1B	7.74	7.75	9796	148	U	148	76	U	76	309.00		307.33	990		1013.33	8.79	8.65	2.2	U	2.20	89.3		78.40	206	197.0
		1C	7.77	0.02		148	U	0.00	76	U	0.00	322		15.57	1000		32.15	8.84	0.29	2.2	U	0.00	61.9		14.53	200.0	10.82
		1D	7.42			2472		-	1340			3.02			166			8.74		2.2	U	,	113		,	216	
4/5/18 8:00	24.0	1E	7.36	7.41	9796	937		1818	1900		2037	2.92		2.87	139		139.33	8.49	8.56	2.2	U	2.20	101		99.57	199	194.6
		1F	7.44	0.04		2045		792.28	2870		774.10	2.67		0.18	113	11	26.50	8.46	0.15	2.2	U	0.00	84.7	ш	14.20	169.0	23.80
	***	1A	7.69			148	U		76	U		311			1070		0.00.00	8.53	0.00	2.2	U		98.2		0.00	221	
4/9/18 8:00	28.0	1B	7.73	7.72	9797	148	U	148	76	U	76	214		282.33	854	1	960.33	8.70	8.70	2.2	U	2.20	97.2	\vdash	96.83	210	212.6
		1C	7.74	0.03	ļ	148	U	0.00	76	U	0.00	322		59.43	957		108.04	8.88	0.18	2.2	U	0.00	95.1		1.58	207	7.37
	20.0	1D	7.38	7.20	0707	1966		1012	1500	-	2460	3.04		2.56	156		112.65	8.6	0.45	2.2	U	2.20	103		00.02	190	102.5
4/9/18 8:00	28.0	1E 1F	7.39 7.39	7.39 0.01	9797	1989 1480		1812 287.46	3390 2490		2460 945.36	2.11 2.53		2.56 0.47	86 99		113.67 37.22	8.37 8.4	8.47 0.12	2.2	U	2.20 0.00	98 99		99.93 2.77	192 196	192.6° 3.06

JBLM2, #1 DUP

SET 1 DUP (A	,B,C= water	.,			Start	4/16/18 8:00													-								\vdash	
10 ⁷ DHC/mL		For	t Lewis J	BML																								
								OC's ug/						ne/Ethe				Anions					1	FA m				
DATE	TIME (hrs)	Bottle	pН	mean/SD	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide Q) mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic		mean
		1A	7.58			148	U		10120			3.33			0.44	U		10.5		572			0.93	U		3.7	U	
4/16/18 8:00	0.0	1B	7.96	7.84	9802	148	U	148	8710		9490	3.51		3.46	0.44	U	0.44	10.7	10.60	587		556.67	0.93	U	0.93	3.7	U	3.70
		1C	7.97	0.22		148	U	0.00	9640		716.87	3.54		0.11	0.44	U	0.00	10.6	0.10	511		40.25	0.93	U	0.00	3.7	U	0.00
		1D	7.75			148	U		10110			3.89			0.44	U		11.8		650			0.93	U		3.7	U	
4/16/18 8:00	0.0	1E	7.82	7.78	9802	148	U	148	11650		10587	3.37		3.50	0.44	U	0.44	12.40	12.00	677		663.67	0.93	U	0.93	3.7	U	3.70
		1F	7.78	0.04		148	U	0.00	10000		922.51	3.24		0.34	0.44	U	0.00	11.8	0.35	664		13.50	0.93	U	0.00	3.7	U	0.00
		1A	7.97			148	U		9150			3.32			0.44	U		9.52		607			0.93	U		3.7	U	
4/16/18 14:00	6	1B	8.12	7.73	9803	148	U	148	8640		9300	3.36		3.31	0.44	U	0.44	9.66	9.73	565		573.67	0.93	U	0.93	3.7	U	3.70
		1C	7.11	0.55		148	U	0.00	10110		746.39	3.24		0.06	0.44	U	0.00	10.0	0.25	549		29.96	0.93	U	0.00	3.7	U	0.00
		1D	7.74			148	U	_	7790			3.21			0.44	U		9.37		524		_	0.93	U		3.7	U	
4/16/18 14:00	6	1E	7.79	7.79	9803	148	U		9350		8710	3.04		3.16	0.44	U	0.44	9.45	9.45	529		530.00	0.93	U		3.7	U	3.70
		1F	7.83	0.05		148	U	0.00	8990		816.82	3.24		0.11	0.44	U	0.00	9.53	0.08	537		6.56	0.93	U	0.00	3.7	U	0.00
		1A	8.06			148	U	_	8850			2.12			0.44	U		9.23		542		_	0.93	U		3.7	U	
4/17/18 8:00	24	1B	8.05	8.06	9804	148	U	148	7850		8517	2.64		2.54	0.44	U	0.44	9.92	9.61	551		544.67	0.93	U	0.93	3.7	U	3.70
		1C	8.06	0.01	,	148	U	0.00	8850		577.35	2.87		0.38	0.44	U	0.00	9.67	0.35	541		5.51	0.93	U	0.00	3.7	U	0.00
		1D	7.78			187	J		6600			2.75			0.74	J		9.42		518			0.93	U		3.7	U	
4/17/18 8:00	24	1E	7.80	7.81	9804	198	J	190	8190		7773	2.16		2.36	0.60	J	0.59	9.57	9.53	529		523.67	0.93	U		3.7	U	3.70
		1F	7.85	0.04		186	J	6.66	8530		1030.26	2.17		0.34	0.44	U	0.15	9.61	0.10	524		5.51	0.93	U	0.00	3.7	U	0.00
		1A	7.96			148	U		8650			2.05			0.44	U		9.11		497			0.93	U		3.7	U	
4/17/18 14:00	30	1B	8.07	8.05	9804	148	U	148	7350		7970	2.06		2.31	0.44	U	0.44	9.40	9.31	518		512.00	0.93	U	0.93	3.7	U	3.70
		1C	8.12	0.08		148	U	0.00	7910		652.07	2.83		0.45	0.44	U	0.00	9.41	0.17	521		13.08	0.93	U	0.00	3.7	U	0.00
		1D	7.77			274			6000			3.09			1.80	J		8.98		489			0.93	U		3.7	U	
4/17/18 14:00	30	1E	7.80	7.81	9804	322		275	6710		6793	2.95		2.89	1.40	J	1.34	9.06	9.03	494		491.33	0.93	U	0.93	3.7	U	3.70
		1F	7.85	0.04		228	J		7670		838.11	2.64		0.23	0.82	J	0.49	9.05	0.04	491		2.52	0.93	U		3.7	U	0.00
		1A	8.04			55	J		7810			1.87			0.44	U		8.73		492			0.93	U		3.7	U	
4/18/18 8:00	48	1B	8.18	8.14	9805	148	U	117	7110		7427	2.13		2.22	0.44	U	0.44	9.10	8.95	531		484,67	0.90	U	0.92	3.7	U	3.70
		1C	8.20	0.09		148	U		7360		354.73	2.65	•	0.40	0.44	U	0.00	9.02	0.19	431		50.40	0.93	U	0.02	3.7	U	0.00
		1D	7.86			512			5130			2.35			4.65	,		8.65		436			0.93	Ū		3.7	Ü	
4/18/18 8:00	48	1E	7.92	7.92	9805	501		474	4560		5260	2.49	_	2.37	3.66		3,68	8.77	8.81	443		439.67	0.93	U	0.93	3.7	U	3.70
4/10/10/0.00	70	1F	7.97	0.06	7005	410		55,99	6090		773.24	2.28	•	0.11	2.73		0.96	9.00	0.18	440		3.51	0.93	U		3.7	U	0.00
		1A	7.82	0.00		49	J	33.77	8000		113.24	2.65		0.11	0.44	U	0.70	9.06	0.10	511	_	3.31	0.93	U	0.00	3.7	U	0.00
4/18/18 14:00	54	1B	8.1	8.02	9805	148	U	115	7010		7767	2.12	•	2.44	0.44	U	0.44	8.89	8.96	498		500.00	0.93	U	0.93	3.7	U	3.70
4710/10/14:00		1C	8.13	0.17	7005	148	U		8290		671.14	2.54	•	0.28	0.44	U	0.00	8.94	0.09	491		10.15	0.93	U		3.7	U	0.00
		1D	7.80	0.17		601		37.10	5620		0/1.14	2.13		0.20	5.28		0.00	8.63	0.07	469		10.13	0.93	U	0.00	3.7	Ū	0.00
4/18/18 14:00	54	1E	7.88	7.87	9805	503		509	6120		5960	2.13	-	2.31	4.18		4.32	8.64	8.68	411		445.33	0.93	U	0.93	3.7	U	3.70
4/18/18 14:00	34	1F	7.94	0.07	7603	423		89.15	6140		294.62	2.47		0.17	3.50		0.90	8.78	0.08	456		30.44	0.93	U		3.7	U	0.00
				0.07		312	-	07.13	8310	-	294.02	2.47	-	0.17	0.66	Ţ	0.90		0.08	498	+-	30.44		U	0.00	3.7	U	0.00
4/19/18 8:00	72	1A 1B	7.94 8.12	8.07	9806	156	J	207	6720		7460	2.43	-	2.56	0.66	J U	0.51	9.10 9.19	9.16	505		476.33	0.93	U	0.93	3.7	U.	3.70
4/19/18 8:00	12	1C	8.16	0.12	2000	150	J		7350		800.69	2.71	-	0.14	0.44	U	0.31	9.19	0.06	426		476.33	0.93	U		3.7	U,	0.00
		1D	7.93	0.12		806		71.24	4250		000.09	2.34		U.14	7.15	<u> </u>	0.13	9.20 8.77	0.06	426		43./3	0.93	U	0.00		U	0.00
4/10/10 0 00	72	1D 1E	7.93	7.99	9806			719	5170		4910	2.11	-	2.33	6.44	\vdash	6.22	8.77	8.91	474	-	472.33	0.93	U	0.93	3.7		3.70
4/19/18 8:00	72	IE IF	8.06	0.07	9800	638 712		84.20	5170		575.85	2.60	-	0.25	5.08		6.22 1.05	8.97	0.12	4/4	-	27.54	0.93	U		3.7	U	0.00
				0.07			\vdash	84.20			313.83		\vdash	0.23		7	1.03		0.12			21.34			0.00			0.00
		1A	7.89	0.01	0007	290		220	6640		(210	3.04	-	2.00	1.26	J	1.00	8.07	0.25	443	-	141.00	0.93	U	0.00	3.7	U	2.70
4/19/18 14:00	78	1B	8.06	8.01	9806	201	J	228	6010		6310	3.38	-	3.08	3.00		1.63	8.50	8.35	434		441.33	0.93	U		3.7	U	3.70
		1C	8.09	0.11		194	J	53.52	6280		316.07	2.82		0.28	0.64	J	1.22	8.49	0.25	447		6.66	0.93		0.00	3.7	U	0.00
	70	1D	7.81	7.00	0007	946		002	4840		2612	2.42	-	2.04	9.92	\vdash		7.74	7.05	403	-	7 200 22	0.93	U	0.00	3.7	U	2.70
4/19/18 14:00	78	1E	7.89	7.89	9806	971		893	6450		5613	2.37	-	2.26	6.94		7.23	7.89	7.85	395	-	388.33	0.93	U	0.93	3.7	U	3.70
		1F	7.97	0.08		763		113.56	5550	_	806.87	1.98		0.24	4.84	\Box	2.55	7.93	0.10	367		18.90	0.93	U	0.00	3.7	U	0.00
		1A	7.86			2390		.	1860			31.55			33.58			8.57		2.23	U		109.14			223.51	\square	r
4/23/18 8:00	168	1B	7.94	7.91	9807	2160		2202	1430		1860	21.08		22.73	23.48		24.18	8.64	8.62	2.23	U		94.96		100.16	195.94	\sqcup	205.84
		1C	7.94	0.05		2055		171.34	2290		430.00	15.55		8.13	15.47		9.08	8.64	0.04	2.23	U	0.00	96.38		7.81	198.1	<u> </u>	15.34
		1D	7.53			1286			2720			2.65			41.27			8.11		2.23	U		98.53			203.44	ш	
4/23/18 8:00	168	1E	7.62	7.60	9807	1140		1292	4080		3803	2.55		2.53	27.88		29.22	8.21	8.17	2.23	U	2.23	92.71		94.50	168.15		186.42
		1F	7.66	0.07		1449		154.58	4610		974.90	2.39		0.13	18.52		11.43	8.2	0.05	2.23	U	0.00	92.25		3.50	187.7		17.68

Mathon M	SET 2 (A,B,C= 10 ⁶ DHC/mL	water only)	Fo	rt Lewis JI	BML	Start	3/12/18 9:00										H											
1. 1. 1. 1. 1. 1. 1. 1.								V	OC's ug/					Meth	nane/Ether	ne ug/L			Ani	ons mg/L				V	FA mg	/L		
Section Sect	DATE	TIME (days)			mean	ATL ID			mean		Q	mean		Q	mean			mean		Q mean		Q	mean			mean		
Section Sect	3/12/18 9:00	0.0		7.85	7.88	9789			148			9163			1.16			0.44		11.87			539.33			0.93		U 3.70
3239000 06 22 25 25 25 25 25 25 2					0.03				0			871			0.10		~~~~	0.00		0.93			15.31			0.00		
1	3/12/18 9:00	0.0			7.51	9789			148			9530			1.04			0.44		11.10			645.67			0.93		
1991 1992			2F	7.60	0.14		148						0.97		0.08	0.44	U		11.0		720			0.93	U		3.7	U 0.00
12	3/15/18 0-00	3.0			8.01	9790			148			8883			1.05			0.44		10.80			624 33			0.93		
Section Sect			2C	8.01			148			8760						0.44			10.6		556							
12 72 70 70 71 72 73 70 71 72 73 73 73 74 74 74 74 74	2/15/10 0.00	2.0			7.90	0700			1.40			0617			1.10			0.44		10.57			614.00			0.02		
Section Sect	3/13/18 9:00	3.0				9790											U											
The color The					6.00	omou			211			0852			2.00			0.44		40.50			045.65			21.02		102.37
	3/19/18 9:00	7.0				9/91																						37.97
29 17.5 0.00 128 0.7 0 900 100 133 113 0.01 0.00 90 70.31 30.9 100.37 20.4 70.11 1.0 1			2D	6.54				U		8520			1.58			0.46	J		10.2		405			43.5			16.1	J
22	3/19/18 9:00	7.0				9791																						
20					0.00			I		07.00	т	1071			0.13		J			0.15			103.67			10.11		0 0.42
20 10 20 17 17 18 18 18 18 18 18	3/22/18 8:35	10.0				9792		J	279								U	0.49										175.00
Section 100 22 7.07 704 772 188 U 18 18 180 705 705 772 178 U 18 180 705 7					0.04				38			1418			0.36		J	0.09		0.26		U	15.21			19.69		32.36
2.24 771 772 795 791 792 792 793	3/22/18 8:35	10.0	2E	7.07		9792	148	U		6590			1.78			0.5	J		9.8			Ē		84			130	155.17
32018580 140 280 771 772 979 259 J 399 9320 9113 921 920 944 0.45 101 10.23 122 U 2.00 112 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00 102 122 12667 244 2.05 2.00					0.16	-			18		\vdash	1060	_		0.22		J	0.18		0.10	2 2 2	11	59.35		H	38.74		73.55
1. 20 23	3/26/18 9:00	14.0			7.72	9793			319			8113			9.80			0.45		10.23			2.30			120.67		255.67
Decision 140 282 742 739 799 181 723 696 743 1990 729 732 732 732 732 733 743			2C	7.73				J					9.48			0.44			10.0		2.5	J		136			278	19.35
3P	3/26/18 9:00	14.0			7.39	9793			203			8430			3.22		J	1.12		9.79			2.33			156.67		271.67
22318900 17.0 28 7.66 7.65 9794 193 1 262 8790 7700 14.00 14.00 14.00 04.44 U 04.44 P, 7 9.99 12.2 U 2.00 118 118.67 255 245 255 2			2F	7.41			260	J		11090			3.22			0.99	J		9.7		2.6	J	0.23	161			280	9.07
Color Colo	2/20/10 0 20	17.0			7.65	0704			262			7610			14.02			0.44		0.00					۱.,	110 67		246.00
September 170 2E 7.36 7.37 7.94 148 U 239 5.996 7153 318 3.34 1.05 J 7.33 9.63 9.62 2.2 U 2.20 120 719.33 216 7.21 229 2.20 7.20	3/29/18 9:30	17.0				7/74																						21.93
Part					,			U	······································			r					J	,					,		,	,		
## ## ## ## ## ## ## ## ## ## ## ## ##	3/29/18 9:30	17.0				9794																						218.67 12.22
Color Colo				7.71			239	J		5680			2.39			0.44	U		10.1		2.2			119			250	
## Color Part Part	4/2/18 9:00	21.0			7.71	9795		J	254			7133					U	0.44				U	2.20					241.33 15.89
4218-980 21.0 2E 7.4 7.41 9795 148 U 148 5950 6880 3.06 3.43 1.06 J 13.2 9.15 9.24 2.2 U 2.20 114 11.33 203 2.00 2.00 2.00 2.00 3.06 2.00 2.00 2.00 3.06 2.00 2.00 3.06 2.00 2.00 2.00 3.06 2.00 2.00 2.00 3.06 2.00 2.00 2.00 2.00 2.00 3.06 2.00					0.01				30			1333			13.33			0.00		0.20			0.00					13.69
## 4518900 24.0 28 7.74 7.73 7.75	4/2/18 9:00	21.0		7.4		9795	148	U									J							114			203	204.67
4918900 240 28 7.74 7.73 9796 269 J 314 7850 7687 37.1 36.27 0.44 U 0.44 8.99 9.16 2.2 U 2.00 105 105.67 234 72.0 105.67 234 22.0 2.0					0.02				0		Н	1268			0.39		U	0.28		0.08			0.00		\vdash	3.06		10.60
	4/5/18 9:00	24.0	2B	7.74		9796	269	J		7830						0.44	U		8.99		2.2	U					234	223.33
4918900 240 2E					0.02				45			348			1.27		U	0.00		0.32			0.00		[4.04		10.07
2F 7.48 0.04 132 J 4 7630 290 3.3 0.31 189 J 0.39 8.53 0.03 2.2 U 0.00 106.00 4.58 191 5.	4/5/18 9:00	24.0			7.45	9796		J	131			7347			3.40		J	1.46		8.51		U	2.20			110.00		195.67
A9918900 28.0 28 7.7 7.73 7977 276 1 308 8880 7650 78.1 6.297 0.44 U 0.44 9.1 9.26 2.2 U 2.20 103 103.3 201 72.1 1.5					0.04		132	J	4			290			0.31		J	0.39		0.03	2.2	U	0.00			4.58		5.69
Column C	4/9/18 9:00	28.0			7.73	9797			308			7650			62.97		11	0.44		9.26		U	2.20			106.33		214.33
\$\begin{array}{c c c c c c c c c c c c c c c c c c c			2C	7.76			311	J					54.7			0.44	U	0.00	9.17		2.2	U		105			211	15.28
12 13 14 15 15 17 18 18 18 18 19 19 18 18	4000000	20.0			7.40	0707			112			6650			2.40		J	1.24		0.40			2.20			105.22		183.67
41218900 31.0 2B 7.65 7.63	49/18900	26.0				9191											J				2.2							17.90
20		21.0			7 77 72	0000			201			#102			65.00			0.44		0.00			2.20			100 50		212.00
131 1 130 130 120 7.45 131 1 130 1	4/12/18 9:00	31.0				9800																						15.13
2F 7.65 0.12 149 J 20 96.30 219 2.7 0.35 1.55 J 0.37 8.37 0.02 2.2 U 0.00 0.111 7.34 193 2.0 149 193 2.0 2.0 1.07 0.44 U 9.33 0.2 2.3 U 0.05 1				7.45						6990							J		8.41									
24 178 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 1 28 28	4/12/18 9:00	31.0				9800											J				2.2	U						176.00 20.66
2C 7.81 0.04 229 J 33 6729 698 82.6 13.55 0.44 U 0.00 8.87 0.31 2.32 U 0.05 105 9.29 224 2.72 41618960 35.0 2E 7.66 7.70 9802 88 J 100 5509 6.383 3.20 3.23 1.17 J 1.49 8.46 8.38 2.23 U 2.23 118 114.00 191 188 41618960 35.0 2E 7.80 0.08 116 J 14 7421 966 3.60 0.36 2.34 0.074 8.23 0.13 2.23 U 0.00 113 3.61 187 9.84 41918960 38.0 2B 7.79 7.76 9806 215 J 264 7220 7000 99.0 99.03 0.44 U 0.44 8.66 8.67 2.23 U 0.05 109 118 118 41918960 38.0 2B 7.79 7.76 9806 215 J 264 7220 7000 99.0 99.03 0.44 U 0.00 8.55 0.17 2.32 U 0.05 109 9.29 234 2.75 41918960 38.0 2E 7.54 7.55 9806 108 J 103 5610 6.387 3.55 2.89 1.33 J 1.27 8.30 8.34 2.23 U 2.23 116 118 114 118 114 118 114 118 114 118 114 114 118 114 11					0.12			J	20			210)	107		0.55		U	0.57		0.02	2.23		0.00			7.07		20.00
20	4/16/18 9:00	35.0				9802																						233.33
41618980 35.0 2E 7.66 7.70					0.04				33			098			13.33		J	0.00		0.31			0.05		+	9.29		27.23
1918 24 771 25 28 28 3930 933 0.44 U 8.86 2.23 U 91 181 182 283 28	4/16/18 9:00	35.0	2E	7.66		9802	88			5509							J					U		118			191	183.67
41918900 38.0 2B 7.79 7.76 9806 215 1 264 7220 7000 99.0 99.08 0.44 U 0.44 8.60 8.67 223 U 2.26 104 101.33 218 21 22 23 23 23 23 23 24 24					0.08	-		J	14		Н	966			0.36		п	0.74		0.13		_	0.00		\vdash	3.61		9.45
20 7.52 7.54 7.55 7.50 7.	4/19/18 9:00	38.0	2B	7.79		9806	215	J		7220			99.0			0.44	U		8.60		2.23	U		104			218	211.00
41918900 38.0 2E 7.54 7.55 9896 108 J 103 5610 6387 3.55 2.89 1.33 J 1.27 8.30 8.34 2.3 U 2.23 125 118.67 194 118 1					0.04	ļ			43		-	979			9.64		U	0.00		0.17			0.05			9.29		27.18
2F 7.58 0.03 111 J 11 7640 1096 2.37 0.60 1.50 0.27 8.32 0.00 2.23 U 0.00 115 5.51 181 7.	4/19/18 9:00	38.0			7.55	9806			103			6387			2.89		J	1.27		8.34			2.23			118.67		186.00
42918900 42.0 2B 7.89 7.88 9807 267 288 7960 7260 89.16 82.14 0.44 U 0.44 8.74 8.90 2.23 U 2.23 94.05 97.24 207.26 21. 2C 7.91 0.04 239 62 6550 705 74.23 7.50 0.44 U 0.00 8.62 0.39 2.23 U 0.00 94.94 4.78 210.63 9.10 0.00 94.94 4.78 210.63 9.10 0.00 94.94 4.78 210.63 9.10 0.00 94.94 4.78 210.63 9.10 0.00 94.94 94.02 0.00 94.04 94.02 94.02 0.00 94.04 94.02 94.02 94.02 94.02 94.00 94.04 94.02 94.02 94.02 94.02 94.02 94.02 94.02 9			2F	7.58			111	J		7640			2.37			1.50			8.32		2.23	U		115				7.00
2C 7.91 0.04 239 62 6550 705 74.23 7.50 0.44 U 0.00 8.62 0.39 2.23 U 0.00 94.94 4.78 210.63 9. 42018.90 42.0 2E 7.72 7.71 9807 107 J 103 5300 6210 3.22 3.13 1.26 J 1.55 8.42 8.34 2.23 U 2.23 92.00 93.44 148.76 14	4/23/18 9-00	42.0			7.88	9807			288			7260			82.14			0.44		8.90			2.23		\vdash	97.24		214.11
2D 7.66 95 J 6270 2.86 1.06 J 8.30 2.23 U 94.02 143.19 423/18/900 42.0 2E 7.72 7.71 9807 107 J 103 5300 6210 3.22 3.13 1.26 J 1.55 8.42 8.34 2.23 U 2.23 92.00 93.44 148.76 144.76	~2,510 7.00	72.0	2C	7.91		2307	239			6550			74.23			0.44			8.62		2.23	U		94.94			210.63	9.10
	4/22/1000	42.0			7.71	0007						6210					J	1.00		0.27			2.22		H		143.19	
	4/23/18 9:00	42.0				9807			7								J											149.29 6.38
	,					•					_																	

SET 3	For	t Lewis JB	ML		Start	3/12/18 11:00																						
10 ⁵ DHC/mL																												
							V	OCs ug/l	L				Meth	ane/Ether	ne ug/L			Anions	mg/L				VI	FA mg	z/L			
DATE	TIME (days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q	mean	Methane	Q	mean	Ethene	Q	mean	Bromide Q	mean	Lactic	Q	mean	Acetic	Q	mean	Proprionic	. Q	mean
		3A	7.71			148	U		9050			0.81	J		0.44	U		11.90	İ	555			0.93	U		3.7	U	
3/12/18 11:00	0.0	3B	7.76	7.75	9789	148	U	148.0	12960		10360	1.11		1.04	0.44	U	0.44	12.00	11.73	607		572.33	0.93	U	0.93	3.7	U	3.70
		3C	7.78	0.04		148	U	0.00	9070		2251.69	1.20		0.20	0.44	U	0.00	11.30	0.38	555		30.02	0.93	U	0.00	3.7	U	0.00
		3A	8.02			148	U		9040			0.90	J		0.44	U		10.40		559			0.93	U		3.7	U	
3/19/18 10:00	7.0	3B	8.04	8.03	9791	148	U	148.00	8730		8980	0.95		0.93	0.44	U	0.44	10.50	10.30	470		502.67	0.93	U	0.93	3.7	U	2.78
		3C	8.02	0.01		148	U	0.00	9170		226.05	0.93	J	0.03	0.44	U	0.00	10.00	0.26	479		48.99	0.93	U	0.00	0.93	U	1.60
		3A	8.02			148	U		8400			1.13			0.44	U		10.30		439			0.93	U		3.7	U	
3/26/18 11:00	14.0	3B	8.03	8.03	9793	148	U	148.00	9680		9040	1.22		1.18	0.44	U	0.44	10.30	10.30	352		395.50	66.8		33.87	74.0		38.85
		3C	8.03	0.01		148	U	0.00	8870		905.10	1.23		0.06	0.44	U	0.00	10.30	0.00	339		61.52	74.4		46.58	96.5		49.71
		3A	7.96			148	U		7340			1.09			0.44	U		9.83	İ	259			71.4			99.5		
4/2/18 11:00	21.0	3B	7.83	7.82	9795	148	U	148.00	9370		8355	1.36		1.23	0.44	U	0.44	10.10	9.97	195		227.00	90.60		81.00	153		126.25
		3C	7.66	0.15		148	U	0.00	8110		1435.43	1.1		0.19	0.44	U	0.00	9.94	0.19	110		45.25	100		13.58	117		37.83
		3A	7.84			148	U		8320			0.99			0.44	U		9.43		213			87.90			138		
4/9/18 11:00	28.0	3B	7.68	7.69	9797	148	U	148.00	9060		8690	1.31		1.15	0.44	U	0.44	9.64	9.54	106		159.50	109		98.45	204		171.00
		3C	7.54	0.15		148	U	0.00	9770		523.26	1.0		0.23	0.44	U	0.00	9.48	0.15	13.2		75.66	123		14.92	258		46.67
		3A	7.75			148	U		9259			1.11			0.44	U		9.56		154			101			173		
4/16/18 11:00	35.0	3B	7.62	7.66	9802	148	U	148.00	9733		9496	1.48		1.30	0.44	U	0.44	9.60	9.58	26.2		90.10	115		108.00	245		209.00
		3C	7.61	0.08		148	U	0.00	7548		335.17	1.50		0.26	0.44	U	0.00	9.28	0.03	2.23	U	90.37	115		9.90	267		50.91
		3A	7.73			148	U		8540			0.93	J		0.44	U		9.65		68.54			98			184		
4/23/18 11:30	42.0	3B	7.73	7.74	9807	148	U	148.00	9280		8910	1.42		1.18	0.44	U	0.44	9.58	9.62	5.94	J	37.24	176		137.11	401		292.69
		3C	7.75	0.01		148	U	0.00	8990		523.26	1.70		0.35	0.44	U	0.00	9.56	0.05	34.94		44.26	101.5		55.01	205.76		153.71

SET 4	F	ort Lewis JBM	L		Start	3/12/18 11:00																				
10 ⁴ DHC/mL																										
								VOC's ug/L				Met	hane/Ethene	ug/L			Anions	mg/L			V	FA mg/l	L			
DATE	TIME(days)	Bottle	pН	mean	ATL ID	VC	Q	mean	cDCE	Q mean	Methane	Q	mean	Ethene	Q	mean	Bromide Q	mean	Lactic (Q mean	Acetic	Q	mean	Proprionic	Q	mean
		4A	7.58			148	U		7950		1.05			0.44	U		11.7		459		0.93	U		3.7	U	
3/12/18 11:00	0.0	4B	7.76	7.71	9789	148	U	148.00	11700	9726.67	1.06		0.94	0.44	U	0.44	11.7	11.67	630	565.33	0.93	U	0.93	3.7	U	3.70
		4C	7.80	0.12		148	U	0.00	9530	1882.72	0.71	J	0.20	0.44	U	0.00	11.6	0.06	607	92.80	0.93	U	0.00	3.7	U	0.00
		4A	8.00			148	U		7260		0.89	J		0.44	U		10.4		501		0.93	U		3.7	U	
3/19/18 10:00	7.0	4B	7.97	7.96	9791	148	U	148.00	10590	8623.33	1.02		0.94	0.44	U	0.44	10.20	10.27	464	533.33	0.93	U	0.93	3.7	U	3.70
		4C	7.95	0.01		148	U	0.00	8020	1745.06	0.91	J	0.07	0.44	J	0.00	10.2	0.12	635	89.97	0.93	U	0.00	3.7	U	0.00
		4A	8.40			148	U		8420		0.79	J		0.44	U		9.92		383		0.93	U		3.7	U	
3/26/18 11:00	14.0	4B	8.42	8.41	9793	148	U	148.00	9800	9110.00	1.05		0.92	0.44	U	0.44	10.2	10.06	562	472.50	0.93	U	0.93	3.7	U	3.70
BROKEN		4C		0.01				0.00		975.81			0.18			0.00		0.20		126.57			0.00			0.00
		4A	8.35			148	U		7500		1.12			0.44	U		9.46		495		0.93	U		3.7	U	
4/2/18 11:00	21.0	4B	8.26	8.31	9795	148	U	148.00	9450	8475.00	1.06		1.09	0.44	U	0.44	10.3	9.88	507	501.00	0.93	U	0.93	4.89	J	4.30
		4C		0.06				0.00		1378.86			0.04			0.00		0.59		8.49			0.00			0.84
		4A	8.13			148	U		7100		0.82	J		0.44	U		4.35		498		0.93	U		16.7	J	
4/9/18 11:00	28.0	4B	8.12	8.13	9797	148	U	148.00	9290	8195.00	0.91	J	0.87	0.44	U	0.44	9.81	7.08	521	509.50	1	U	0.93	22.8	J	19.75
		4C		0.01				0.00		1548.56			0.06			0.00		3.86		16.26			0.00			4.31
		4A	7.98			148	U		7197		0.93	J		0.44	U		8.91		341		60.6			71.3		
4/16/18 11:00	35.0	4B	8.01	8.00	9802	148	U	148.00	9791	8494.00	0.97		0.95	0.44	U	0.44	9.57	9.24	357	349.00	73.8		67.20	63.5		67.40
		4C		0.02				0.00		1834.23			0.03			0.00		0.47		11.31			9.33			5.52
		4A	7.78			148	U		7610		0.87	J		0.44	U		9.57		196.64		78.46			114.65		
4/23/18 11:30	42.0	4B	7.97	7.88	9807	148	U	148.00	9660	8635.00	1.31		1.09	0.44	U	0.44	9.46	9.52	230.3	213.47	71.61		75.04	93.7		104.18
		4C		0.13				0.00		1449.57	1.01		0.31			0.00		0.08		23,80			4.84			14.81

SET 5 Live	For	t Lewis JB	ML		Start	3/12/18 11:30																						
Live Control																								Ш				
								OC's ug/			-			ane/Ethe					s mg/L					FA mg				
DATE	TIME (days)		pН	mean	ATL ID	VC	Q		cDCE	Q		Methane	e Q	mean	Ethene	Q	mean	Bromide	` _	Lactic	Q		Acetic	Q	mean	Proprionic		mean
3/12/18 11:30	0.0	5A 5B	7.66 7.69	7.68	9789	148 148	U	148.00	10850 11080		10965 163	1.07 0.98	J	1.03 0.064	0.44	U	0.44	11.7 11.7	11.70 0.00	600 632	-	616.00 22.63	0.93	U	0.93	3.7	U	3.70 0.00
3/12/18 11:30	0.0	5C	7.60	7.61	9789	148		148.00	9030		9360	0.51	<u></u>	0.004	0.44	U	0.44	11.7	11.65	567		578.00	0.93	Ū,	0.00	3.7	U	3.70
3/12/18 11:30	0.0	5D	7.61	0.01	7/07	148	U	0	9690		467	1.11	,	0.424	0.44	U	0.44	12.0	0.49	589		15.56	0.93	U		3.7	U	0.00
3/15/18 11:30	3.0	5A	8.01	8.01	9790	148	U	148.00	10400		10000	0.99		0.99	0.44	U	0.44	10.9	10.42	537	_	518.00	0.93	U	0.93	3.7	U	3.70
		5B	8.01	0.00		148	U		9600		566	0.98		0.007	0.44	U	0	9.9	0.68	499		26.87	0.93	U		3.7	U	0.00
3/15/18 11:30	3.0	5C	7.8	7.81	9790	148	Ü	148.00	8900		8825	1.17		1.18	0.44	U	0.44	10.9	10.75	536		476.50	0.93	U	0.93	3.7	U	3.70
		5D	7.82	0.01		148	U	0	8750		106	1.19		0.014	0.44	U	0	10.6	0.21	417		84.15	0.93	U	0.00	3.7	U	0.00
3/19/18 11:00	7.0	5A	7.97	7.97	9791	148	U	148.00	9270		9465	0.96		0.97	0.44	U	0.44	10.3	10.20	426		507.50	0.93	U	0.93	3.7	U	3.70
		5B	7.97	0.00		148	U	0	9660		276	0.97		0.007	0.44	U	0	10.1	0.14	589		115.26	0.93	U	0.00	3.7	U	0.00
3/19/18 11:00	7.0	5C	7.78	7.78	9791	148	U	148.00	7490		7910	1.4		1.44	0.44	U	0.55	9.9	9.60	383		335.50	0.93	U	0.93	3.7	U	3.70
		5D	7.77	0.01		148	U	0	8330		594	1.47		0.049	0.65	J	0	9.3	0.42	288		67.18	0.93	U	0.00	3.7	U	0.00
3/22/18 9:50	9.9	5A	7.83	7.82	9792	148		148.00	10130		10035	0.94	J	0.97	0.44	U	0.44	10.1	10.05	516		443.00	0.93	U	0.93	3.7	U	3.70
		5B	7.81	0.01	0700	148	U	0	9940		134	1.00		0.042	0.44	U	0	10.0	0.07	370		103.24	0.9	U		3.7	U	0.00
3/22/18 9:50	9.9	5C 5D	7.11 7.21	7.16	9792	148 148	U	148.00	7960 8570		8265 431	1.66		1.50 0.233	0.79	J	0.81	9.7 9.7	9.70	28 175	-	101.30 104.23	84.6 76.7		80.65 5.59	132 101		116.50 21.92
3/26/18 11:30	14.0	5A	8.40	8.41	9793	148	U	148.00	10450		10285	1.07		1.07	0.83	U	0.03	10.0	9.96	545	_	551.50	0.93	U	0.93	3.7	U	3.70
3/20/18 11:30	14.0	5B	8.42	0.01	7173	148	U		10120		233	1.06		0.007	0.44	U	0.44	10.0	0.01	558		9.19	0.93	U,		3.7	U	0.00
3/26/18 11:30	14.0	5C	7.36	7.35	9793	148	U	148.00	8330		8190	1.60		1.91	1.14	J	1.14	9.6	9.42	64		33.00	151	·	121.35	239		196.00
3/20/10/11:30	14.0	5D	7.33	0.02	7175	148	U	0	8050		198	2.22		0.438	1.13	J	0	9.3	0.24	2	U		91.7		41.93	153		60.81
3/29/18 11:30	17.0	5A	8.36	8.34	9794	148	U	148.00	8930		8915	1.00	_	0.99	0.44	U	0.44	9.6	9.80	497	Ť	487.00	0.93	U	0.93	3.7	U	3.70
		5B	8.32	0.03		148	U	0	8900		21	0.98		0.014	0.44	U	0	10.0	0.29	477		14.14	0.93	U	0.00	3.7	U	0.00
3/29/18 11:30	17.0	5C	7.33	7.34	9794	148	Ü	148.00	6800		6865	2.09		1.78	1.15	J	1.11	9.6	9.60	12	J	7.10	128	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	117.50	214	,,,,,,,,,,	200.50
		5D	7.34	0.01		148	U	0	6930		92	1.47		0.438	1.07	J	0	9.6	0.04	2	U	6.93	107		14.85	187		19.09
4/2/18 11:30	21.0	5A	8.22	8.30	9795	148	U	148.00	9040		9205	0.88	J	0.95	0.44	U	0.44	9.6	9.55	448		450.00	0.93	U	0.93	12.6	J	8.15
		5B	8.38	0.11		148	U	0	9370		233	1.02		0.099	0.44	U	0	9.5	0.07	452		2.83	0.9	U	0.00	3.7	U	6.29
4/2/18 11:30	21.0	5C	7.83	7.59	9795	148	U	148.00	9030		8255	2.9		2.94	2.13	J	2.32	9.0	8.99	10	J	6.20	116		116.00	196		197.00
		5D	7.34	0.35		148	U	0	7480		1096	2.98		0.057	2.51		0	9.0	0.05	2	U		116		0.00	198.0		1.41
4/5/18 11:30	24.0	5A	8.16	8.22	9796	148	U	148.00	8950		9055	0.91	J	0.92	0.44	U	0.44	9.3	9.28	443		457.50	0.93	U	0.93	20.2		12.39
	24.0	5B 5C	8.28	0.08	0706	148	U	0	9160		148	0.92	J	0.007	0.44	U	0	9.2	0.08	472		20.51	0.9	U		4.58	J	11.05
4/5/18 11:30	24.0	5D	7.47	7.42 0.08	9796	148 148	U	148.00	7550 6580		7065 686	2.63		2.51 0.170	1.86	J	1.58	8.8 8.8	8.79 0.02	11	J	6.70 6.36	111 110.0		110.50 0.71	181 185.0		183.00
4/9/18 11:30	28.0	5A	8.13	8.15	9797	148	U	148.00	8770	_	9030	0.96	-	0.170	0.44	U	0.44	9.1	9.21	454	-	458.50	0.93	U	0.71	28.1	T	20.85
4/9/18 11:30	26.0	5B	8.16	0.02	2121	148	U	0	9290		368	0.90		0.021	0.44	U	0.44	9.3	0.19	463		6.36	0.93	U		13.6	J	10.25
4/9/18 11:30	28.0	5C	7.42	7.80	9797	148	U	148.00	7180		7240	2.69		2.76	1.83	J	1.64	8.8	8.85	15	J	8.45	125	·	124.00	199		202.00
		5D	8.18	0.54		148	U	0	7300		85	2.82		0.092	1.44	J	0	8.9	0.08	2	U		123		1.41	205.0		4.24
4/12/18 11:30	31.0	5A	8.09	8.11	9800	148	U	148.00	8740		9355	0.85	J	0.82	0.44	U	0.44	9.0	9.06	432		418.50	0.93	U	0.93	26.3		21.85
		5B	8.13	0.03		148	U	0	9970		870	0.79	J	0.042	0.44	U	0	9.1	0.05	405		19.09	0.9	U	0.00	17.4	J	6.29
4/12/18 11:30	31.0	5C	7.34	7.35	9800	148	U	148.00	7200		7240	2.41		2.45	1.69	J	1.49	8.7	8.78	15	J		120		120.50	193	,	193.50
		5D	7.36	0.01		148	U	0	7280		57	2.48		0.049	1.29	J	0	8.8	0.09	2	U		121.0		0.71	194.0		0.71
4/16/18 11:30	35.0	5A	8.12	8.14	9802	148	U	148.00	8229		8353	0.97		0.95	0.44	U	0.44	8.8	8.94	375		380.00	55.3		57.30	29.9		27.45
		5B	8.15	0.02		148	U	0	8476		175	0.93	J	0.028	0.44	U	0	9.0	0.14	385		7.07	59.3	[2.83	25.0		3.46
4/16/18 11:30	35.0	5C	7.53	7.50	9802	148	U	148.00	7364		7175	2.91		2.83	2.81		2.20	8.7	8.75	12	J	6.87	128		118.00	192		184.50
	20.0	5D	7.46	0.05	0006	148	U	0	6985	_	268	2.75	-	0.113	1.58	J	0.87	8.8	0.03	2	U	_	108	Н,	14.14	177	Щ,	10.61
4/19/18 11:30	38.0	5A 5B	8.09 8.1	8.10 0.01	9806	148 148	U	148.00	8490 8640		8565 106	0.74	J	0.82	0.44	U	0.44	9.0 9.6	9.33	365		375.00 14.14	56.0 51.8	.	53.90 2.97	34.4 29.7	-	32.05
4/10/19 11:20	38.0	5B 5C			9806	148	Ü				6345		J	2.37		U	1.48		8.70	385	J	5.68	125	ļ,	124.00		ļ,	
4/19/18 11:30	38.0	5D	7.49	7.46 0.04	9800	148	U		6530 6160		262	1.97 2.76		0.559	1.35	T	0	8.7 8.7	0.02	2	U		123		1.41	187 186.00	-	186.50 0.71
4/23/18 11:30	42.0	5A	8.07	8.08	9807	148	_	148.00	8610		8595	1.15		0.339	0.44	U	0.44	9.0	8.93	288	10	290.87	59.94	\vdash	58.62	37.15	 	36.27
4/23/18 11:30	42.0	5B	8.08	0.01	700/	148	U	0	8580		21	0.82	J	0.99	0.44	U	0.44	8.9	0.06	294		3.95	57.3		1.87	35.4		1.24
4/23/18 11:30	42.0	5C	7.62	7.59	9807	148	U	148.00	6960		6740	2.68		3.51	2.27		3,60	8.6	8.58	8	J	5.05	103.49		99.84	161.28		158.65
1 20 10 11:00	12.0	5D	7.55	0.05	2007	148	U	0	6520		311	4.34	-	1.174	4.92		2	8.6	0.02	2	U		96.2	-	5.17	156.0	-	3.72

SET 6 Killed	I	ort Lewis JBM	ſL		Start	3/12/18 12:	00																		
Live Control						_	_	voci a				M 4		_							*****			—	
DATE	TIME (days)	Bottle	рН	mean	ATL ID	VC	Q	VOC's ug/L	cDCE	O mean	Methane	Methane/Ethene	e ug/L Ethene	0	mean	Bromide (mg/L mean	Lactic	O mean	Acetic	VFA m		Boundard.	0	mean
3/12/18 12:00	0.0	6A	6.10	6.12	9789	148	U		9920	Q mean 9245	1.12	1.09	0.44	U	0.44	9.4	9,95	615	612.50	0.93	U	mean 0.93	Proprionic 3.7	U	3.70
3/12/18 12:00	0.0	6B	6.13	0.12	7/07	148	U		8570	955	1.06	0.042	0.44	U	0.44	10.5	0.78	610	3.54	0.93	U		3.7	U	0.00
3/12/18 12:00	0.0	6C	6.30	6.34	9789	148	U		10860	9775	0.59	J 0.89	0.44	U	0.44	8.84	9.77	501	519.50	0.93	U		3.7	U	3.70
		5D	6.38	0.06		148	U		8690	1534	1.19	0.424	0.44	U	0	10.7	1.32	538	26.16	0.93	U		3.7	U	0.00
3/15/18 12:15	3.0	6A	6.17	6.17	9790	148	U	148.00	8820	8330	0.98	0.99	0.44	U	0.44	9.48	10.04	421	420.50	0.93	U	0.93	3.7	U	3.70
		6B	6.17	0.00		148	U	0	7840	693	0.99	0.007	0.44	U	0	10.6	0.79	420	0.71	0.93	U	0.00	3.7	U	0.00
3/15/18 12:15	3.0	6C	6.57	6.61	9790	148	U		9980	9080	2.39	2.11	0.44	U	0.44	9.68	9.12	367	358.50	0.93	U	0.93	3.7	U	3.70
		6D	6.65	0.06		148	U		8180	1273	1.82	0.403	0.44	U	0	8.56	0.79	350	12.02	0.93	U		3.7	U	0.00
3/19/18 12:00	7.0	6A	6.19	6.17	9791	148	U		8670	8095	1.05	1.02	0.44	U	0.44	9.62	8.94	625	618.50	0.93	U	0.93	3.7	U	3.70
	7.0	6B	6.15	0.03	0701	148	U		7520	813	0.99	0.042	0.44	U	0	8.25	0.97	612	9.19	0.93	U		3.7	U	0.00
3/19/18 12:00	7.0	6C 6D	6.52	6.59 0.09	9791	148	U	148.00	9590 7920	8755 1181	3.13	2.68 0.643	0.44	U	0.44	7.24 5.19	6.22	464 515	489.50 36.06	0.93	U	0.93	3.7	U	3.70 0.00
3/22/18 10:50	10.0	6A	6.23	6.22	9792	148	U		8730	8140	0.81	J 0.75	0.44	U	0.44	7.78	8.59	520	519.50	0.93	U		3.7	U	3.70
3/22/18 10:30	10.0	6B	6.20	0.02	9192	148	U		7550	834	0.69	J 0.085	0.44	U	0.44	9.39	1.14	519	0.71	0.93	U		3.7	U	0.00
3/22/18 10:50	10.0	6C	6.56	6.61	9792	148	U		9100	8370	3.6	2,68	0.53	J	0.49	6.80	6.03	504	511.00	0.93	U		3.7	U	3.70
		6D	6.65	0.06		148	U		7640	1032	1.76	1.301	0.44	U	0	5.25	1.10	518	9.90	0.93	U	0.00	3.7	U	0.00
3/26/18 12:00	14.0	6A	7.39	7.39	9793	148	U		9140	8460	1.05	0.98	0.44	U	0.44	7.38	8.36	443	487.00	0.93	U	0.93	3.7	U	3.70
		6B	7.39	0.00		148	U	0	7780	962	0.90	J 0.106	0.44	U	0	9.34	1.39	531	62.23	0.93	U	0.00	3.7	U	0.00
3/26/18 12:00	14.0	6C	7.12	7.14	9793	148	U		10140	8940	3.96	3.35	0.44	U	0.44	7.09	5.92	520	478.00	0.93	U	0.93	3.7	U	3.70
		6D	7.15	0.02		148	U		7740	1697	2.73	0.870	0.44		0	4.74	1.66	436	59.40	0.93	U		3.7	U	0.00
3/29/18 12:00	17.0	6A	7.35	7.36	9794	148	U		8320	7785	0.93	J 0.93	0.44	U	0.44	7.13	8.03	518	512.50	0.93	U	0.93	3.7	U	3.70
		6B	7.36	0.01	ļ	148	U		7250	757	0.93	J 0.000	0.44	U	0	8.92	1.27	507	7.78	0.93	U	***************************************	3.7	U	0.00
3/29/18 12:00	17.0	6C 6D	7.12	7.15 0.04	9794	148 148	U		8150 6440	7295 1209	3.54	3.07 0.672	0.44	U	0.44	7.24 4.05	5.65	508 473	490.50 24.75	0.93	U	0.93	3.7	U	3.70 0.00
4/2/18 12:00	21.0	6A	7.17	7,40	9795	148	U		7380	7520	1.03	1.03	0.44	U	0.44	6.83	7.80	473	491.50	0.93	U	0.00	3.7	U	3.70
4/2/18 12:00	21.0	6B	7.39	0.01	9193	148	U		7660	198	1.03	0.007	0.44	U	0.44	8.77	1.37	513	30.41	0.93	U		3.7	U	0.00
4/2/18 12:00	21.0	6C	7.18	7.21	9795	148	U	·*····	9520	8765	4.28	3,62	0.44	U	0.44	6.66	5.33	466	481.50	0.93	U	0.93	3.7	U	3.70
		6D	7.23	0.04		148	U		8010	1068	2.96	0.933	0.44		0	4.00	1.88	497	21.92	0.93	U	0.00	3.7	U	0.00
4/5/18 12:00	24.0	6A	7.38	7.38	9796	148	U	148.00	7960	7710	0.86	J 0.88	0.44	U	0.44	6.98	7.47	513	481.50	0.93	U	0.93	3.7	U	3.70
		6B	7.38	0.00		148	U	0	7460	354	0.9	J 0.028	0.44	U	0	7.96	0.69	450	44.55	0.93	U	0.00	3.7	U	0.00
4/5/18 12:00	24.0	6C	7.19	7.21	9796	148	U		8640	8210	3.88	3.20	0.44	U	0.44	6.18	4.97	534	508.50	0.93	U	0.93	3.7	U	3.70
		6D	7.23	0.03		148	U	_	7780	608	2.52	0.962	0.44	U	0	3.76	1.71	483	36.06	0.93	U		3.7	U	0.00
4/9/18 12:00	28.0	6A	7.39	7.40	9797	148	U		8070	7740	1.15	1.07	0.98	J	0.71	6.88	7.63	490	496.50	0.93	U	0.93	3.7	U	3.70
		6B	7.4	0.01	0707	148	U		7410 8840	467	0.98	0.120	0.44	U	0	8.37 6.44	1.05	503 471	9.19 483.00	0.93	U	0.00	3.7	U	0.00
4/9/18 12:00	28.0	6C 6D	7.22	7.24	9797	148	U		7730	8285 785	3.76 2.80	3.28 0.679	0.44	U	0.44	3.80	5.12 1.87	4/1	483.00 16.97	0.93	U	0.93	3.7	U	3.70 0.00
4/12/18 12:00	31.0	6A	7.20	7.32	9800	148	U		8800	8455	0.80	J 0.83	0.44	U	0.44	7.01	7.78	493	491.50	0.93	U		6.1	ī	4.92
4/12/18/12/00	31.0	6B	7.32	0.01	7800	148	U		8110	488	0.85	J 0.035	0.44	U	0.44	8.55	1.09	491	0.71	0.93	U		3.7	U	1.73
4/12/18 12:00	31.0	6C	7.1	7.15	9800	148	U	148.00	9730	8840	3.63	3.00	0.44	U	0.44	6.36	5.07	485	477.00	0.93	U	0.93	3.7	U	3.70
		6D	7.2	0.07		148	U	0	7950	1259	2.37	0.891	0.44	U	0	3.77	1.83	469	11.31	0.93	U	0.00	3.7	U	0.00
4/16/18 12:00	35.0	6A	7.39	7.40	9802	148	U		8156	7779	0.95	0.95	0.44	U	0.44	7.25	8.03	488	483.00	0.93	U	0.93	3.7	U	3.70
		6B	7.4	0.01		148	U		7402	533	0.95	0.000	0.44	U	0	8.81	1.10	478	7.07	0.93	U		3.7	U	0.00
4/16/18 12:00	35.0	6C	7.23	7.28	9802	148	U		8543	7823	4.17	3.64	0.44	U	0.44	6.08	4.75	400	427.50	0.93	U	0.93	3.7	U	3.70
	20.0	6D	7.33	0.07	0000	148	U		7102	1019	3.10	0.757	0.44	U	0	3.41	1.89	455	38.89	0.93	U		3.7	U	0.00
4/19/18 12:00	38.0	6A 6B	7.35	7.36	9806	148	U		8020 6530	7275	0.77	J 0.84	0.44	U	0.44	9.31	8.71	615 544	579.50	0.93	U	0.93	3.7	U	3.70
4/19/18 12:00	38.0	6B 6C	7.36 7.17	0.01 7.22	9806	148	U	- w	6530 9590	1054 8055	3.10	J 0.092 3.08	0.44	U	0.48	8.11 5.83	0.85 4.24	544 473	50.20 468.00	0.93	U	0.00	3.7	U	0.00 3.70
4/19/18 12:00	30.0	6D	7.17	0.06	7000	148	U		6520	2171	3.05	0.035	0.44	1	0.48	2.64	2.26	463	7.07	0.93	U		3.7	U	0.00
4/23/18 12:00	42.0	6A	7.38	7.39	9807	148	U		7930	7520	0.82	J 0.83	0.44	U	0.44	7.69	8.11	425	430,27	0.93	U		3.7	U	3.70
423101230	12.0	6B	7.39	0.01	,,,,,	148	U		7110	580	0.84	J 0.014	0.44	U	0.44	8.52	0.59	435	7.23	0.93	U	0.00	3.7	U	0.00
4/23/18 12:00	42.0	6C	7.22	7.29	9807	148	U		8410	7560	3.49	3.00	0.44	U	0.44	5.76	4.32	421	420.35	0.93	U	0.93	3.7	U	3.70
		6D	7.36	0.10		148	U		6710	1202	2.5	0.700	0.44	U	0	2.87	2.04	420	0.31	0.93	U	0.00	3.7	U	0.00

JBLM1, Compiled rate coefficients and biomarker abundances

							LOG + 0 Transformed for Analysis																			
Fro	m RATES_07AU	G18.xls					Rates			Peptides								DHC gene		Function	al Gene	s				
	TIME ZERO	n	Kcis	Kcis 95%CI	Kvc	Kvc 95%CI	Log Kcis	KcisSD	Log Kvc LogKvcSD	FdhA	FdhA std	PceA	PceA std	TceA	TceA std	VcrA	VcrA std	DHC 16S gene	DHC std	tceA	tceA s	td vcrA	vcrA st	d fdhA	fdhA std	pceA pceA:
JBLM1 TIMEZERO	Set1A	21	6.26E+01	7.31E+01	1.02E+01	3.91E+00	1.80E+00		1.01E+00 9.53E-01		13.54		12.35		12.97		11.92	8.88	8.07							7.44234 6.5136
JBLM1 TIMEZERO	Set1B	21	5.70E+01	5.37E+01	1.02E+01	3.61E+00	1.76E+00	2.09E+00	1.01E+00 9.18E-01	13.76	13.54	12.80	12.35	13.31	12.97	13.10	11.92	8.72	8.07							7.326558 6.5136
JBLM1 TIMEZERO	Set1C	21	7.36E+01	1.46E+02	1.13E+01	5.87E+00	1.87E+00	2.52E+00	1.05E+00 1.13E+00	13.23	13.54	12.82	12.35	13.17	12.97	13.14	11.92	8.80	8.07	8.902009	9 8.2628	71 8.8236	25 8.36167	3 8.5420	18 7.727589	7,400075 6,5136
JBLM1 TIMEZERO	Set2A	21	1.48E+00	4.99E-01	3.07E-01	7.25E-02	1.70E-01	5.84E-02	-5.13E-01 -7.80E-01	11.28	11.45	11.82	11.52	11.64	11.50	11.42	11.27	7.30	6.53	7.469212	2 6.6798	81 7.4489	51 6.64766	9 7.1364	6 6.264624	5.843198 4.905
JBLM1 TIMEZERO	Set2B	21	1.49E+00	4.84E-01	3.42E-01	7.78E-02	1.72E-01	4.53E-02	-4.66E-01 -7.49E-01	11.80	11.45	11.89	11.52	11.72	11.50	11.54	11.27	7.14	6.53	7.373399	6,679	81 7.3114	46 6.64766	9 7.0544	32 6.264624	5,749007 4,905
JBLM1 TIMEZERO	Set2C	21	1.68E+00	5.28E-01	3.37E-01	7.04E-02	2.26E-01	8.24E-02	-4.73E-01 -7.92E-01	11.83	11.45	11.94	11.52	11.91	11.50	11.79	11.27	7.28	6.53	7.520002	2 6.6798	81 7.450	93 6.64766	9 7.1749	55 6.264624	5.847296 4.905
JBLM1 TIMEZERO	Set3A	33	2.41E-02	2.60E-03	1.54E-03	1.84E-03	-1.62E+00	-2.13E+00	-2.81E+00 -2.28E+00	10.58	10.16	10.51	9.84	11.24	10.95	10.71	10.28	5.05	5.49	5.252395	5 5.6218	64 5.1664	47 5.5505	3 5.0450	74 5.377145	3.842067 4.167
JBLM1 TIMEZERO	Set3B	33	1.57E-02	1.18E-08	5.81E-04	6.57E-08	-1.81E+00	-7.47E+00	-3.24E+00 -6.72E+00	10.48	10.16	10.49	9.84	10.84	10.95	10.38	10.28	4.36	5.49	4.60722	3 5.6218	4.567	45 5.5505	4.3035	06 5.377145	3.023122 4.167
JBLM1 TIMEZERO	Set3C	33	2.60E-02	2.18E-07	3.66E-03	6.64E-07	-1.58E+00		-2.44E+00 -5.72E+00	10.23	10.16	#DIV/0!	9.84	10.90	10.95	10.19	10.28	5.78	5.49	5.916362	2 5.6218	5.844	98 5.5505	5.6727	71 5.377145	4.461371 4.167
JBLM1 TIMEZERO	Set4A	24								10.23	9.95			9.91	9.43	9.48	9.39	3.58	3.21	3.670815	3.5926	33 3.6864	25 3.55458	6 3.3442	3.487099	1.683909 1.692
JBLM1 TIMEZERO	Set4B	24	1.34E-03	1.32E-04	,		-2.87E+00	-3.49E+00		10.47	9.95			10.07	9.43	9.89	9.39	3.69	3.21	3.998031	3.5926	3.9785	03 3.55458	6 3.8410	54 3.487099	2.121097 1.692
JBLM1 TIMEZERO	Set4C	24	1.36E-03	1.36E-03			-2.87E+00	-2.48E+00		#DIV/0!	9.95			9.84	9.43	9.78	9.39	3.24	3.21	3.362912	3.5926	3.3919	93 3.55458	6 3.0713	13 3.487099	1.656167 1.692
	Set5A	30		•																						
	Set5B	30																								
	Set5C	30																								
							Transformed for Analysis																			
							Rates			Peptides								DHC gene		Function	al Gene	s				
	ME LANDROUNT		Water	W-1- 050/51		W 050/GI		w.t.co			545.4.44					14			DUG 44						fill a stat	
	ME MIDPOINT	n	Kcis	Kcis 95%CI		Kvc 95%CI	Log Kcis	KcisSD	Log Kvc LogKvcSD	FdhA	FdhA std	PceA	PceA std	TceA	TceA std	VcrA	VcrA std	DHC_16S gene	DHC std	tceA	tceA s	td vcrA				
JBLM1 TIMETWO	Set1A	21	6.26E+01	7.31E+01	1.02E+01	3.91E+00	Log Kcis 1.80E+00	2.22E+00	1.01E+00 9.53E-01	FdhA 12.44	12.65	12.41	12.36	12.73	12.88	12.13	12.55	DHC_16S gene 8.709371652	7.626064285	tceA 8.781646	tceA s	td vcr#	7.07809	1 8.4741	12 7.2764	7.255479 6.52
JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B	21 21	6.26E+01 5.70E+01	7.31E+01 5.37E+01	1.02E+01 1.02E+01	3.91E+00 3.61E+00	Log Kcis 1.80E+00 1.76E+00	2.22E+00 2.09E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01	FdhA 12.44 12.22	12.65 12.65	12.41 12.43	12.36 12.36	12.73 13.11	12.88 12.88	12.13 11.94	12.55 12.55	DHC_16S gene 8.709371652 8.742700785	7.626064285 7.626064285	tceA 8.781646 8.88760	tceA s	td vcr/	7.07809 85 7.07809	1 8.4741 1 8.5259	7.2764 7.2764	7.255479 6.52 7.338236 6.52
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C	21 21 21	6.26E+01 5.70E+01 7.36E+01	7.31E+01 5.37E+01 1.46E+02	1.02E+01 1.02E+01 1.13E+01	3.91E+00 3.61E+00 5.87E+00	Log Kcis 1.80E+00 1.76E+00 1.87E+00	2.22E+00 2.09E+00 2.52E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00	FdhA 12.44 12.22 12.95	12.65 12.65 12.65	12.41 12.43 12.73	12.36 12.36 12.36	12.73 13.11 12.78	12.88 12.88 12.88	12.13 11.94 12.86	12.55 12.55 12.55	DHC_16S gene 8.709371652 8.742700785 8.670652654	7.626064285 7.626064285 7.626064285	tceA 8.781646 8.887602 8.89114	tceA s 5 7.9928 2 7.9928 3 7.9928	td vcr/ 341 8.8413 341 8.8555 341 8.8451	7.07809 685 7.07809 642 7.07809	1 8.4741 1 8.5259 1 8.4985	7.2764 7.2764 7.2764 7.2764	7.255479 6.52 7.338236 6.52 7.393151 6.52
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A	21 21 21 21 21	6.26E+01 5.70E+01 7.36E+01 1.48E+00	7.31E+01 5.37E+01 1.46E+02 4.99E-01	1.02E+01 1.02E+01 1.13E+01 3.07E-01	3.91E+00 3.61E+00 5.87E+00 7.25E-02	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01	2.22E+00 2.09E+00 2.52E+00 5.84E-02	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01	FdhA 12.44 12.22 12.95 11.08	12.65 12.65 12.65 11.27	12.41 12.43 12.73 11.37	12.36 12.36 12.36 11.63	12.73 13.11 12.78 11.39	12.88 12.88 12.88 11.57	12.13 11.94 12.86 10.26	12.55 12.55 12.55 11.02	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172	7.626064285 7.626064285 7.626064285 5.418067973	tceA 8.781646 8.88760 8.89114 7.371944	tceA s 5 7.9928 2 7.9928 3 7.9928 4 5.745	td vcr4 841 8.8413 841 8.855 841 8.8451 61 7.2606	7.07809 685 7.07809 642 7.07809 628 6.23248	1 8.4741 1 8.5259 1 8.4985 3 7.0450	7.2764 7.2764 7.2764 7.2764 5.4.943032	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B	21 21 21 21 21 21	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02	1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01	FdhA 12.44 12.22 12.95 11.08 11.46	12.65 12.65 12.65 11.27 11.27	12.41 12.43 12.73 11.37 11.94	12.36 12.36 12.36 11.63 11.63	12.73 13.11 12.78 11.39 11.81	12.88 12.88 12.88 11.57 11.57	12.13 11.94 12.86 10.26 11.32	12.55 12.55 12.55 11.02 11.02	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973	tceA 8.781646 8.887602 8.891143 7.371944 7.388871	tceA s 5 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745	td vcr 841 8.8413 841 8.8555 841 8.8451 61 7.2606 61 7.3214	7.07809 685 7.07809 642 7.07809 628 6.23248 66.23248	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513	7.2764 7.2764 33 7.2764 05 4.943032 4.943032	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.914457 4.669
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C	21 21 21 21 21 21 21	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 1.68E+00	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01	FdhA 12.44 12.22 12.95 11.08 11.46 11.37	12.65 12.65 12.65 11.27 11.27 11.27	12.41 12.43 12.73 11.37 11.94 11.59	12.36 12.36 12.36 11.63 11.63	12.73 13.11 12.78 11.39 11.81 11.71	12.88 12.88 12.88 11.57 11.57	12.13 11.94 12.86 10.26 11.32 10.57	12.55 12.55 12.55 11.02 11.02 11.02	DHC_165 gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973	tceA 8.781646 8.887602 8.89114 7.371944 7.388872 7.389834	tceA s 5 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 4 5.745	td vcr4 841 8.8413 841 8.8555 841 8.8451 661 7.2606 661 7.3214 661 7.3295	7.07809 685 7.07809 642 7.07809 628 6.23248 684 6.23248	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459	7.2764 7.2764 33 7.2764 05 4.943032 4.943032 4.943032	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.914457 4.669 5.869386 4.669
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B	21 21 21 21 21 21	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02	1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00	FdhA 12.44 12.22 12.95 11.08 11.46	12.65 12.65 12.65 11.27 11.27	12.41 12.43 12.73 11.37 11.94 11.59 10.01	12.36 12.36 12.36 11.63 11.63	12.73 13.11 12.78 11.39 11.81 11.71 10.23	12.88 12.88 12.88 11.57 11.57 11.57	12.13 11.94 12.86 10.26 11.32	12.55 12.55 12.55 11.02 11.02	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973 5.698525843	tceA 8.781646 8.887602 8.89114 7.371944 7.388872 7.389834 6.154825	tceA s 5 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 4 5.745 5 5.896	td vcr/ 841 8.8413 841 8.8553 841 8.8451 661 7.2606 661 7.3214 661 7.3295 823 6.1409	7.07809 7.0	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306	7.2764 7.2764 33 7.2764 05 4.943032 4.943032 4.943032 4.943032 5.67668	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.914457 4.669
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A Set3B	21 21 21 21 21 21 21 21 33 33	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 1.68E+00 2.41E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01 -1.62E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00 -3.24E+00 -6.72E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56	12.65 12.65 12.65 11.27 11.27 11.27 9.97	12.41 12.43 12.73 11.37 11.94 11.59 10.01 9.93	12.36 12.36 12.36 11.63 11.63 11.63 10.40	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58	12.88 12.88 12.88 11.57 11.57 11.57 10.21	12.13 11.94 12.86 10.26 11.32 10.57	12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0!	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973 5.698525843	tceA 8.781646 8.887602 8.89114 7.371944 7.38887 7.389834 6.154825 4.635994	tceA s 5 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 4 5.745 5 5.896 4 5.896	ttd vcr/ 841 8.8413 841 8.855 841 8.8451 661 7.2606 661 7.3214 661 7.3295 823 4.5870	7.07809 7.0	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0459 5 5.9306 5 4.3532	7.2764 7.2764 33 7.2764 05 4.943032 32 4.943032 4.943032 4.943032 5.67668 5.67668	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.914457 4.669 5.869386 4.669 4.489716 4.22 2.945365 4.22
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A	21 21 21 21 21 21 21 21 33	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 1.68E+00 2.41E-02 1.57E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03 1.18E-08	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03 5.81E-04	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03 6.57E-08	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01 -1.62E+00 -1.81E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56 9.82	12.65 12.65 12.65 11.27 11.27 11.27 9.97 9.97	12.41 12.43 12.73 11.37 11.94 11.59 10.01	12.36 12.36 12.36 11.63 11.63 11.63 10.40 10.40	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58 10.57	12.88 12.88 12.88 11.57 11.57 11.57	12.13 11.94 12.86 10.26 11.32 10.57 #VALUE!	12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0! #DIV/0!	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093 4.452861088	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973 5.698525843 5.698525843 5.698525843	tceA 8.781646 8.887602 8.891143 7.371944 7.388871 7.389834 6.154825 4.635994 4.934293	tceA s 7.9928 7.9928 7.9928 7.9928 7.9928 5.745 5.745 5.745 5.745 5.8963 5.8963 5.8963	ttd vcr/ 841 8.8413 841 8.855 841 8.8451 661 7.2606 661 7.3214 661 7.3295 823 6.1405 823 4.8954	875 7.07809 885 7.07809 828 6.23248 836 6.23248 836 6.23248 837 6.23248 839 6.23248 830 6.23248 830 6.23248 831 6.23248 832 5.88370 833 6.83370 834 6.83370 835 7.07809 836 6.23248 837 6.23248 837 6.23248 838 6.23248 838 6.23248 838 6.23248 839 6.23248 840 6	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306 5 4.3532 5 4.5682	42 7.2764 47 7.2764 33 7.2764 05 4.943032 32 4.943032 44.943032 5.67668 25 5.67668 44 5.67668	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.869386 4.669 5.869386 4.22 2.945365 4.22 3.43388 4.22
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A Set3B Set3C	21 21 21 21 21 21 21 21 33 33 33	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 1.68E+00 2.41E-02 1.57E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03 1.18E-08	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03 5.81E-04	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03 6.57E-08	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01 -1.62E+00 -1.81E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00 -3.24E+00 -6.72E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56 9.82 10.32	12.65 12.65 12.65 11.27 11.27 11.27 9.97 9.97 9.97	12.41 12.43 12.73 11.37 11.94 11.59 10.01 9.93 10.72	12.36 12.36 12.36 11.63 11.63 11.63 10.40 10.40	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58	12.88 12.88 12.88 11.57 11.57 10.21 10.21 10.21	12.13 11.94 12.86 10.26 11.32 10.57 #VALUE! #VALUE!	12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0! #DIV/0! #DIV/0!	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093 4.452861088	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973 5.698525843 5.698525843 5.698525843	tceA 8.781646 8.887602 8.891143 7.371944 7.388871 7.389834 6.154825 4.635994 4.934293 #VALUE	tceA s 7.9928 7.9928 7.9928 7.9928 5.745 5.745 5.745 5.896 5.896 5.896 5.896 1.24938	std vcr/ 841 8.8413 841 8.855 841 8.8451 661 7.2606 661 7.3214 661 7.3295 823 4.5870 823 4.8954 885 #VALU	875 7.07809 885 7.07809 828 6.23248 838 6.23248 839 6.23248 840 6.23248 850 6.23248 861 6.23248 862 6.23248 863 6.23248 864 6.23248 865 7.07809 867 7.07809 868 7	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306 5 4.3532 5 4.5682 3 #VALU	7.2764 7.2764 33.7.2764 35.4.943032 32.4.943032 34.943032 35.5.67668 35.5.67668 36.5.67668 36.5.67668	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.665 5.914457 4.665 5.869386 4.665 4.489716 4.22 2.945365 4.22 3.43388 4.22
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A Set3B Set3C Set4A	21 21 21 21 21 21 21 21 33 33 33 24	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 2.41E-02 1.57E-02 2.60E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03 1.18E-08 2.18E-07	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03 5.81E-04	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03 6.57E-08	Log Kcis 1.80E+00 1.76E+00 1.70E+01 1.72E-01 2.26E-01 -1.62E+00 -1.81E+00 -1.58E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00 -6.20E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00 -3.24E+00 -6.72E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56 9.82 10.32 #DIV/0!	12.65 12.65 12.65 11.27 11.27 11.27 9.97 9.97 9.97 #DIV/0!	12.41 12.43 12.73 11.37 11.94 11.59 10.01 9.93 10.72 #DIV/0!	12.36 12.36 12.36 11.63 11.63 11.63 10.40 10.40 10.40 #DIV/0!	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58 10.57 #DIV/0!	12.88 12.88 12.88 11.57 11.57 11.57 10.21 10.21 10.21 9.58 9.58	12.13 11.94 12.86 10.26 11.32 10.57 #VALUE! #VALUE! #VALUE! #VALUE!	12.55 12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0! #DIV/0! #DIV/0! #DIV/0!	DHC_16S gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093 4.452861088 4.699565761	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.418067973 5.698525843 5.698525843 2.75119299	tceA 8.781646 8.887602 8.89114 7.371944 7.388872 7.389834 6.154822 4.635994 4.934293 #VALUE 3.386912	tceA s 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 5 5.896 4 5.896 3 5.896 2 2.4938 2 2.4938	ktd vcrA 441 8.8413 441 8.8453 441 8.8453 441 8.8453 441 8.8453 461 7.2606 461 7.3214 461 7.3293 423 6.1403 423 4.5870 423 4.885 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444	875 7.07809 885 7.07809 842 7.07809 828 6.23248 886 6.23248 884 6.23248 823 5.88370 824 5.88370 824 5.88370 825 6.8370 826 6.23248 827 6.23248 828 6.	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306 5 4.3532 5 4.5682 3 #VALU 3 3.3464	7.2764 7.2764 33.7.2764 35.4.943032 32.4.943032 34.943032 35.5.67668 35.5.67668 36.5.67668 36.5.67668 37.5.67668 38.5.67668 39.5.67668 39.5.67668 39.5.67668	7.255479 6.52 7.338236 6.52 7.3393151 6.52 5.914457 4.665 5.914457 4.665 5.869386 4.665 4.489716 4.22 2.945365 4.22 3.43388 4.22 4.489716 4.22 4.489716 4.22 4.489716 4.22
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A Set3B Set3C Set3A Set3B Set3C Set4A Set4B	21 21 21 21 21 21 21 21 33 33 33 24 24	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 2.41E-02 1.57E-02 2.60E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03 1.18E-08 2.18E-07	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03 5.81E-04	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03 6.57E-08	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01 -1.62E+00 -1.81E+00 -1.58E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00 -6.20E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00 -3.24E+00 -6.72E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56 9.82 10.32 #DIV/0! 10.30	12.65 12.65 12.65 11.27 11.27 11.27 9.97 9.97 9.97 #DIV/0!	12.41 12.43 12.73 11.37 11.94 11.59 10.01 9.93 10.72 #DIV/0!	12.36 12.36 12.36 11.63 11.63 11.63 10.40 10.40 10.40 #DIV/0!	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58 10.57 #DIV/0! 9.93	12.88 12.88 12.88 11.57 11.57 11.57 10.21 10.21 10.21 9.58 9.58	12.13 11.94 12.86 10.26 11.32 10.57 #VALUE! #VALUE! #VALUE!	12.55 12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0! #DIV/0! #DIV/0! #DIV/0!	DHC 165 gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093 4.452861088 4.699565761	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.698525843 5.698525843 5.698525843 2.75119299 2.75119299	tceA 8.781646 8.887602 8.89114 7.371944 7.388872 7.389834 6.154822 4.635994 4.934293 #VALUE 3.386912	tceA s 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 5 5.896 4 5.896 3 5.896 2 2.4938 2 2.4938	ktd vcrA 441 8.8413 441 8.8453 441 8.8453 441 8.8453 441 8.8453 461 7.2606 461 7.3214 461 7.3293 423 6.1403 423 4.5870 423 4.885 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444	875 7.07809 885 7.07809 842 7.07809 828 6.23248 886 6.23248 884 6.23248 823 5.88370 824 5.88370 824 5.88370 825 6.8370 826 6.23248 827 6.23248 828 6.	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306 5 4.3532 5 4.5682 3 #VALU 3 3.3464	7.2764 7.2764 33.7.2764 35.4.943032 32.4.943032 34.943032 35.5.67668 35.5.67668 36.5.67668 36.5.67668 37.5.67668 38.5.67668 39.5.67668 39.5.67668 39.5.67668	7.255479 6.52 7.338236 6.52 7.3393151 6.52 5.914457 4.665 5.914457 4.665 5.869386 4.665 4.489716 4.22 2.945365 4.22 3.43388 4.22 4.489716 4.22 4.489716 4.22 4.489716 4.22
JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO JBLM1 TIMETWO	Set1A Set1B Set1C Set2A Set2B Set2C Set3A Set3B Set3C Set4A Set4A Set4A Set4B	21 21 21 21 21 21 21 21 33 33 33 24	6.26E+01 5.70E+01 7.36E+01 1.48E+00 1.49E+00 2.41E-02 1.57E-02 2.60E-02	7.31E+01 5.37E+01 1.46E+02 4.99E-01 4.84E-01 5.28E-01 2.60E-03 1.18E-08 2.18E-07	1.02E+01 1.02E+01 1.13E+01 3.07E-01 3.42E-01 3.37E-01 1.54E-03 5.81E-04	3.91E+00 3.61E+00 5.87E+00 7.25E-02 7.78E-02 7.04E-02 1.84E-03 6.57E-08	Log Kcis 1.80E+00 1.76E+00 1.87E+00 1.70E-01 1.72E-01 2.26E-01 -1.62E+00 -1.81E+00 -1.58E+00	2.22E+00 2.09E+00 2.52E+00 5.84E-02 4.53E-02 8.24E-02 -2.13E+00 -7.47E+00 -6.20E+00	1.01E+00 9.53E-01 1.01E+00 9.18E-01 1.05E+00 1.13E+00 -5.13E-01 -7.80E-01 -4.66E-01 -7.49E-01 -4.73E-01 -7.92E-01 -2.81E+00 -2.28E+00 -3.24E+00 -6.72E+00	FdhA 12.44 12.22 12.95 11.08 11.46 11.37 9.56 9.82 10.32 #DIV/0! 10.30	12.65 12.65 12.65 11.27 11.27 11.27 9.97 9.97 9.97 #DIV/0!	12.41 12.43 12.73 11.37 11.94 11.59 10.01 9.93 10.72 #DIV/0!	12.36 12.36 12.36 11.63 11.63 11.63 10.40 10.40 10.40 #DIV/0!	12.73 13.11 12.78 11.39 11.81 11.71 10.23 10.58 10.57 #DIV/0! 9.93	12.88 12.88 12.88 11.57 11.57 11.57 10.21 10.21 10.21 9.58 9.58	12.13 11.94 12.86 10.26 11.32 10.57 #VALUE! #VALUE! #VALUE! #VALUE!	12.55 12.55 12.55 12.55 11.02 11.02 11.02 #DIV/0! #DIV/0! #DIV/0! #DIV/0!	DHC 165 gene 8.709371652 8.742700785 8.670652654 7.179655172 7.166144123 7.179078359 5.956243093 4.452861088 4.699565761	7.626064285 7.626064285 7.626064285 5.418067973 5.418067973 5.698525843 5.698525843 5.698525843 2.75119299 2.75119299	tceA 8.781646 8.887602 8.89114 7.371944 7.388872 7.389834 6.154822 4.635994 4.934293 #VALUE 3.386912	tceA s 7.9928 2 7.9928 3 7.9928 4 5.745 1 5.745 5 5.896 4 5.896 3 5.896 2 2.4938 2 2.4938	ktd vcrA 441 8.8413 441 8.8453 441 8.8453 441 8.8453 441 8.8453 461 7.2606 461 7.3214 461 7.3293 423 6.1403 423 4.5870 423 4.885 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444 485 47444	875 7.07809 885 7.07809 842 7.07809 828 6.23248 886 6.23248 884 6.23248 823 5.88370 824 5.88370 824 5.88370 825 6.8370 826 6.23248 827 6.23248 828 6.	1 8.4741 1 8.5259 1 8.4985 3 7.0450 3 7.0513 3 7.0459 5 5.9306 5 4.3532 5 4.5682 3 #VALU 3 3.3464	7.2764 7.2764 33.7.2764 35.4.943032 32.4.943032 34.943032 35.5.67668 35.5.67668 36.5.67668 36.5.67668 37.5.67668 38.5.67668 39.5.67668 39.5.67668 39.5.67668	7.255479 6.52 7.338236 6.52 7.393151 6.52 5.914457 4.669 5.914457 4.669 5.869386 4.669 4.489716 4.22 2.945365 4.22

JBLM2, Compiled rate coefficients and biomarker abundances

						LOG + 0 Transfo	med for Ana	lysis													_						
			\vdash			Rates				Peptides								DHC gene		Functional	Genes						
	TIME ZERO	Kcis	Kcis 95%CI	Kvc	Kvc 95%CI	Log Kcis	KcisSD	Log Kvc	LogKvcSD	FdhA	FdhA std	PceA I	ceA std	TceA	TceA std	VcrA	VcrA std	DHC_16S gene	DHC std	tceA	ce A std	vcrA	vcrA std	fdhA	fdhA std	pceA	pceA st
JBLM2 TZERO	Set1A	1.05E+00	9.37E-01	1.13E-01	1.82E-02	0.021	0.386	-0.946	-1.326	10.38	9.96	10.77	10.67	10.55	10.58	#VALUE!	9.58	7.13	7.06	7.24	6.38	7.17	6.74		4 6.78	6.06	6 4.0
JBLM2 TZERO	Set1B		1.14E+00		2.78E-02	0.021	0.472	-0.880	-1.141	10.16	9.96	10.35	10.67	10.07	10.58	10.05	9.58	7.19	7.06	7.16	6.38	7.04	6.74				
JBLM2 TZERO	Set1C		1.28E+00		4.06E-02	0.051	0.522	-0.759	-0.977	10.52	9.96	10.74	10.67	10.75	10.58	9.76	9.58	7.54	7.06	7.29	6.38	7.34	6.74				
JBLM2 TZERO	Set1A_Du				3.07E-02	-0.550	-1.249		-1.337	10.64	10.14	10.76	10.73	10.63	10.55	#VALUE!	9.70	7.16	6.76	7.06	6.48	7.03	6.49				
JBLM2 TZERO JBLM2 TZERO	Set1B_Du				2.28E-02 2.11E-02	-0.527 -0.656	-1.328 -1.591	-4.583 -2.173	-1.467 -1.501	10.31	10.14 10.14		10.73	10.41	10.55	10.20 9.95	9.70 9.70	6.62	6.76 6.76	6.77	6.48	6.67 6.91	6.49 6.49				
JBLM2 TZERO	Set1C_Du				8.56F-03	-0.656	-2.901	-2.173	-1.573	10.63	10.14	11.77	11.52	11.64	11.55	12.03	11.27	4.51	4.38	4.32	4.02	4.32	4.22				
JBLM2 TZERO	Set2B			0.202 00	8.29E-03	-2.755	-3.118	-2.590	-1.587			11.84	11.52	11.72	11.55	12.00	11.27	3.79	4.38	3.66	4.02	3.66	4.22				
JBLM2 TZERO	Set2C		_		9.89E-03	-2.620	-2.913	-2.374	-1.510			11.85	11.52	11.91	11.55	11.92	11.27	3.30	4.38	3.06	4.02	3.06	4.22				-
JBLM2 TZERO	Set3/			2.79E-03	1.35E-02																						
JBLM2 TZERO	Set3l	8.75E-04	2.18E-04	3.31E-03	1.31E-02																						
JBLM2 TZERO	Set3	9.61E-04	2.52E-04	1.71E-03	1.33E-02																						
JBLM2 TZERO	Set4A				1.37E-02																						
JBLM2 TZERO	Set4B				1.32E-02																						
JBLM2 TZERO IBI M2 TZERO	Set4C				e not sampled rate not compu									-													-
JBLM2 TZERO JBLM2 TZERO					rate not compu																						_
JBLM2 TZERO	Set6A				rate not compu																						_
JBLM2 TZERO	Set6B	rate not co	rate not co	rate not co	rate not comput	ted																					
JBLM2 TZERO	Set1l		1.57E-02			-8.48E-01	-1.52E+00	-1.72E+00	-1.80E+00	9.55	9.20		9.46	9.13	9.25	8.56	8.41	5.98	6.29	6.01	6.31	6.10					
JBLM2 TZERO			1.33E-02			-1.06E+00		-1.75E+00		8.92	9.20		9.46	#N/A	9.25	8.78	8.41	5.85	6.29	6.00	6.31	6.12					
JBLM2 TZERO			1.50E-02		1.51E-02	-1.02E+00		-1.68E+00		9.61	9.20		9.46	9.60	9.25	7.96	8.41	6.62	6.29	6.66	6.31	6.74	6.38				
JBLM2 TZERO	Set1D_Dup				1.64E-02	-1.11E+00		-5.00E+00		9.86	9.55	9.84	10.19	9.86	10.08	9.39	9.35	7.01	6.08	6.91	6.32	7.09	6.30				
JBLM2 TZERO JBLM2 TZERO	Set1E_Dup Set1F Dup		3.78E-03 3.50E-03		1.98E-02 1.83E-02	-1.23E+00 -1.25E+00		-1.74E+00 -2.57E+00		9.99	9.55 9.55	10.29 10.17	10.19	10.21	10.08	9.39 9.80	9.35 9.35	6.92 7.02	6.08	6.95 7.08	6.32	6.92 7.00	6.30 6.30				
JBLM2 TZERO			2.61E-04		1.52E-02	-1.25E+00 -3.00E+00		-2.57E+00 -4.05E+00		8.53		#DIV/0!	8.15	8.10	8.13	9.80 8.94	8.73	5.20	4.60	5.28	4.46	5.41	4.85				
JBLM2 TZERO			2.86E-04			-3.00E+00		-5.00E+00		8.60		#DIV/0!	8.15	8.34	8.13	#VALUE!	8.73	5.36	4.60	5.39	4.46	5.60					
JBLM2 TZERO			4.84E-04			-5.00E+00		-4.00E+00		8.85	8.29	8.68	8.15	8.51	8.13	8.01	8.73	5.35	4.60	5.36	4.46	5.54	4.85				
JBLM2 TZERO	Set5C	rate not co	rate not co	rate not co	rate not compu	ted																					
JBLM2 TZERO	Set5D	rate not co	rate not co	rate not co	rate not compu	ted																					
JBLM2 TZERO	Set6	rate not co	rate not co	rate not co	rate not compu	ted																					
JBLM2 TZERO	Set6l	rate not co	rate not co	rate not co	rate not compu	ted																					
						LOG + 0 Transfo	mad for Ana	hoic																			
						Rates	illed for Alla	iiysis		Peptides								DHC gene		Functional	Genes						
	TIME TWO	Kcis	Kcis 95%CI		Kvc 95%CI	Log Kcis	KcisSD	Log Kvc		FdhA	FdhA std		ceA std		rceA std			DHC_16S gene	DHC std		ce A std		vcrA std		fdhA std		pceA st
JBLM2 TTWO	Set1A				0.018179135	0.021	0.386	-0.946	-1.326	10.40	9.84	10.72	10.26	10.65	10.22	#VALUE!	9.00	6.73	5.97	6.79	6.14	6.86	6.21				
JBLM2 TTWO JBLM2 TTWO	Set1B Set1C		1.141049 1.279709			0.021	0.472 0.522	-0.880 -0.759	-1.141 -0.977	10.14 10.38	9.84 9.84	10.34 10.43	10.26 10.26	10.25 10.37	10.22 10.22	9.60 9.41	9.00 9.00	6.68	5.97 5.97	6.60	6.14	6.71 6.92	6.21 6.21				
JBLM2 TTWO	Set1C Set1A_Dup		3 0.037581			-0.550	-1.249		-0.977	10.38	9.84	10.43	10.26	10.37	9.98	9.41	9.00	6.82	6.37	6.82	6.54	6.89	6.21				
JBLM2 TTWO	Set1B_Dup		0.05/301			-0.527	-1.328		-1.467	10.57	9.75	10.75	10.14	10.41	9.98	10.17	9.82			6.58	6.54						
JBLM2 TTWO	Set1C_Dup		0.021250					-4.505												0.50		0.55	0.55		0.20		
JBLM2 TTWO	Scere_bup		3 0.031359 3 0.017099	0.006713				-2 173	-1 501									6.94	6.37	7.03	6 54	7 04	6.55	7.02	6.28		
	Set2A		0.017099		0.02105224	-0.656	-1.591		-1.501 -1.573	10.61	9.75		10.14	10.19	9.98	10.14	9.82	7.11	6.37	7.03	6.54	7.04	6.55	7.02	6.28	5.97	
	Set2A Set2B	0.0027742	0.017099 0.000403	0.0030997	0.02105224 0.008559355	-0.656 -2.557	-1.591 -2.901	-2.509	-1.573											7.03	6.54	7.04	6.55	7.02	6.28	5.9	
JBLM2 TTWO	Set2B	0.0027742 0.0017588	0.017099 0.000403 0.000244	0.0030997 0.0025729	0.02105224 0.008559355 0.008290165	-0.656 -2.557 -2.755	-1.591	-2.509	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
		0.0027742 0.0017588 0.002399	0.017099 0.000403	0.0030997 0.0025729 0.0042237	3 0.02105224 7 0.008559355 9 0.008290165 7 0.009893947	-0.656 -2.557	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C	0.0027742 0.0017588 0.002399 0.0010045	3 0.017099 2 0.000403 3 0.000244 9 0.000391	0.0030997 0.0025729 0.0042237 0.0027909	3 0.02105224 7 0.008559355 9 0.008290165 7 0.009893947 9 0.013478322	-0.656 -2.557 -2.755	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A	0.0027742 0.0017588 0.002399 0.0010045 0.0008753	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000218	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135	3 0.02105224 7 0.008559355 9 0.008290165 7 0.009893947 9 0.013478322	-0.656 -2.557 -2.755	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009609	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000218 9 0.000252 L 0.000295	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189	3 0.02105224 7 0.008559355 9 0.008290165 7 0.009893947 9 0.013478322 5 0.013063268 L 0.013283913 9 0.013659581	-0.656 -2.557 -2.755	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009609 0.001121 0.0008245	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000218 9 0.000252 1 0.000299	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426	3 0.02105224 7 0.008559355 9 0.008290165 7 0.009893947 9 0.013478322 5 0.013063268 1 0.013283913 9 0.013659581 5 0.013224546	-0.656 -2.557 -2.755	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.03	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009609 0.001121 0.0008245 not sample	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000218 9 0.000252 1 0.000295 5 0.000209	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426 not sample	3 0.02105224 7 0.008559355 9 0.008290165 7 0.003893947 9 0.013478322 5 0.013063268 L 0.013283913 9 0.013659581 5 0.013224546 enot sampled	-0.656 -2.557 -2.755 -2.620	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.03	6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009609 0.001121 0.0008245 not sample	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000252 1 0.000295 5 0.000209 e not sample	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426 not sample	8 0.02105224 7 0.00859355 9 0.008290165 7 0.00893947 9 0.013478322 5 0.013063268 1 0.01363268 1 0.013659581 5 0.013224546 1 ont sampled orate not comput	-0.656 -2.557 -2.755 -2.620	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009609 0.001121 0.0008245 not sample rate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000252 1 0.000259 6 0.000209 e not sample or rate not co	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426 not sample rate not co	3 0.02105224 7 0.008559355 9 0.008290165 9 0.013478322 5 0.013063268 1 0.013283913 9 0.01324546 1 0.013224546 1 0.013224546 1 0.013224546 1 0.01324546 1 0.0132466 1 0.01324666 1 0.0132466 1 0.0132466 1 0.0132466 1 0.0132466 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.013246666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.013246666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.01324666 1 0.	-0.656 -2.557 -2.755 -2.620	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set4C Set5A Set5B Set6A	0.0027742 0.0017588 0.0010045 0.0010045 0.0008752 0.001121 0.0008245 not sample rate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000218 0 0.000255 5 0.000209 e not sample or rate not co	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426 not sample rate not co	3 0.02105224 7 0.008559355 9 0.008290165 7 0.008839347 9 0.013478322 6 0.013063268 0 0.013665981 5 0.013224546 e not sampled orate not computate no	-0.656 -2.557 -2.755 -2.620	-1.591 -2.901 -3.118	-2.509 -2.590	-1.573 -1.587											7.03	6.54	7.04	6.55	7.02	2 6.28	5.9.	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set6B	0.0027742 0.0017588 0.002399 0.0010045 0.0008752 0.000121 0.0008245 not sample rate not co	8 0.017099 2 0.000403 3 0.000244 5 0.000262 8 0.000252 1 0.000252 1 0.000299 9 not sample 9 orate not co	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037189 0.0037426 not sample rate not co	3 0.02105224 0.008559355 7 0.00859355 7 0.008893947 9 0.013478322 0.013063268 1 0.013283913 9 0.01365981 5 0.013224546 e not sampled o rate not compuly rate not compuly	-0.656 -2.557 -2.755 -2.620 tted tted tted	-1.591 -2.901 -3.118 -2.913	-2.509 -2.590 -2.374	-1.573 -1.587 -1.510	10.61	9.75	10.64	10.14	10.19	9.98	10.14	9.82	7.11	6.37								
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set5B Set6B Set1D	0.0027742 0.0017588 0.002399 0.0010045 0.0008753 0.0009605 0.001121 0.0008245 not sample rate not co rate not co rate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000252 1 0.000295 5 0.000209 e not sample or rate not co	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0017131 0.0037426 ont sample rate not co- rate not co- rate not co- rate not co- rate not co- rate not co-	3 0.02105224 7 0.008559355 9 0.008290165 9 0.008893947 9 0.013478322 0.013063268 1 0.0132283913 9 0.013629581 5 0.013224546 enot sampled rate not compular at the compular of the computation of the	-0.656 -2.557 -2.755 -2.620 tted tted tted tted tted -8.48E-01	-1.591 -2.901 -3.118 -2.913 -1.52E+00	-2.509 -2.590 -2.374 -1.72E+00	-1.573 -1.587 -1.510	9.60	9.75	9.42	9.24	10.19	9.98	10.14	9.82	7.11	6.37	5.74	5.53	5.84	5.63	5.74	4 5.30	4.23	
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B	0.0027742 0.0017588 0.002399 0.0010045 0.0008755 0.000825 0.001121 0.0008245 not sample rate not co rate not co rate not co rate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000252 3 0.000252 1 0.000252 1 0.000299 9 0.000299 9 or ate not contrate n	0.0030997 0.0042237 0.0042237 0.0027909 0.003133 0.0037139 0.00371426 not sample rate not co rate not co orate not co	3 0.02105224 0.008559355 7 0.00859355 7 0.008893947 9 0.013478322 5 0.013263983 1 0.013659881 5 0.013224546 enot sampled orate not compuly orate not compuly	-0.656 -2.557 -2.755 -2.620 tted ted ted ted ted ted -8.48E-01 -1.06E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00	9.60 9.91	9.75 9.31 9.31	9.42 9.74	9.24 9.24	10.19	9.89 9.89 9.89	9.68 9.21	9.82 9.82 9.21 9.21	7.11 5.55 5.70	6.37 5.35 5.35	5.74 5.93	5.53 5.53	5.84	5.63 5.63	5.74 5.93	4 5.30 3 5.30	4.23	3 3.
JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4B Set5B Set5B Set5B Set6B Set6B Set6B Set1D Set1D Set1E Set1E	0.0027742 0.0017588 0.002399 0.0010048 0.00008752 0.0008245 0.0008245 not sample rate not co rate not co rate not co 0.0141780 0.087545 0.0961964	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000252 8 0.000252 1 0.000295 5 0.000209 e not sample or rate not co or rate not co or rate not co	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0037189 0.0037426 not sample rate not corrate not correct not	3 0.02105224 7 0.008559355 9 0.008290165 7 0.00839347 9 0.013478322 5 0.013063268 1 0.013283913 9 0.013659581 5 0.013224546 e not sampled orate not compute rate not not compute rate not compute rate not compute rate not compute	-0.656 -2.557 -2.755 -2.620 ted ted ted ted -8.48E-01 -1.02E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00 -1.54E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00	9.60 9.91 9.83	9.75 9.31 9.31 9.31	9.42 9.74 9.66	9.24 9.24 9.24 9.24	10.19 10.02 10.31 10.21	9.89 9.89 9.89	9.68 9.21 9.58	9.82 9.21 9.21 9.21	7.11 5.55 5.70 5.90	6.37 5.35 5.35 5.35	5.74 5.93 6.09	5.53 5.53 5.53	5.84 6.04 6.19	5.63 5.63 5.63	5.74 5.93 5.96	4 5.303 3 5.305 5 5.30	4.23 4.43 4.47	3 3. 7 3.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set5B Set1D Set1E Set1F Set1D_Dup	0.0027742 0.0017588 0.002395 0.0010044 0.0008753 0.00008605 0.001121 0.0008244 not sample rate not corate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000252 9 0.000252 9 0.000295 9 0 0.000295 9 0 0.000299 9 ort sample orate not contrate	0.0030997 0.0025729 0.0042237 0.0042237 0.0027909 0.0033135 0.0017131 0.0037426 orate not corrate not	3 0.02105224 7 0.008559355 9 0.008290165 7 0.00839347 9 0.013478322 6 0.013063268 1 0.013283913 9 0.013659581 6 not sampled o rate not computor rate not com	-0.656 -2.557 -2.755 -2.620 tted ted ted ted -8.48E-01 -1.00E+00 -1.11E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00 -1.54E+00 -1.92E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -5.00E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00	9.60 9.91 9.83 9.83	9.75 9.31 9.31 9.31 9.31	9.42 9.74 9.66 10.21	9.24 9.24 9.24 9.24 9.73	10.19 10.02 10.31 10.21 9.95	9.89 9.89 9.89 9.89 9.89	9.68 9.21 9.58	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73	5.35 5.35 5.35 6.47	5.74 5.93 6.09 6.29	5.53 5.53 5.53 6.24	5.84 6.04 6.19 6.35	5.63 5.63 5.63 6.20	5.74 5.93 5.96 6.79	4 5.30 3 5.30 5 5.30 5 6.44	4.23 4.43 4.47 5.58	3 3. 7 3. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4B Set4C Set5A Set5B Set6A Set6B Set1D Set1E Set1L	0.0027742 0.0017588 0.002395 0.001004 0.00008752 0.0009605 0.001121 orate not corate not	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000255 5 0.000295 5 0.000295 6 0.000295 6 0.000295 6 0.000295 7 0.00045 8 0 0.00469 9 0.015692 9 0.013327 9 0.004629 9 0.004629 9 0.004629 9 0.004629	0.0030997 0.0025729 0.002237 0.002290 0.0027909 0.0037189 0.0037426 not sample rate not co rate not co	3 0.02105224 7 0.008559355 9 0.008290165 7 0.008893947 9 0.013478322 5 0.013063268 0.013283913 5 0.013659581 5 0.013224546 enot sampled rate not compute rate not compute rate not compute rate not compute rate not compute rate not compute rate rate rate rate rate rate rate ra	-0.656 -2.557 -2.755 -2.620 ted ted ted ted -1.00E+00 -1.12E+00 -1.23E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00 -1.54E+00 -1.92E+00 -2.01E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -1.68E+00 -1.74E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.29E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3. 7 3. 8 5. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set5B Set1D Set1E Set1F Set1D_Dup	0.0027742 0.0017588 0.001309 0.001309 0.0010045 0.0008752 0.0001621 0.0001621 0.0001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621 0.001621	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000252 1 0.000252 1 0.000259	0.0030997 0.0025729 0.0025729 0.002237 0.002237 0.0027909 0.0033135 0.0017131 0.0037189 orate not corate not cor	3 0.02105224 7 0.008559355 9 0.008290165 7 0.008893947 9 0.013478322 5 0.013063268 9 0.013659581 9 0.013224546 6 enot sampled or rate not computed or rate not rate not rate not rate not rate not rate not rate not rate not rate not rate not	-0.656 -2.557 -2.755 -2.620 tted ted ted ted -8.48E-01 -1.00E+00 -1.11E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00 -1.54E+00 -1.92E+00 -2.01E+00 -2.04E+00	-2.599 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -5.00E+00 -1.74E+00 -2.57E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.29E+00 -1.32E+00	9.60 9.91 9.83 9.83	9.75 9.31 9.31 9.31 9.31	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.24 9.24 9.73	10.19 10.02 10.31 10.21 9.95	9.89 9.89 9.89 9.89 9.89	9.68 9.21 9.58	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73	5.35 5.35 5.35 6.47	5.74 5.93 6.09 6.29	5.53 5.53 5.53 6.24	5.84 6.04 6.19 6.35	5.63 5.63 5.63 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3. 7 3. 8 5. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set6B Set6B Set1E Set1E Set1E Set1E Set1E Dup	0.0027742 0.0017588 0.002399 0.0010045 0.0003953 0.0009605 0.000122 0.0000224 not sample rate not corate not corate not corate not corate not co.0.1417804 0.087545 0.0961964 0.0782688 0.05966964 0.05666665 0.0001	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000262 3 0.000255 5 0.000295 5 0.000295 6 0.000295 6 0.000295 6 0.000295 7 0.00045 8 0 0.00469 9 0.015692 9 0.013327 9 0.004629 9 0.004629 9 0.004629 9 0.004629	0.0030997 0.0025729 0.0025729 0.0025729 0.003135 0.0017131 0.0037189 0.0037189 0.0037189 0.0037189 0.0037426 not sample rate not co rate not co rate not co rate not co rate not co rate not co rate not co rate not co 0.0188711 0.0168781 0.0206977 1E-05 0.0008875 0.000095	3 0.0215224 7 0.00859355 9 0.008290165 7 0.00889347 7 0.00889347 9 0.013478322 5 0.01326326 0 0.013283913 5 0.013224546 0 rate not compurate not compurate not compurate not compurate not compurate not compurate not compu	-0.656 -2.557 -2.755 -2.620 tted tted tted tted -1.06E+00 -1.11E+00 -1.125E+00 -1.125E+00 -1.125E+00	-1.591 -2.901 -3.118 -2.913 -1.52E+00 -1.59E+00 -1.54E+00 -2.01E+00 -2.04E+00 -3.09E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -5.00E+00 -1.74E+00 -2.57E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.37E+00 -1.37E+00 -1.32E+00 -1.32E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3. 7 3. 8 5. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6B Set1D Set1E Set1E Set1E Set1E Set1E Set1F Dup Set1E_Dup Set1F_Dup	0.0027742 0.0017588 0.002392 0.0010045 0.0003952 0.00008752 0.0001021 0.00008245 not sample rate not corate no	3 0.017099 2 0.000403 3 0.000244 9 0.000391 5 0.000218 9 0.000252 L 0.000295 or rate not contrate	0.0030997 0.0025729 0.0025729 0.00227909 0.0033135 0.0017131 0.0037188 0.0037188 0.0037426 not sample rate not co rate not co rate not co rate not co 0.0188711 0.0176388 0.0206977 1E-05 0.0183197 0.0026875 0.00000	3 0.0105224 7 0.00859397 7 0.00859397 7 0.00859397 7 0.00859397 8 0.013478322 8 0.01367838 9 0.01367838 9 0.013679381 9 0.013689381 9 0.01369581 9 0.01369581 9 0.01369581 9 0.013638863 9 0.013638863 9 0.015823781 9 0.015818954 0 0.01582781	-0.656 -2.557 -2.755 -2.620 ted ted ted ted ted ted -1.06E+00 -1.02E+00 -1.11E+00 -1.23E+00 -1.25E+03	-1.591 -2.901 -3.118 -2.913 -2.913 -1.52E+00 -1.59E+00 -1.92E+00 -2.01E+00 -2.04E+00 -3.09E+00 -3.09E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -1.74E+00 -2.57E+00 -4.05E+00 -4.05E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.32E+00 -1.32E+00 -1.32E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3. 7 3. 8 5. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set5B Set1D Set1E Set1D_Dup Set1E_Dup Set1E_Dup Set1F_Dup Set2D Set2D Set2D Set3D Se	0.0027742 0.001758 0.002395 0.002095 0.0010045 0.000875 0.0009605 0.001121 0.0008245 0.006245 0.006166666 0.00782688 0.005666666 0.0000000000000000000000000	3 0.017099 2 0.000403 3 0.000244 9 0.000391 9 0.000252 9 0.000255 0 0.000295 10 0.000295 10 0.000296 10 0.000296 10 0.000299	0.0030997 0.0025729 0.0025729 0.0022939 0.0033135 0.0017131 0.0037188 0.0037188 0.0037426 not sample rate not co r	3 0.0215224 7 0.00859355 9 0.008290155 7 0.00859347 7 0.00859367 9 0.013478322 6 0.013263268 9 0.013878321 9 0.013824546 0 rate not computor rate not comput	-0.656 -2.557 -2.755 -2.620 tted ted ted ted -1.066-00 -1.126-00 -1.226-00 -1.226-00 -3.006-00 -5.006-00	-1.591 -2.901 -3.118 -2.913 -2.913 -1.52E+00 -1.59E+00 -1.92E+00 -2.01E+00 -2.04E+00 -3.09E+00 -3.09E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -5.00E+00 -1.74E+00 -2.57E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.32E+00 -1.32E+00 -1.32E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3. 7 3. 8 5. 8 5.
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5A Set5B Set6A Set6B Set1D Set1E Set1E Dup Set1E_Dup Set1E_Dup Set2D Set2D Set2D Set2D Set2D Set2D Set3D Set4D Set4D Set5D	0.0027742 0.001758 0.002392 0.002095 0.0010045 0.0008752 0.0000605 0.001121 0.0008245 0.001121 0.0008245 0.001121 0.0008245 0.01121 0.0008245 0.0961960 0.0961960 0.0982965 0.0992448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448 0.09692448	3 0.017099 3 0.000403 3 0.000244 9 0.000391 9 0.000252 8 0.000252 9 0.000259 6 0.000269 6 0.000269 6 0.000269 6 0.000269 6 0.000269 7 0.000269 1 0.000269 1 0.000269 1 0.000269 1 0.000269 1 0.000261 1 0.000261 1 0.000264	0.0030997 0.0025729 0.0025729 0.0042237 0.0042237 0.0027909 0.0033135 0.0037189 0.0037426 not sample rate not corate not	3 0.015224 7 0.008593937 7 0.008593937 7 0.008593937 7 0.008593937 8 0.013478325 8 0.013269331 9 0.013659581 9 0.013269331 9 0.013269331 9 0.013283931 9 0.013283931 9 0.013283931 9 0.013283931 9 0.013283931 9 0.015283931 9 0.015283931 9 0.015283931 9 0.015283931 9 0.015283931	-0.656 2.557 2.755 2.2620 tted ted ted ted ted ted 1.026-00 1.116-00 1.1254-00 3.006-00 3.006-00 5.006-00 tted	-1.591 -2.901 -3.118 -2.913 -2.913 -1.52E+00 -1.59E+00 -1.92E+00 -2.01E+00 -2.04E+00 -3.09E+00 -3.09E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -1.74E+00 -2.57E+00 -4.05E+00 -4.05E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.32E+00 -1.32E+00 -1.32E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3.1 7 3.1 8 5.0 8 5.0
JBLM2 TTWO JBLM2 TTWO	Set2B Set2C Set3A Set3B Set3C Set4A Set4B Set4C Set5B Set5B Set5B Set5B Set1D Set1E Set1E Set1E Set1E Set1E Set1E, Dup Set1E, Dup Set2E Set2E Set2E Set3E Set3E Set5B Set5B	0.0027742 0.0017588 0.002392 0.0010045 0.0008752 0.0010050 0.001121 0.0008245 not sample rate not corate not co	3 0.017099 2 0.000403 3 0.000244 9 0.000391 9 0.000252 9 0.000252 5 0.000262 5 0.000262 6 0.00027 6 0.00027 7 0.0002	0.0030997 0.0025729 0.0042237 0.0027909 0.0033135 0.0037138 0.00371426 not sample rate not corrate not correct not	3 0.0215224 7 0.00859355 9 0.008290155 7 0.00859347 7 0.00859367 9 0.013478322 6 0.013263268 9 0.013878321 9 0.013824546 0 rate not computor rate not comput	-0.656 2.557 -2.755 -2.620 -2.	-1.591 -2.901 -3.118 -2.913 -2.913 -1.52E+00 -1.59E+00 -1.92E+00 -2.01E+00 -2.04E+00 -3.09E+00 -3.09E+00	-2.509 -2.590 -2.374 -1.72E+00 -1.75E+00 -1.68E+00 -1.74E+00 -2.57E+00 -4.05E+00 -4.05E+00	-1.573 -1.587 -1.510 -1.80E+00 -1.53E+00 -1.53E+00 -1.37E+00 -1.32E+00 -1.32E+00 -1.32E+00	9.60 9.91 9.83 9.87	9.75 9.31 9.31 9.31 9.46 9.46	9.42 9.74 9.63 10.21 10.22	9.24 9.24 9.27 9.73	10.19 10.02 10.31 10.21 9.95 10.16	9.89 9.89 9.89 9.81 9.81	9.68 9.21 9.53 9.24	9.82 9.21 9.21 9.21 9.61	7.11 5.55 5.70 5.90 6.73 7.00	5.35 5.35 5.35 6.47 6.47	5.74 5.93 6.09 6.29 6.70	5.53 5.53 5.53 6.24 6.24	5.84 6.04 6.19 6.35 6.71	5.63 5.63 5.63 6.20 6.20	5.74 5.96 6.79 6.92	4 5.30 3 5.30 6 5.30 9 6.449 2 6.449	4.23 4.43 4.47 5.58 5.48	3 3.8 7 3.8 8 5.0 8 5.0

JBLM2, Compiled rate coefficients and biomarker abundances (continued)

					LO	G + 0 Transfor	med for Ana	lysis																			
					Ra	tes			P	eptides								DHC gene		Functional	Genes						
	TIME THRE	E Kcis	Kcis 95%CI	Kvc	Kvc 95%CI	Log Kcis	KcisSD	Log Kvc	LogKvcSD	FdhA	FdhA std	PceA I	ceA std	TceA	ceA std	VcrA	VcrA std	DHC_16S gene	DHC std	tceA	tceA std	vcrA	vcrA std	fdhA	fdhA std	pceA	pceA sto
JBLM2 TTHREE	Set1A	1.0486058	0.937007	0.1131681	0.018179135	0.021	0.386	-0.946	-1.326	10.54	9.87	10.76	10.25	10.82	10.37	8.34	9.47	6.62	5.63	6.61	4.55	6.66	5.11	6.71	5.97	5.36	4.5
JBLM2 TTHREE	Set1B	1.0497445	1.141049	0.1317895	0.027812426	0.021	0.472	-0.880	-1.141	10.46	9.87	10.57	10.25	10.63	10.37	9.72	9.47	6.68	5.63	6.60	4.55	6.66	5.11	6.70	5.97	5.45	4.5
JBLM2 TTHREE	Set1C	1.1256485	1.279709	0.1741944	0.040566336	0.051	0.522	-0.759	-0.977	10.38	9.87	10.35	10.25	10.49	10.37	9.72	9.47	6.70	5.63	6.60	4.55	6.64	5.11	6.54	5.97	5.33	4.5
JBLM2 TTHREE	Set1A_Dup	0.2817833	0.037581	0.012639	0.03067384	-0.550	-1.249	-1.898	-1.337	10.57	9.75	10.59	10.10	10.18	9.90	9.72	9.53	6.65	5.88	6.62	5.53	6.33	5.29	6.60	6.02	5.84	4.9
JBLM2 TTHREE	Set1B_Dup	0.2973343	0.031359	2.61E-05	0.022750745	-0.527	-1.328	-4.583	-1.467	10.58	9.75	10.70	10.10	10.35	9.90	10.02	9.53	6.77	5.88	6.67	5.53	6.26	5.29	6.74	6.02	5.84	4.9
JBLM2 TTHREE	Set1C_Dup	0.2206698	0.017099	0.006713	0.02105224	-0.656	-1.591	-2.173	-1.501	10.48	9.75	10.52	10.10	10.17	9.90	10.06	9.53	6.76	5.88	6.68	5.53	6.33	5.29	6.77	6.02	5.72	4.9
JBLM2 TTHREE	Set2A	0.0027742	0.000403	0.0030997	0.008559355	-2.557	-2.901	-2.509	-1.573																		
JBLM2 TTHREE	Set2B	0.0017588	0.000244	0.0025729	0.008290165	-2.755	-3.118	-2.590	-1.587																		
JBLM2 TTHREE	Set2C	0.002399	0.000391	0.0042237	0.009893947	-2.620	-2.913	-2.374	-1.510																		
JBLM2 TTHREE	Set3A	0.0010045	0.000262	0.0027909	0.013478322																						
JBLM2 TTHREE	Set3B	0.0008753	0.000218	0.0033135	0.013063268																						
JBLM2 TTHREE	Set3C	0.0009609	0.000252	0.0017131	0.013283913																						
JBLM2 TTHREE	Set4A	0.001121	0.000295	0.0037189	0.013659581																						
JBLM2 TTHREE	Set4B	0.0008245	0.000209	0.0037426	0.013224546																						
JBLM2 TTHREE	Set4C	not sample	not sample	not sample	not sampled																						
JBLM2 TTHREE	Set5A	rate not cor	rate not co	rate not co	rate not compute	d																					
JBLM2 TTHREE	Set5B				rate not compute																						
JBLM2 TTHREE	Set6A				rate not compute																						
JBLM2 TTHREE	Set6B	rate not cor	rate not co	rate not co	rate not compute	d																					
JBLM2 TTHREE	Set1D	0.1417804	0.015692	0.0188711	0.008121799	-8.48E-01	-1.52E+00	-1.72E+00	-1.80E+00	9.55	9.23	9.08	8.96	10.05	9.93	9.79	9.36	5.87	5.49	5.81	5.47	5.81	5.48	5.84	5.49	4.86	4.48
JBLM2 TTHREE	Set1E	0.087549	0.013327	0.0176388	0.015232098	-1.06E+00	-1.59E+00	-1.75E+00	-1.53E+00	9.81	9.23	9.36	8.96	10.31	9.93	9.26	9.36	5.74	5.49	5.63	5.47	5.70	5.48	5.69	5.49	4.71	4.48
JBLM2 TTHREE	Set1F	0.0961964	0.015042	0.0206977	0.015125851	-1.02E+00	-1.54E+00	-1.68E+00	-1.53E+00	9.77	9.23	9.46	8.96	10.31	9.93	9.49	9.36		5.49	4.76	5.47	4.85		4.95	5.49	4.10	4.48
JBLM2 TTHREE	Set1D Dup	0.0782689	0.004629	1E-05	0.016380863	-1.11E+00	-1.92E+00	-5.00E+00	-1.37E+00	9.45	9.54	9.74	10.05	9.58	9.73	9.98	9.64		6.45	6.37	6.02	6.41		6.75	6.55	5.41	5.15
JBLM2 TTHREE	Set1E Dup		0.003784	0.0183197		-1.23E+00	-2.01E+00	-1.74E+00	-1.29E+00	9.08	9.54	10.38	10.05	10.09	9.73	9.02	9.64		6.45	5.36	6.02	4.63		5.01	6.55	3.25	5.15
JBLM2 TTHREE	Set1F Dup	0.0566669	0.003497	0.0026875	0.018287361	-1.25E+00	-2.04E+00	-2.57E+00	-1.32E+00	9.08	9.54	9.38	10.05	9.17	9.73	9.64	9.64	6.61	6.45	6.15	6.02	6.25	6.11	6.83	6.55	5.36	5.15
JBLM2 TTHREE	Set2D	0.001	0.000261	0.00009	0.015181954	-3.00E+00	-3.09E+00	-4.05E+00	-1.32E+00																		
JBLM2 TTHREE	Set2E		0.000286	0.00001	0.016602235	-3.00E+00	-3.05E+00		-1.29E+00																		
JBLM2 TTHREE	Set2F		0.000484	0.0001		-5.00E+00		-4.00E+00																			
JBLM2 TTHREE	Set5C				rate not compute																						
JBLM2 TTHREE	Set5D				rate not compute																						
JBLM2 TTHREE	Set6C				rate not compute																						
JBLM2 TTHREE	Set6D				rate not compute								_														

APPENDIX E KEY POINTS OF CONTACT

KEY POINTS OF CONTACT

The key personnel involved in this project and their contact information is summarized in Table 8-1 below. The Principal Investigator (PI) and all co-PIs share responsibility for the overall execution and delivery of this project, including data quality, analysis, interpretation and preparation of deliverables. Mandy Michalsen, PI, is responsible for the overall execution of this project and identification/coordination with cVOC-contaminated DoD field sites. Ember Korver, USACE Project Manager, is responsible for contractual oversight and general project support. Paul Hatzinger, co-PI, is responsible for microcosm testing, SDC-9TM culture growth and application, chemical analyses for microcosms, and data interpretation. Frank Löffler, co-PI, is responsible for qPCR, gene-transcript-protein correlation factors, data interpretation. Kate Kucharzyk, co-PI, is responsible for proteomic and genomic analysis and data interpretation. John Wilson and Jack Istok, co-PIs, are responsible for technical reviews and data interpretation.

Table 8-1. Project points of contact

POINT OF	ORGANIZATION	Phone	Dala in Duais at
CONTACT Name	Name Address	Fax E-mail	Role in Project
Mandy Michalsen	U.S. Army Corps of	(p) 206-764-3324	Principal Investigator,
	Engineers, Seattle	mandy.m.michalsen@usace.army.mil	Field Support
Ember Korver	U.S. Army Corps of	(p) 206-764-6792	Project Management,
	Engineers, Seattle	ember.e.korver@usace.army.mil	Contract Administration
Paul Hatzinger	CB&I	(p) 267-337-4003	Co-Principal
		paul.hatzinger@cbifederalservices.com	Investigator
Frank Löffler	University of	(p) 865-974-4933	Co-Principal
	Tennessee	frank.loeffler@utk.edu	Investigator
Kate Kucharzyk	Battelle Memorial	(p) 614-424-5489	Co-Principal
	Institute	kucharzyk@battelle.org	Investigator
John Wilson	Scissortail	(p) 580-421-3551	Co-Principal
	Environmental	john@sissortailenv.com	Investigator
	Solutions		
Jack Istok	Oregon State	(p) 541-619-3996	Co-Principal
	University	jack.istok@oregonstate.edu	Investigator