Final Technical Report - December 2014

Proposal Title: MURMoT: Design and Application of Microbial Uranium Reduction Monitoring Tools

Funding Opportunity Announcement Number: DE-PS02-07ER08-09

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Performance Period: 07/01/2009-12/31/14 (no-cost extension granted in August 2013). The award was initially made in 2009 but funding to the PI was delayed because of Dr. Löffler's transition from the Georgia Institute of Technology to the University of Tennessee/Oak Ridge National Laboratory. The Co-Investigators were funded directly and no subcontracts were established. This report summarizes the progress from the entire collaborative project across three separately funded University efforts and one National Laboratory project (University of Tennessee – Frank Löffler (Lead PI) – award number ER65019; University of Illinois Urbana/Champaign – Robert Sanford (Co-PI) – award number ER64781; Tufts University - Kurt Pennell (Co-PI) – award number ER64780; Argonne National Laboratory – Ken Kemner (Co-PI) FWP# - FWP 66286).

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DOE/Office of Science Program Office: Environmental Remediation Science Program

DOE/Office of Science Program Office Technical Contact: Dr. Todd Anderson

Students trained: Dr. Gillian Walshe, postdoc, 3/2012-12/2014
Ms. Jenny Onley (formerly Merryfield), Ph.D. student
Dr. Anirban Basu, Ph.D. student, graduated 12/2013
Mr. Theodore Grimm, M.S. student
Dr. Kelly Fletcher, Ph.D. student, graduated 12/2010
Dr. Darlene (formerly Ryan) Wagner, Ph.D. student, graduated 09/2012
Dr. Jarrod Pollock, postdoc 6/2010-11/2011
Mr. Alessandro Scandurra, M.S. student, University of Trieste, Italy (6/2010-9/2010)
Kevin Hade, undergraduate student 5/2009-12/2010

Deliverables

Peer-Reviewed Journal Publications (published)

1. Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. 2014. Uranium isotopic fractionation factors during U(VI) reduction by bacterial isolates. Geochim. Cosmochim. Acta. 136:100-113. doi.org/10.1016/j.gca.2014.02.041
2. Im, I., J. Lee, and F. E. Löffler. 2013. Interference of ferric ions with ferrous ion quantification using the ferrozine assay. J. Microbiol. Methods. 95:366-367. doi: 10.1016/j.mimet.2013.10.005
3. 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
4. Yoon, S. R. A. Sanford, and F. E. Löffler. 2013. *Shewanella* spp. use acetate as electron donor for denitrification but not ferric iron or fumarate reduction. Appl. Environ. Microbiol. 79: 2818-2822. doi: 10.1128/AEM.03872-12
5. 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
6. Boyanov, M. I., K. E. Fletcher, M. Jae Kwon, X. Rui, E. J. O'Loughlin, F. E. Löffler, and K. M. Kemner. 2011. Solution and microbial controls on the formation of reduced U(IV) species. Environ. Sci. Technol. 45:8336-8344. doi**:** 10.1021/es2014049
7. Wu, W., J. Carley, S. J. Green, J. Luo, S. D. Kelly, J. van Nostrand, K. Lowe, T. Mehlhorn, S. Carroll, B. Boonchayanant, F. E. Löffler, D. Watson, K. M. Kemner, J. Zhuo, P. K. Kitanidis, J. E. Kostka, P. M. Jardine, and C. S. Criddle. 2010. Effects of nitrate on the stability of uranium in a bioreduced region of the subsurface. Environ. Sci. Technol. 44:5104-5111. doi: 10.1021/es100408r
8. Fletcher, K. E., M. I. Boyanov, S. H. Thomas, Q. Wu, K. M. Kemner, and F. E. Löffler. 2010. Uranium reduction to mononuclear U(IV) by *Desulfitobacterium* spp. Environ. Sci. Technol. 44:4705-4709. doi: 10.1021/es903636c
9. Thomas, S. H., R. A. Sanford, M. B. Leigh, E. Cardenas, and F. E. Löffler. 2010. Unique ecophysiology among U(VI)-reducing bacteria as revealed by evaluation of oxygen metabolism in *Anaeromyxobacter dehalogenans* strain 2CP-C. Appl. Environ. Microbiol. 76:176-183. doi: 10.1128/AEM.01854-09
10. Bopp IV, C.J., C. Lundstrom, T. Johnson, R. A. Sanford, P. E. Long, and K. H. Williams. 2010. Uranium isotope ratios as indicators of reduction: results from an *in situ* biostimulation experiment at Rifle, CO. Environ. Sci. Technol., 44:5927-5933.

Peer-Reviewed Journal Publications (In Preparation)

11. Walshe, G., K. D. Pennell, and F. E. Löffler. One-dimensional column experiments demonstrate competition between distinct ferric iron-reducing bacteria in oxic-anoxic transition zones. In preparation.
12. 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.
13. Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. Effect of U(VI) concentration variation on U isotope fractionation during U(VI) reduction by a novel *Shewanella* isolate. In Preparation.
14. Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. Isotopic fractionation of ^{235}U, ^{236}U, and ^{238}U during microbial U(VI) reduction. In Preparation.

Oral Presentations

1. 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.
2. Kemner, K. M. Uranium in Nanostructures Resulting from Biogeochemical Interactions. Materials Research Society Spring Meeting, San Francisco, California, April 3, 2013.
3. Boyanov, M., D. Latta, B. Mishra, E.O'Loughlin, and K. Kemner. Reduction of U(VI) at Biological and Mineral Surfaces: Mechanisms and Factors Controlling the Speciation of U(IV). Session Chemical Pictures of Environmental Interfaces: Advances in Molecular-Level Understanding and Quantitative Analysis of Species. ACS Meeting New Orleans, LA, April 8, 2013
4. Boyanov, M. Understanding Contaminant and Iron Biogeochemistry: The X-Ray Advantage. Colloquium, Department of Geology, University of Illinois at Urbana-Champaign, February 1, 2013.
5. Löffler, F. E. Immobilizing a Legacy: Bacterial Reduction of Hexavalent Uranium (Keynote Lecture). 8th International Symposium of Subsurface Microbiology, September 11, 2011.
6. Löffler, F. E. Radionuclide Contamination in Subsurface Environments: How Can Microbes Help? Energy*Solutions*, University of Tennessee, Knoxville, TN. April 14, 2011
7. Löffler, F. E. Radionuclide Bioremediation. Energy*Solutions*, Oak Ridge, TN. November 17, 2010.
8. Löffler, F., M. Boyanov, T. Johnson, C. Lundstrom, K. Kemner, K. Pennell, K. Ritalahti, and R. Sanford. Microbial Uranium Reduction and Monitoring Tools. DOE-SBR PI Meeting, Washington D.C. April 27, 2011.
9. 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.
10. Boyanov, M. I., B. Mishra, D. E. Latta, X. Rui, M.-J. Kwon, K. E. Fletcher, F. E. Löffler, E. J. O'Loughlin, and K. M. Kemner. Bioreduction of U(VI) in the Presence of Phosphate. European Geosciences Union General Assembly, Vienna, Austria, April 22-27, 2012.
11. 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.
12. Löffler, F. E. MURMoT: Design and Application of Microbial Uranium Reduction Monitoring Tools. Argonne National Laboratory, Argonne, IL. September 16. 2010.
13. Löffler, F. E. New Insights into Microbial Radionuclide Reduction and Immobilization. Argonne National Laboratory, Argonne, IL. October 2009.
14. Kemner, K. M. Investigating Mineral-Microbe Interactions with Hard x-Ray Radiation. University of Illinois, Champaign-Urbana Geology Department, Champaign-Urbana, Illinois, September 11, 2009.
15. Boyanov, M. I., K. E. Fletcher, E. J. O'Loughlin, M. J. Kwon, F. E. Löffler, and K. M. Kemner. Bioreduction of U(VI): Factors Controlling the Speciation of U(IV). Session Uranium Biogeochemistry: Transformations and Applications, Ascona, Switzerland, March 11-16, 2012.
16. Boyanov, M., K. Fletcher, E. O'Loughlin, M. Kwon, B. Mishra, K. Skinner, D. Sholto-Douglas, F. Löffler, and K. Kemner. Differences in the Electron Transfer Mechanisms of Gram-Negative vs. Gram-Positive Bacteria Suggested by the Products of Uranyl Reduction. Biosciences Division seminar, Argonne National Laboratory, December 3, 2009.

Poster Presentations

1. Chourey, K., R. Hettich, K.M. Ritalahti, B. Simsir, and F.E. Löffler. Development of a metaproteomics platform to detect reductive dechlorination biomarker proteins in environmental samples. Battelle 9th International Conference in Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, USA, May 19-22, 2014.
2. Kemner, K. M., M. I. Boyanov, B. Mishra, E. J. O'Loughlin, and D. Latta. Controls on the products of uranium reduction: Are we modeling the correct species?", Radioactive working group breakout meeting, Annual DOE TES/SBR PI Meeting, Washington, DC, May 6-7, 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. Poster presented at the 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, Poster presented at the 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 conditons. 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/ 2012. 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 7th Annual PI Meeting, Washington, DC, April 30-May 2, 2012.
12. Boyanov, M., E. O'Loughlin, D. Latta, B. Mishra, K. Skinner, M. Scherer, W.-M. Wu, C. Criddle, F. Yang, T. Marsh, R. Sanford, F. Löffler, M. Mueller, T. Mehlhorn, K. Lowe, D. Watson, S. Brooks, and K. Kemner. Understanding uranium transformations in reduced sediments: An integrated bottom-up and top-down X-ray spectroscopy approach. DOE-SBR 7th Annual PI Meeting, Washington, DC, April 30-May 2, 2012.
13. Sanford, R., A. Basu, C. Lundstrom, T. Johnson, J. Merryfield, G. Walshe, K. Kemner, M. Boyanov, K. Pennell, K. Ritalahti, and F. Löffler (PI). 2012. Monitoring microbial uranium reduction at the oxic-anoxic interface. DOE-SBR 7th Annual PI Meeting, Washington, DC, April 30-May 2, 2012.

14. Kemner, K., E. O'Loughlin, M. Boyanov, D. Antonopoulos, D. Latta, T. Flynn, S. Brooks, E. Carpenter, C. Criddle, J. Fredrickson, F. Löffler, T. Marsh, M. McCormick, B. Mishra, R. Sanford, C. Segre, M. Scherer, W. Wu, J. Zachara, C. Giometti. The Argonne Subsurface Biogeochemical Research Program Scientific Focus Area. DOE-SBR 7th Annual PI Meeting, Washington, DC, April 30-May 2, 2012.
15. Wagner, D. D. and F. E. Löffler. Heme counts of *c*-type cytochromes as a factor in Proteobacterial respiratory physiology. In: Abstracts of the 8th Georgia Tech - Emory - ORNL Conference on Bioinformatics, Atlanta, GA, USA, November 10-12, 2011.
16. Sanford, R., K. Fletcher, S. Thomas, K. Kemner, M. Boyanov, K. Ritalahti, and F. Löffler. 6/18/2010. Uranium Biogeochemistry: Novel insights from a microbe's prospective. Goldschmidt, Earth, Energy and Environment, Knoxville, TN., June 13-18, 2010.
17. 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.
18. Boyanov, M., K. Fletcher, M. Kwon, X. Rui, E. O'Loughlin, F. Löffler, and K. Kemner. U(IV) Products suggest distinct U(VI) bioreduction mechanisms in *Desulfitobacterium*, *Anaeromyxobacter*, and *Shewanella*. In Abstracts of the 111th General Meeting of the American Society for Microbiology, New Orleans, LA, USA, May 21-24, 2011.
19. Sanford, R., C. Lundstrom, T. Johnson, K. Kemner, M. Boyanov, K. Pennell, K. Ritalahti, and F. E Löffler. 2011. Microbial Uranium Reduction and Monitoring Tools. DOE-SBR PI Meeting, Washington D.C., 26-28 April 2011,
20. 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 PI Meeting, Washington D.C., 26-28 April 2011
21. Kemner, K., E. O'Loughlin, M. Boyanov, D. Antonopoulu, S. Brooks, E. Carpenter, C. Criddle, J. Fredrickson, T. Henne, M.-J. Kwon, B. Lai, D. Latta, F. Löffler, T. Marsh, M. McCormick, B. Mishra, R. Sanford, C. Segre, M. Scherer, D. Sholto-Douglas, K. Skinner, W.M. Wu, and C. Giometti. The Argonne Subsurface Scientific Focus Area. DOE-SBR PI Meeting, Washington D.C., 26-28 April 2011.
22. Marshall, M.J., J. Pollock, E.S. Shelobolina, O.V. Geydebrekht, E.E. Roden, and F. E. Löffler. Isolation and characterization of metal redox-transforming microorganisms from the Hanford Site 300 Area. DOE-SBR PI Meeting, Washington D.C., 26-28 April 2011.
23. Capiro, N.L., J. K. Hatt, Y. Wang, F. E. Löffler, and K. D. Pennell. Distribution of dechlorinating and metal-reducing bacteria between aqueous and solid phases. American Geophysical Union, Fall Meeting; San Francisco, CA, December 13-17, 2010.
24. Boyanov, M., E. O'Loughlin, M-J. Kwon, K. Skinner, B. Mishra, C. Criddle, W-M. Wu, F. Yang, T. Marsh, K. Fletcher, F. Löffler, K. Kemner. The influence of ligands on the formation of non-uraninite U(IV) phases during biotic and abiotic U(VI) reduction, DOE-ERSP PI Meeting, Washington, D.C., March 28-31, 2010.
25. Sanford, R., C. Lundstrom, T. Johnson, K. Kemner, M. Boyanov, K. Pennell, K. Ritalahti, and F. Löffler. Design and application of microbial uranium reduction monitoring tools. DOE-ERSP PI Meeting, Washington, D.C., March 28-31, 2010.
26. Bopp, I.V., C.J., C. Lundstrom, T. Johnson, R. Sanford, K.H. Williams, P.E. Long. 2010. $^{238}U/^{235}U$ isotope ratios as tracers of chemical reduction: Integrating observations from the Rifle field site and U ore deposits. DOE-ERSP PI Meeting, Washington, D.C., March 28-31, 2010.
27. Fletcher, K. E., M. I. Boyanov, S. H. Thomas, Q. Wu, M. J. Beazley, K. M. Kemner, and F. E. Löffler. 2009. Uranium reduction is a common trait of *Desulfitobacterium* spp. 109th annual meeting of the American Society for Microbiology, Philadelphia, PA, May 17-21 2009.

Objectives

The overarching project goal of the MURMoT project was the design of tools to elucidate the presence, abundance, dynamics, spatial distribution, and activity of metal- and radionuclide-transforming bacteria. To accomplish these objectives, an integrated approach that combined nucleic acid-based tools, proteomic workflows, uranium isotope measurements, and U(IV) speciation and structure analyses using the Advanced Photon Source (APS) at Argonne National Laboratory was developed.

Accomplishments

Objective 1. Detection and quantification of 16S rRNA genes of U(VI)-reducing bacteria.

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. The application of these tools to samples collected from Area 3 at the uranium-contaminated Oak Ridge Integrated Field Research Challenge (IFRC) site demonstrated that (i) *A. dehalogenans* strains were unevenly distributed in the contaminated subsurface, (ii) the predominant *A. dehalogenans* strains in a biostimulation area belonged to a clade with no cultured representatives, (iii) the *Anaeromyxobacter* population increased in size following biostimulation in zones with active U(VI) reduction to U(IV), and (iv) the *Anaeromyxobacter* population responded positively to the intrusion of oxygenated groundwater suggesting the strains present at the IFRC can grow aerobically. Laboratory studies determined that *A. dehalogenans* strains indeed consumed (i.e., detoxified) oxygen at higher concentrations (i.e., air) and respired oxygen at partial pressures below 0.18 atm.

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 below).

Manuscript:

Thomas, S. H., R. A. Sanford, M. B. Leigh, E. Cardenas, and F. E. Löffler. 2010. Unique ecophysiology among U(VI)-reducing bacteria as revealed by evaluation of oxygen metabolism in *Anaeromyxobacter dehalogenans* strain 2CP-C. Appl. Environ. Microbiol. 76:176-183. doi: 10.1128/AEM.01854-09

Objective 2. $^{238}U/^{235}U$ isotope measurements as indicator of microbial U(VI) reduction activity.
A novel method involving uranium isotopic measurements by high precision mass spectrometry was developed and validated. During microbial U(VI) reduction, ^{238}U is enriched relative to ^{235}U in the reaction products, rendering the residual dissolved U(VI) enriched in ^{235}U. The unusual sense of fractionation, with a heavier isotope reacting faster, can be rationalized by invoking a nuclear field shift effect.

The $^{238}U/^{235}U$ ratio in groundwater collected from the Rifle IFRC site decreased by ~1 per mil during stimulated U(VI) bioreduction. Similarly sized isotopic fractionation occurs during U(VI) reduction in cultures of *Geobacter sulfurreducens* and *A. dehalogenans* strain FRC-W, an isolate obtained from the Oak Ridge IFRC site. Interestingly, *Desulfovibrio vulgaris* grown under pyruvate fermentative conditions did not reduce or fractionate the U(VI) carbonate.

To better understand fractionation mechanisms, the ε values for U(VI) reduction were determined for two *Geobacter sulfurreducens* isolates (strain PCA and strain Criddle), two *Anaeromyxobacter dehalogenans* isolates (strain FRC-W and strain FRC-R5), a novel *Shewanella* isolate (*Shewanella* sp. strain NR), and the Gram-positive bacterium *Desulfitobacterium* sp. strain Viet1. The results demonstrated that microbial U(VI) reduction induces isotopic fractionation for all bacterial cultures tested. The ε values determined for *Geobacter sulfurreducens* strain PCA and strain Criddle were 0.68‰ and 0.99‰, respectively. The ε values for *A. dehalogenans* strain FRC-W, *A. dehalogenans* strain FRC-R5, *Shewanella* sp. strain NR, and *Desulfitobacterium* sp. strain Viet1 were 0.72‰, 0.99‰, 0.96‰ and 0.86‰, respectively. The data revealed that ε increases with decreasing cell-specific reduction rate, suggesting that ε varies with the kinetics of individual reduction reactions. The ε values reached maxima of ~1‰ under low biomass and electron donor-limiting conditions. These findings reveal a fundamental relationship between metabolism and isotopic fractionation, and should prove useful for monitoring U(VI) under various geochemical settings where microbial U(VI) reduction is expected to occur.

Manuscripts:

Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. 2014. Uranium isotopic fractionation factors during U(VI) reduction by bacterial isolates. Geochim. Cosmochim. Acta. 136:100-113. doi.org/10.1016/j.gca.2014.02.041

Bopp IV, C.J., C. Lundstrom, T. Johnson, R. A. Sanford, P. E. Long, and K. H. Williams. 2010. Uranium isotope ratios as indicators of reduction: results from an *in situ* biostimulation experiment at Rifle, CO. Environ. Sci. Technol., 44:5927-5933.

Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. Effect of U(VI) concentration variation on U isotope fractionation during U(VI) reduction by a novel *Shewanella* isolate. In Preparation.

Basu, A., R. A. Sanford, T. M. Johnson, C. C. Lundstrom, and F. E. Löffler. Isotopic fractionation of ^{235}U, ^{236}U, and ^{238}U during microbial U(VI) reduction. In Preparation.

Objective 3. Characterization of reduced U(IV) phases. Uranium L_{III}-edge X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) analyses were performed to determine the valence state and the average local environment of uranium in the hydrated solid phase. The XANES spectra obtained from samples prepared from five *Desulfitobacterium* spp. cultures demonstrated that at least 95% of all solid phase uranium was present as U(IV), confirming the spectrofluorescence measurements and U(VI) reduction. EXAFS analyses were performed on these samples to determine the atomic environments of the solid phase uranium. Interestingly, while bioreduction of U(VI) is almost always reported to yield the uraninite mineral (UO_2), extended X-ray absorption fine structure (EXAFS) analysis demonstrated that the U(IV) produced by the *Desulfitobacterium* spp. was not UO_2. The EXAFS data indicated that the U(IV) product was a phase or mineral composed of mononuclear U(IV) atoms closely surrounded by light element shells. This atomic arrangement likely results from inner-sphere bonds between U(IV) and C/N/O or P/S-containing ligands, such as carbonate or phosphate. The formation of a distinct U(IV) phase is a relevant discovery because the characteristics of the reduced material affect uranium stability and fate in the contaminated subsurface.

Manuscripts:

Fletcher, K. E., M. I. Boyanov, S. H. Thomas, Q. Wu, K. M. Kemner, and F. E. Löffler. 2010. Uranium reduction to mononuclear U(IV) by *Desulfitobacterium* spp. Environ. Sci. Technol. 44:4705-4709. doi: 10.1021/es903636c

Boyanov, M. I., K. E. Fletcher, M. Jae Kwon, X. Rui, E. J. O'Loughlin, F. E. Löffler, and K. M. Kemner. 2011. Solution and microbial controls on the formation of reduced U(IV) species. Environ. Sci. Technol. 45:8336-8344. doi: 10.1021/es2014049

Objective 4. Identification of metal reduction biomarkers. 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 *A. dehalogenans* strain 2CP-C 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, theses 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.

The proteomics approach 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:

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Objective 5. Competition between metal-reducing populations near the oxic-anoxic interface. Oxic-anoxic transitions zones are hotspots for microbial activity and function as key controls of radionuclide mobility. Under anoxic conditions, U(VI) is reduced to U(IV) by metal-reducing bacteria, resulting in uranium immobilization. In oxic environments (e.g., following oxygen intrusion), reduced and immobile U(IV) is oxidized to soluble and mobile U(VI). Monitoring of *Anaeromyxobacter* and *Geobacter* populations at the Oak Ridge IFRC site suggested that members of these bacterial groups responded differently to oxygen intrusion. To explore the effect of fluctuating redox conditions on metal-reducing populations in more detail, laboratory one-dimensional (1-D) continuous flow experiments were performed. A novel column design was needed to control an oxic/anoxic transition zone to explore the effects of fluctuating redox conditions on U(VI)-reducing populations and uranium mobility. These columns were manufactured using plexiglass and were equipped with multiple valves for manipulating influent and effluent streams. The manipulation of flow gave operational control of an oxic-anoxic transition zone. The columns were equipped with multiple sampling ports to monitor the abundance of *Anaeromyxobacter* and *Geobacter* 16S rRNA genes in different sections of the column. In addition, sacrificial sampling of the columns revealed the distribution of *Anaeromyxobacter* and *Geobacter* cells attached to the solids. These experiments revealed the dynamic changes in *Anaeromyxobacter* and *Geobacter* population sizes in response to fluctuation redox conditions.

Manuscript:

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