

Title: An Integrated Assessment of Geochemical and Community Structure Determinants of Metal Reduction Rates in Subsurface Sediments

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Summary of Project Results:

This project represented a joint effort between Oak Ridge National Laboratory (ORNL), the University of Tennessee (UT), and Florida State University (FSU). ORNL served as the lead institution with Dr. A.V. Palumbo responsible for project coordination, integration, and deliverables. *In situ* uranium bioremediation is focused on biostimulating indigenous microorganisms through a combination of pH neutralization and the addition of large amounts of electron donor. Successful biostimulation of U(VI) reduction has been demonstrated in the field and in the laboratory. However, little data is available on the dynamics of microbial populations capable of U(VI) reduction, and the differences in the microbial community dynamics between proposed electron donors have not been explored. In order to elucidate the potential mechanisms of U(VI) reduction for optimization of bioremediation strategies, structure-function relationships of microbial populations were investigated in microcosms of subsurface materials cocontaminated with radionuclides and nitrate from the Oak Ridge Field Research Center (ORFRC), Oak Ridge, Tennessee.

At least 10 microcosm experiments were conducted involving the three laboratories listed above. From the FSU portion, a total of 3 publications and 19 seminars or meeting presentations were completed under this project. Several other publications are in preparation. A listing of papers and other products delivered follows the summary of our results.

The primary objective of year 1 under Task 1 was the development and optimization of microcosm experiments designed to manipulate uranium reduction activity (Edwards et al., 2007). Under the remaining tasks in years 2 and 3, we proposed to further define experimental conditions for the perturbation of microbial community structure by manipulating electron donor, electron shuttle, and nutrient concentrations. Based on work conducted under Task 1, we hypothesized that the activity of nitrate- and Fe(III)-reducing microbial populations, catalyzing the reductive immobilization of U(VI) in subsurface radionuclide-contaminated sediments, would be dependent on the choice of electron donor. The objectives for the remainder of the project were to: 1) characterize structure-function relationships for microbial groups likely to catalyze or limit U(VI) reduction in radionuclide-contaminated sediments; and 2) to further develop a molecular proxy for the metabolic activity of U(VI)-reducers. Microbial activity was assessed by monitoring terminal-electron-accepting processes (TEAPs), electron donor utilization, and Fe(III) mineral transformations in microcosms conducted with subsurface materials cocontaminated with high levels of U(VI) and nitrate. In parallel, microbial functional groups were enumerated and characterized using a combination of cultivation-dependent and -independent methods.

A polyphasic approach was used to assess the functional diversity of microbial populations likely to catalyze electron flow under conditions proposed for in-situ uranium bioremediation (Akob et al., 2008). The addition of ethanol and glucose as supplemental electron donors stimulated microbial nitrate and Fe(III) reduction as the predominant TEAPs. U(VI), Fe(III), and sulfate reduction overlapped in the glucose treatment, whereas U(VI) reduction was concurrent with sulfate reduction but preceded Fe(III) reduction in the ethanol treatments. Phyllosilicate clays were shown to be the major source of Fe(III) for microbial respiration using variable-temperature Mössbauer spectroscopy. Nitrate- and Fe(III)-reducing bacteria (NRB and FeRB) were abundant throughout the shifts in TEAPs observed in biostimulated microcosms and were affiliated with the genera *Geobacter*, *Tolumonas*, *Clostridium*, *Arthrobacter*, *Dechloromonas*, and *Pseudomonas*. Up to two orders of magnitude higher counts of FeRB and enhanced U(VI) removal were observed in ethanol-amended as compared to glucose-amended treatments. Quantification of citrate synthase (*gltA*) levels demonstrated a stimulation of *Geobacteraceae* activity during metal reduction in carbon amended microcosms with the highest expression observed in the glucose treatment. Phylogenetic analysis indicated that the active FeRB share high sequence identity with *Geobacteraceae* members cultivated from contaminated subsurface environments. Our results show that the functional diversity of populations capable of U(VI) reduction is dependent upon the choice of electron donor.

Ethanol and glucose have been considered as the primary electron donors to stimulate the reductive immobilization of U(VI) in bioremediation practices at the ORFRC. This study revealed that the addition of ethanol stimulated rapid nitrate reduction followed by the complete reduction of U(VI) concurrent with sulfate reduction (Akob et al., 2008). We therefore propose that ethanol is a more appropriate electron donor than glucose for future *in situ* bioremediation practices, and that sulfate-reducing bacteria are most likely a critical microbial functional group involved in the reductive immobilization of U(VI). The accumulation of acetate and thereafter slow oxidation may serve as a long-term electron donor to support the maintenance of a reduced subsurface environment and promoting the insolubility and of U(IV) species. The microbial community within the ethanol treatment was composed of members of the *Proteobacteria*, *Actinobacteria*, and *Firmicutes* including phylogenetic groups not previously associated with uranium bioremediation, e.g., *Arthrobacter* and *Dechloromonas*. Members of the *Geobacteraceae* family are substantial contributors to metal reduction in uranium-contaminated subsurface sediments and are important for the long-term stability of reduced uranium. We speculate that these groups are well adapted for the extremes of contaminated subsurface sediments and are a good target for effective *in situ* uranium bioremediation.

The project has provided substantial insight into optimization of uranium reduction by manipulation of communities and of the importance of microbial community structure in uranium bioremediation outcomes.

Papers and Other Products Delivered:

The following papers, presentations and other products were completed by ORNL, FSU, and UT researchers in conjunction with this ERSP research.

Kostka, J.E. 2005. "Metal Reducing Microbial Communities in the Acidic Subsurface," University of Tubingen, Tubingen, Germany, March.

Akob, D.M., H. J. Mills, L. Edwards, D. L. Balkwill, J. E. Kostka. 2005. Metabolically-Active Microbial Communities in Acidic Uranium-Contaminated Subsurface Sediments. DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April.

Palumbo, A.V., C. W. Schadt, C. C. Brandt, J. E. Kostka, S. M. Pfiffner. 2005. An integrated assessment of geochemical and community structure determinants of metal reduction rates in subsurface sediments. DOE-NABIR Principal Investigator Meeting, Warrenton, VA, 18-20 April.

Kostka, J.E. 2005. "The Global Significance of Metal Reduction and Metal-Reducing Bacteria," Korea Ocean Research and Development Institute, Seoul, Korea, May.

Akob, D.M., H. J. Mills, L. Edwards, J. E. Kostka. 2005. The "Active" Microbial Communities found in Acidic Uranium-Contaminated Subsurface Sediments. Annual Meeting of the American Society for Microbiology, Atlanta, GA, June.

Palumbo, A. V., S.M. Pfiffner, L. Fagan, M. McNeilly, S. Rishell, J.E. Kostka, and C.C. Brandt. 2005. Substrate and Community Structure Determinants of Uranium Reduction Rates in Subsurface Sediments. In: N. Kalogerakis (Ed.), Proceedings of the Third European Bioremediation Conference (Chania, Greece, July 2005), (on CD).

Bottomly, S., A. Smithgall, A. Parsons, S.M. Pfiffner, L. Fagan, C. Brandt, and A. Palumbo. 2005. Effects of Carbon Source Additions on Microbial Community Structure in Uranium Contaminated Sediment Microcosms. Kentucky-Tennessee Branch of the American Society of Microbiology, Nashville, TN, September.

Palumbo, A.V., C.C. Brandt, S.M. Pfiffner, L.A. Fagan, A.S. Madden, T.J. Phelps, J.C. Schryver, M.S. McNeilly, C.W. Schadt, J.R. Tarver, and J.E. Kostka. 2006. Reduction processes and community structure in remediation of uranium. 231st American Chemical Society National Meeting, Division of Geochemistry, Atlanta, GA, March.

Kostka, J.E. 2006. Microbial Fe(III) reduction: Coupling biogeochemistry with microbiology to determine process controls in aquatic sediments. 231st American Chemical Society National Meeting, Division of Geochemistry, Atlanta, GA, March.

Palumbo, A.V., C.W. Schadt, C.C. Brandt, J.E. Kostka, and S.M. Pfiffner. 2006. An integrated assessment of geochemical and community structure determinants of metal reduction rates in subsurface sediments. DOE-ERSP Principal Investigator Meeting, Warrenton, VA, April 2-5.

Akob, D.M., H.J. Mills, T.M. Gihring, and J.E. Kostka. 2006. Structure and Function of Metal- and Nitrate-reducing Microbial Communities in the FRC Subsurface. DOE-ERSP Principal Investigator Meeting, Warrenton, VA, 2-5 April.

Akob, D.M., H.J. Mills, T.M. Gihring, L. Kerkhof, and J.E. Kostka. 2006. Structure and Function of Metal- and Nitrate-reducing Microbial Communities in Subsurface Sediments Contaminated with Radionuclides. Annual Meeting of the American Society for Microbiology, Orlando, FL, May.

Kerkhof, L.J., D. Watson, J.E. Kostka, and L. McGuinness. 2006. Active Microorganisms in Groundwater Along a Contamination Plume at the Field Research Center in Oak Ridge, TN, USA. Annual Meeting of the American Society for Microbiology, Orlando, FL, May.

Palumbo, A.V., C.W. Schadt, C.C. Brandt, J.E. Kostka, S.M. Pfiffner, L.A. Fagan, J.R. Tarver, and M.S. McNeilly. 2006. Influence of the Addition of Humic Acid on Reduction Rates of Nitrate, Sulfate, and Uranium in Sediment Slurries from a Uranium Contaminated Site. Annual Meeting of the American Society for Microbiology, Orlando, FL, May.

Kostka, J.E. 2006. "Linking the structure and function of subsurface microbial communities for the assessment of bioremediation potential at the Oak Ridge Field Research Center," U.S. Department of Energy Environmental Remediation Sciences Program Fall Principal Investigator Meeting, Oak Ridge, Tennessee, October.

D. M. Akob, H. J. Mills, L. Kerkhof, A. Palumbo, K. Kuesel, and J. E. Kostka. 2007. Determination of the Metabolically Active Microbial Groups in Contaminated Subsurface Sediments of the Oak Ridge Field Research Center Using Stable Isotope Probing (SIP), ERSP Principal Investigator Meeting, Lansdowne, VA, April.

H. Mills, J. E. Kostka, D. Akob, L. Kerkhof, K.-J. Chin. 2007. Cultivation independent quantification and characterization of metal and nitrate-reducing prokaryotes in radionuclide contaminated subsurface sediments. Annual Meeting of the American Society for Microbiology, Toronto, Canada, May.

Kostka, J.E. 2007. "Metal reduction and metal-reducing microorganisms in the radionuclide-contaminated subsurface," Institute for Geosciences, Friedrich-Schiller University, Jena, Germany, July.

Kostka, J.E., 2007. "Metabolically-active Iron- and Sulfate-reducing Prokaryotes in Shallow Subsurface Sediments," International Workshop on Microbial Life under Extreme Energy Limitation, Aarhus, Denmark, October.

L. Edwards, K. Kuesel, H. Drake, and J.E. Kostka. 2007. Electron flow in acidic subsurface sediments cocontaminated with nitrate and uranium during nuclear weapons production. *Geochimica et Cosmochimica Acta* 71: 643-654.

M. Bloethe, D.M. Akob, J.E. Kostka, K. Walter, H.L. Drake, and K. Kuesel. 2008. Heterogeneity of Fe(III)-reducing microorganisms in coal mining lake sediments caused by a pH gradient. *Applied and Environmental Microbiology* 74: 1194-1197.

D. M. Akob, H. J. Mills, T. M. Gihring, L. Kerkhof, J. W. Stucki, Kuk-Jeong Chin, Kirsten Kuesel, Anthony V. Palumbo, David B. Watson, and Joel E. Kostka. 2008. Functional diversity and electron donor dependence of microbial populations capable of U(VI) reduction in radionuclide contaminated subsurface sediments. *Applied and Environmental Microbiology* (in press).

D. M. Akob, L. Kerkhof, K-J. Chin, K. Kuesel, D. B. Watson, A. V. Palumbo, J. E. Kostka. Characterization of the Metabolically Active Iron(III)- and Sulfate-Reducing Bacteria in Uranium(VI) Contaminated Subsurface Sediments Using DNA-SIP Analysis. *Environmental Microbiology* (in preparation).