Final Technical Report – August 2015

Proposal Title: Metaproteomics Identifies the Protein Machinery Involved in Metal and Radionuclide Reduction in Subsurface Microbiomes and Elucidates Mechanisms and U(VI) Reduction Immobilization

Funding Opportunity Announcement Number: DE-PS02-09ER09-07

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Performance Period: 07-01-2010 to 05-31-2015 (no-cost extensions were granted in March 2013, July 2014, and December 2014). The Co-Investigators were funded directly and no subcontracts were established. This report summarizes the progress from the entire collaborative project across one University effort and one National Laboratory project (University of Tennessee – Susan Pfiffner (Lead PI) Registration # ER65014; and Oak Ridge National Laboratory – Robert Hettich (Co-PI) Registration # ERKP780).

DOE ERSP-BER Grant No.: DE-SC0004793

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Deliverables

Peer-Reviewed Journal Publications (Published)

1. Nissen, S., X. Liu, K. Chourey, R. L. Hettich, D. D. Wagner, S. M. Pfiffner, and F. E. Löffler. 2012. Comparative *c*-type expression analysis in *Shewanella oneidensis* strain MR-1 and *Anaeromyxobacter dehalogenans* strain 2CP-C grown with soluble and insoluble oxidized metal electron acceptors. Biochem. Soc. Trans. 40:1204-1210. doi: 10.1042/BST20120182
2. Chourey, K., S. Nissen, T. Vishnivetskaya, M. Shah, S. Pfiffner, R. L. Hettich, and F. E. Löffler. 2013. Environmental proteomics reveals early microbial community responses to biostimulation at a uranium- and nitrate-contaminated site. Proteomics. 13:2921-2930. doi: 10.1002/pmic.201300155
3. Liu, X. 4015. Comparative proteomics reveals core vs. unique molecular signatures for dissimilatory metal reducing bacteria grown with various electron acceptors. Doctoral Dissertation. Trace: Tennessee Research and Creative Exchange 2014-08-01T07:00:00Z.

Peer-Reviewed Journal Publications (In Preparation)

4. Nissen, S., K. Chourey, X. Liu, R. L. Hettich, and F. E. Löffler. Identification of a *c*-type cytochrome with specific function in electron transfer to insoluble manganese oxide. In preparation.
5. Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. Delineation of microbial *c*-type cytochromes. In preparation.
6. Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. MS-based proteomic characterization of *Anaeromyxobacter dehalogenans* strain 2CP-C reveals elevated energy metabolism during growth with metal electron acceptors. In preparation.

Oral Presentations

1. Pfiffner, S. M., F. E. Löffler, S. Nissen, K. M. Ritalahti, T. Vishnivetskaya, A. Layton, G. Sayler, K. Chourey, X. Liu, M. Shah, and R.L. Hettich. Metaproteomics of metal and radionuclide bioreduction. TES/SBR Joint Investigators Meeting, Potomac, MD, USA May 15, 2013.
2. Löffler, F. E. Immobilizing a Legacy: Bacterial reduction of hexavalent Uranium. China-US Joint Workshop "Systems Biology for Environmental Sustainability". Shenyang University, Shenyang, China, May 27, 2013.
3. Löffler, F. E. Immobilizing a Legacy: Bacterial reduction of hexavalent Uranium (Keynote Lecture). 8th International Symposium of Subsurface Microbiology, September 11, 2011.
4. Löffler, F. E. Radionuclide contamination in subsurface environments: How can microbes help? Energy*Solutions*, University of Tennessee, Knoxville, TN. April 14, 2011
5. Löffler, F. E. Radionuclide Bioremediation. Energy*Solutions*, Oak Ridge, TN. November 17, 2010.
6. Sanford, R., K. Fletcher, S. Thomas, K. Kemner, M. Boyanov, K. Ritalahti, and F. Löffler. Uranium Biogeochemistry: novel insights from a microbe's prospective. Goldschmidt 2010, Earth, Energy and Environment, Knoxville, TN, June 18, 2010.
7. Löffler, F. E. Bioremediation: Science-based engineering of the contaminated subsurface. State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resource and Environmental Engineering, East China University of Science and Technology (ECUST), Shanghai, China. October 23, 2010.
8. Löffler, F. E. New insights into microbial radionuclide reduction and immobilization. Argonne National Laboratory, Argonne, IL. October 2009.

Poster Presentations

1. Liu, X., K. Chourey, S. Nissen, R. Hettich, S. Pfiffner, and F. Löffler. *Anaeromyxobacter dehalogenans* strain 2CP-C employs distinct metabolic activities for growth with metal vs. non-metal electron acceptors. TES/SBR Joint Investigators Meeting, Potomac, MD, USA, April 28-29, 2015.
2. Pfiffner, S. M., S. Nissen, X. Liu, K. Chourey., T. A. Vishnivetskaya, R. Hettich, and F. Löffler. Identification of a *c*-type cytochrome specific for manganese dioxide (mno_2) reduction in *Anaeromyxobacter dehalogenans* strain 2CP-C. American Geophysical Union 2014.
3. Nissen, S., X. Liu, K. Chourey, K. M. Ritalahti, R. Hettich, S. Pfiffner, and F. E. Löffler. *c*-Type cytochrome profiling of metal-reducing bacteria and identification of a *c*-type cytochrome involved in Mn(IV) reduction in *Anaeromyxobacter dehalogenans* strain 2CP-C. TES/SBR Joint Investigators Meeting, Potomac, MD, USA, May 6-7, 2014.
4. Nissen S., X. Liu, K. Chourey, R. L. Hettich, S. M. Pfiffner, and F. E. Löffler. Identification of a *c*-type cytochrome involved in Mn(IV) reduction in *Anaeromyxobacter dehalogenans* strain 2CP-C. ORNL Postdoc Research Symposium, Oak Ridge, TN, USA, July 18, 2013.
5. Nissen, S., X. Liu, K. Chourey, K. M. Ritalahti, R. Hettich, S. Pfiffner, and F. E. Löffler. Identification of a *c*-type cytochrome involved in Mn(IV) reduction in *Anaeromyxobacter dehalogenans* strain 2CP-C. Abstract 1672. 113th General Meeting of the American Society for Microbiology, Denver, CO, USA, May 18-21, 2013.
6. Liu, X., S. Nissen, K. Chourey, F. E. Löffler, S. M. Pfiffner, and R. L. Hettich. Proteomics reveals growth-dependent *c*-type cytochrome expression in dissimilatory metal-reducing bacteria. Abstract 1671, 113th General Meeting of the American Society for Microbiology, Denver, CO, USA, May 18-21, 2013.
7. Walshe, G., J. Merryfield, K. Ritalahti, S. Nissen, K. Chourey, R. Hettich, K. Pennell, A. Basu, R. Sanford, C. Lundstrom, T. Johnson, K. Kemner, E. O'Loughlin, M. Boyanov, and F. Löffler. Exploring the responses of metal-reducing bacteria to fluctuating redox conditions. DOE TES/SBR Joint Investigators Meeting, Potomac, MD, USA, May 13-15, 2013.
8. Liu X., S. Nissen, K. Chourey, F. E. Löffler, and R. L. Hettich. Proteomic characterization of *c*-type cytochrome profiles of dissimilatory metal reducing bacteria. GST Retreat, Oak Ridge, TN, USA, March 1, 2013.
9. Liu, X., K. Chourey, S. Nissen, F. Löffler, and R. Hettich. Proteome characterization of metal-reducing bacteria reveals varying *c*-type cytochrome expression in response to different electron acceptors. Abstract 2822, 160th ASMS Conference on Mass Spectrometry and Allied Topics, Vancouver, BC, Canada, May 20-24, 2012.
10. Nissen, S., X. Liu, K. Chourey, R. Hettich, F. E. Löffler. Identification of *c*-type cytochrome activity biomarkers. A Biochemical Society Focused Meeting – Electron transfer at the microbe-mineral interface. University of East Anglia, UK, April 2-4, 2012.
11. Chourey, K., X. Liu, S. Nissen, F. E. Löffler, M. Shah, R. Hettich, K. Ritalahti, T. Vishnivetskaya, A. Layton, G. Sayler, and S. Pfiffner. Design and application of proteomics workflows to monitor and predict in situ activity of metal-reducing bacteria. DOE-SBR Annual PI Meeting, Washington, DC, April 30-May 2, 2012.
12. Liu, X., K. Chourey, S. Nissen, A. Green, S. Sun, S. Connon, V. Orphan, F. Löffler, and R. Hettich. Enhanced protein extraction for microbial (meta)proteomics of defined laboratory and environmental samples. 59th ASMS Conference on Mass Spectrometry, June 5-9, 2011.
13. Chourey, K., S. Nissen, F. Löffler, R. Hettich, K. Ritalahti, T. Vishnivetskaya, A. Layton, G. Sayler, S. Pfiffner. Comprehensive proteome characterization and cytochrome *c* expression in *Anaeromyxobacter dehalogenans* 2CP-C as a function of electron acceptor growth conditions. DOE-SBR Annual PI Meeting, Washington D.C., 26-28 April 2011.
14. Pfiffner, S. M., A. C. Layton, G. S. Sayler, F. E. Loeffler. Metaproteomics identifies the protein

machinery involved in metal and radionuclide reduction in subsurface microbes and elucidates mechanisms. DOE-SBR Annual PI Meeting, Washington D.C., March 29–31, 2010.

Objectives

The overall goal for this collaborative research project was to design innovative proteomics tools, validate the new tools with defined laboratory cultures, and apply proteomics workflows to study proteome profiles of soil microbial communities. To accomplish these objectives a three-pronged approach were used. Geochemistry indicated conditions prior and after treatment, while genomics (ii) indicated collective genotype (i.e., the potential for activity). The proteomics analysis (iii) measured proteins, and hence gene activity with a focus on those proteins and pattern recognition that were linked with metal-reducing activity.

Accomplishments

Objective 1. Establish a sequence library comprised of *c*-type cytochromes implicated in metal reduction and other redox processes.

c-Type cytochrome libraries were comprised using available genomic sequences from relevant environmental isolates.. FASTA protein databases specific to target organisms: *Geobacter*, *Anaeromyxobacter*, and *Shewanella,* were utilized to develop corresponding predicted proteome database obtained from Joint Genome Institute (JGI, http://genome.jgi.doe.gov/). *A. dehalogenans* strain 2CP-C had 69 putative c-type cytochromes predicted, whereas *S. oneidensis* strain MR-1 and *G. daltonii* strain FRC-32 had respectively ,40, and 72 putative c-type cytochromes predicted. In addition to these microorganisms, computationally assembled databases were comprised which included genome sequences of 152 representative soil microbes (rifle_jgidb145_geo7_ contams_ psm_sept2008) and an Area 2 groundwater database (OakRidgeIFRC_Feb2013).

Manuscript:

Nissen, S., X. Liu, K. Chourey, R. L. Hettich, D. D. Wagner, S. M. Pfiffner, and F. E. Löffler. 2012. Comparative *c*-type expression analysis in *Shewanella oneidensis* strain MR-1 and *Anaeromyxobacter dehalogenans* strain 2CP-C grown with soluble and insoluble oxidized metal electron acceptors. Biochem. Soc. Trans. 40:1204-1210. doi: 10.1042/BST20120182

Chourey, K., S. Nissen, T. Vishnivetskaya, M. Shah, S. Pfiffner, R. L. Hettich, and F. E. Löffler. 2013. Environmental proteomics reveals early microbial community responses to biostimulation at a uranium- and nitrate-contaminated site. Proteomics. 13:2921-2930. doi: 10.1002/pmic.201300155

Objective 2. Investigate U(VI) reduction under controlled conditions to refine metaproteomics and metagenomics analyses.

While the quantitative assessment of 16S rRNA genes can provide information about the abundance of metal-reducing bacteria, this measure does not necessarily correlate with specific metal reduction activity. The goal was to identify biomarkers that are directly involved in the process of interest. A shared characteristic among *Shewanella* spp., *Geobacter* spp., and *Anaeromyxobacter dehalogenans* is the large number of c-type cytochrome genes encoded on the genomes of these metal reducers. Protein biomarkers were explored for gaining information of the presence and activity of metal- and radionuclide-transforming bacteria. Proteomic workflows have been applied to these organisms grown with different electron acceptors including oxygen, fumarate, nitrate, ferric citrate, goethite, and manganese oxide. Following biomass collection, trypsin proteolysis, solid-phase extraction, and solvent exchange, the peptides were analyzed with via 2-D-LC-MS/MS on a linear ion trap mass spectrometer (LTQ XL) or a dual pressure Linear Ion trap (LTQ Velos). Up to 2,000, or about half of the predicted open reading frames (ORFs) were identified, and distinct c-type cytochrome expression profiles were determined in cells grown with different electron acceptors. While several c-type cytochromes were expressed under all growth conditions, the analysis identified c-type cytochromes that were only expressed under specific growth conditions. For example, these efforts identified a c-type cytochrome *A. dehalogenans* that was only expressed when the organisms were grown with manganese oxide as electron acceptor. Consequently, the detection of this specific c-type cytochrome serves as an indicator of cells that actively use manganese oxide as electron acceptor. This proteomics approach (see Objective 6) was expanded and applied to biomass collected from the uranium-contaminated IFRC site. Environmental metaproteomics revealed specific responses of the microbial community to biostimulation, and demonstrated the utility of this approach for monitoring microbial activities related to radionuclide reduction and immobilization in groundwater.

Manuscripts:

Nissen, S., X. Liu, K. Chourey, R. L. Hettich, D. D. Wagner, S. M. Pfiffner, and F. E. Löffler. 2012. Comparative *c*-type expression analysis in *Shewanella oneidensis* strain MR-1 and *Anaeromyxobacter dehalogenans* strain 2CP-C grown with soluble and insoluble oxidized metal electron acceptors. Biochem. Soc. Trans. 40:1204-1210. doi: 10.1042/BST20120182

Chourey, K., S. Nissen, T. Vishnivetskaya, M. Shah, S. Pfiffner, R. L. Hettich, and F. E. Löffler. 2013. Environmental proteomics reveals early microbial community responses to biostimulation at a uranium- and nitrate-contaminated site. Proteomics. 13:2921-2930. doi: 10.1002/pmic.201300155

Nissen, S., K. Chourey, X. Liu, R. L. Hettich, and F. E. Löffler. Identification of a c-type cytochrome with specific function in electron transfer to insoluble manganese oxide. In preparation.

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. Delineation of microbial c-type cytochromes. In preparation.

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. MS-based proteomic characterization of Anaeromyxobacter dehalogenans strain 2CP-C reveals elevated energy metabolism during growth with metal electron acceptors. In preparation.

Objective 3. Monitor the fate of uranium and changes of the metaproteome following perturbation with oxidants including nitrate and oxygen and apply metaproteomic analysis to correlate the change in protein expression pattern with uranium (IV) oxidation and mobilization.

Proteomic workflows have been applied to *A. dehalogenans* strain 2CP-C, *G. daltonii* strain FRC-32, and *S. oneidensis* MR-1 grown with either oxygen or nitrate. Strain 2CP-C and strain MR-1 grown with nitrate as electron acceptor yielded the highest number of identified c-type cytochromes (37 and 20, respectively). Strain FRC-32 did not grow under these electron accepting conditions. All hierarchical clustering approaches reveal that the expression profiles of c-type cytochromes can be grouped into three categories: "core" (present in all conditions), "unique" (represented in only one condition), and non-detected. Under oxygen growth conditions, six *c*-type cytochromes were uniquely expressed that were no expressed under nitrate growth conditions. Likewise, three *c*-type cytochromes were uniquely expressed under nitrate growth conditions, but not with oxygen. For *S. oneidensis* MR-1 three *c*-type cytochromes were expressed under nitrate growth that was not under oxygen, while one uniquely expressed .

Geobacter spp. have also been implicated in U(VI) reduction, and tools targeting the 16S rRNA gene of this bacterial group have been designed. Quantitative monitoring of *Geobacter* spp. 16S rRNA gene abundances at the Oak Ridge IFRC site suggested that the *Geobacter* population declined following oxygen intrusion, which was consistent with the known *Geobacter* physiology. *Geobacter* spp. do not respire oxygen and are considered strict anaerobes. These observations suggested that microbes active in oxic-anoxic transition zones play relevant roles for controlling radionuclide mobility, and focused laboratory experiments were conducted (see above).

Manuscript:

Nissen, S., X. Liu, K. Chourey, R. L. Hettich, D. D. Wagner, S. M. Pfiffner, and F. E. Löffler. 2012. Comparative *c*-type expression analysis in *Shewanella oneidensis* strain MR-1 and *Anaeromyxobacter*

dehalogenans strain 2CP-C grown with soluble and insoluble oxidized metal electron acceptors. Biochem. Soc. Trans. 40:1204-1210. doi: 10.1042/BST20120182

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. Delineation of microbial c-type cytochromes. In preparation.

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. MS-based proteomic characterization of Anaeromyxobacter dehalogenans strain 2CP-C reveals elevated energy metabolism during growth with metal electron acceptors. In preparation.

Objective 4. Apply qRT-PCR to quantify gene transcription and expression and correlate this information with 16S rRNA abundance and U(VI) reduction activity or lack thereof.

Anaeromyxobacter dehalogenans strains were characterized as versaphiles with the ability to couple energy conservation to a variety of electron acceptors, including hexavalent uranium, U(VI). PCR-based tools targeting the 16S rRNA gene of U(VI)-reducing *A. dehalogenans* strains were designed and validated (Thomas et al., 2010). Using the developed PCR-based tools, the 16S rRNA gene copies and transcript copies for Adeh_1278 genes were tracked when *A. dehalogenan*s strain 2CP-C were grown in medium amended with acetate and manganese oxide. Expression of the Adeh_1278 gene was identified as a more abundant cytochrome under manganese oxide growth and was expressed more in the initial stages of growth when manganese oxide was reduced.

Manuscripts:

Nissen, S., K. Chourey, X. Liu, R. L. Hettich, and F. E. Löffler. Identification of a c-type cytochrome with specific function in electron transfer to insoluble manganese oxide. In preparation.

Objective 5. Apply comparative metaproteomics to identify proteins that correlate with metal reduction activity.

In order to understand the cellular mechanisms that enable these target microorganisms to survive under different environmental conditions, a mass spectrometry-based proteomics approach was implemented to characterize the proteome profiles of target microooorganisms grown with various electron acceptors. A total of eight growth conditions were tested, providing a global survey of the proteome-wide responses to different electron acceptors. The pan-proteome For *A. dehalogenans* consists of 2,846 proteins, representing 65% of predicted open reading frames. The results also revealed a core proteome of 710

proteins that comprise the fundamental cellular machinery needed regardless of varying electron-accepting environments. For each target microooorganism, the predicted *c*-type cytochromes were generally two fold higher than the detected *c*-type cytochromes. The cellular localization distribution varied by microorganism and by growth condition. To visualize proteins significantly changed between growth conditions, differentially abundant proteins were mapped to metabolic pathways in KEGG database using iPath 2.0. Significantly abundant proteins in metal electron acceptor growth mapped to metabolic pathways participating in the TCA cycle, and amino acid, nucleotide and carbohydrate metabolism, whereas significantly abundant proteins in non-metal electron acceptor growth mostly involved in regulatory pathways for translation and cell motility. Metabolic pathways mapping clearly indicated elevated expression of energy production pathways in growth with metal electron acceptors. Amino acid and nucleotide metabolism also demonstrated significant higher abundance levels in metal electron acceptor- growth, which could be a result of overall higher expression of energy-generating pathways in cells grown with metal electron acceptors. For each target microorganism, the core proteome covered almost all metabolic pathways represented by their corresponding pan-proteomes. Unique proteins were detected for each target organisms, and their expression and possible functionalities were linked to specific growth conditions through proteomics measurements.

Manuscripts:

Nissen, S., X. Liu, K. Chourey, R. L. Hettich, D. D. Wagner, S. M. Pfiffner, and F. E. Löffler. 2012. Comparative *c*-type expression analysis in *Shewanella oneidensis* strain MR-1 and *Anaeromyxobacter dehalogenans* strain 2CP-C grown with soluble and insoluble oxidized metal electron acceptors. Biochem. Soc. Trans. 40:1204-1210. doi: 10.1042/BST20120182

Nissen, S., K. Chourey, X. Liu, R. L. Hettich, and F. E. Löffler. Identification of a c-type cytochrome with specific function in electron transfer to insoluble manganese oxide. In preparation.

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. Delineation of microbial c-type cytochromes. In preparation.

Liu, X., S. Nissen, K. Chourey, S. M. Pfiffner, F. E. Löffler and R. L. Hettich. MS-based proteomic characterization of Anaeromyxobacter dehalogenans strain 2CP-C reveals elevated energy metabolism during growth with metal electron acceptors. In preparation.

Objective 6: The application of metaproteomics approaches to field site samples provided information about the U(VI)-reducing community and its (in)activity, which could be used to guide site management decisions to achieve efficient and lasting uranium immobilization.

Proteome profiling and *c*-type cytochrome libraries comprised earlier in this project were applied to archived ORIFRC groundwater filters from the emulsified vegetable oil (EVO) biostimulation demonstration. Filters were extracted for proteins as described earlier. Peptide measurements were performed via 2-dimensional liquid chromatography coupled with tandem mass spectrometry (2D-LC-MS/MS). Data obtained from each analysis were matched up using two databases using DBDigger software. Similarly to pyrosequencing and qPCR data, the metaproteomics analyses confirmed the quick biomass increase and community structure alteration following 4 days EVO biostimulation. These results indicate an increase in biomass following EVO amendment, which likely leads to stimulated microbial growth and the formation of cell aggregates or the attachment and growth of cells to soil particles or colloids. These aggregates and colloids were captured by a higher pore size (8.0 μm) filter and single cells (plantonic) reached the smaller pore size (0.2 μm) filter. The data sets obtained for the 8.0 μm pre-filter and 0.2 μm filter were combined for statistical analysis to represent the demonstration site groundwater collected from both the up-gradient and the down-gradient wells. Results from proteome characterization show higher number of proteins being identified in 4 days post-EVO amended FW216 sample (1,192 proteins) compared to the up-gradient FW 215 sample (548 proteins).

The proportion of proteins from Betaproteobacteria was reduced whereas the proteins belonging to members of the Firmicutes and Deltaproteobacteria increased in abundance in the FW216 sample influenced by EVO biostimulation. However, members of the Betaproteobacteria (i.e., *Dechloromonas*, *Ralstonia*, *Rhodoferax*, *Polaromonas*, *Delftia*, *Chromobacterium*) and Firmicutes (*Pelosinus*) dominated the biostimulated aquifer community. In particular, proteins involved in ammonium assimilation, EVO degradation, and polyhydroxybutyrate (PHB) granule formation were prominent following biostimulation. Proteins for the TCA/Glyoxylate pathway, the EVO degradation pathway, and the nitrogen cycle were identified. These pathways were linked to *Dechloromonas aromatica*, and *Dechloromonas*-like microbes (e.g., Rhodocyclaceae) which were shown to dominated the groundwater 4-days after EVO amendment. *c*-Type cytochromes such as citrate synthase, a biomarker for hexavalent uranium reduction activity, were detected at low level suggesting that metal reduction has not commenced 4 days post-EVO delivery. Five annotated heme proteins were also identified, of which enhanced expression of four of the proteins could be a response correlated with nutritional amendment. Expression of Geob3403 appears to be novel in response to biostimulation of the groundwater community. The detection of this heme protein corresponds to the increased acetate levels observed at well FW216 and presence of *Geobacter* detect in the 4-day time point (Gihring et al 2011). A no cost extension was requested to analyze more EVO field samples and to collaborate with ENIGMA. Plans were made to analyze more EVO archived samples and potentially to collaborate with D. Elias on IFRC bioreactors.

Unfortunately the archived samples from the EVO field demonstration were lost when a -80°C freezer went down. Furthermore, the bioreactor experiments did not come on-line during the time frame of this project.

Manuscripts:

Chourey, K., S. Nissen, T. Vishnivetskaya, M. Shah, S. Pfiffner, R. L. Hettich, and F. E. Löffler. 2013. Environmental proteomics reveals early microbial community responses to biostimulation at a uranium- and nitrate-contaminated site. Proteomics. 13:2921-2930. doi: 10.1002/pmic.201300155