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Title: Integrated Nucleic Acid System for In-Field Monitoring of Microbial Community Dynamics and Metabolic Activity – Subproject to Co-PI Eric E. Roden

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Executive Summary

A. Overview

This report summarizes research conducted in conjunction with a project entitled “Integrated Nucleic Acid System for In-Field Monitoring of Microbial Community Dynamics and Metabolic Activity”, which was funded through the Integrative Studies Element of the former NABIR Program (now the Environmental Remediation Sciences Program) within the Office of Biological and Environmental Research. Dr. Darrell Chandler (originally at Argonne National Laboratory, now with Akonni Biosystems) was the overall PI/PD for the project. The overall project goals were to (1) apply a model iron-reducer and sulfate-reducer microarray and instrumentation systems to sediment and groundwater samples from the Scheibe et al. FRC Area 2 field site, UMTRA sediments, and other DOE contaminated sites; (2) continue development and expansion of a 16S rRNA/rDNA-targeted probe suite for microbial community dynamics as new sequences are obtained from DOE-relevant sites; and (3) address the fundamental molecular biology and analytical chemistry associated with the extraction, purification and analysis of functional genes and mRNA in environmental samples. Work on the UW subproject focused on conducting detailed batch and semicontinuous culture reactor experiments with uranium-contaminated FRC Area 2 sediment. The reactor experiments were designed to provide coherent geochemical and microbiological data in support of microarray analyses of microbial communities in Area 2 sediments undergoing biostimulation with ethanol. A total of four major experiments were conducted (one batch and three semicontinuous culture), three of which (the batch and two semicontinuous culture) provided samples for DNA microarray analysis. A variety of other molecular analyses (clone libraries, 16S PhyloChip, RT-PCR, and T-RFLP) were conducted on parallel samples from the various experiments in order to provide independent information on microbial community response to biostimulation.

B. Batch sediment slurry experiment

A set of 16S rRNA samples was isolated from a batch laboratory sediment slurry incubation experiment conducted with U(VI)-contaminated sediment from FRC Area 2 field site (Mohanty et al., 2008) as part of the Scheibe et al. FRC Area 2 field project. A coherent series of terminal electron-accepting processes (TEAPs) was observed in the experiment, and rRNA samples were obtained from each of the major TEAP phases. These samples were analyzed using the same 16S rRNA-targeted tunable bead microarray applied previously to UMTRA sediments (Chandler et al., 2006). The results were compared to clone libraries constructed from reverse-transcribed 16S rRNA (i.e. the same rRNA samples analyzed on the bead array) as well as a high-density 16S rDNA microarray (16S PhyloChip) (Wilson et al., 2002; Brodie et al., 2006) analysis of the reverse-transcribed 16S rRNA. For reasons that remain unclear, the signals from the bead microarray did not match in any consistent manner the community composition and temporal trends evident from the clone library and 16S PhyloChip data, the latter of which were consistent with the sequence of TEAPs observed during the incubation. We have a manuscript in preparation that described in detail the comparison between the clone library and 16S PhyloChip results (Mohanty et al., 2009).

C. Semicontinuous culture reactor experiments

Three long-term (80-140 d) semicontinuous culture reactor (SRC) experiments were conducted (with additional support from the Burgos et al. Integrative Studies project) with U(VI)-contaminated sediment from the FRC Area 2 field site. The first two experiments (SCR1 and SCR2) were conducted with Fe(III) (oxide and phyllosilicate) rich sediment from just below the gravel layer at Area 2, whereas the last experiment (SCR3) used more reduced, fine-grained (< 0.5 mm) material from the gravel layer. The overall goal of these experiments was to provide high-quality datasets for parameterization of reaction-based models of the biogeochemistry (including microbial population dynamics) of ethanol-stimulated Area 2 sediments. The SCR systems support relatively long hydraulic residence times (10-20 days) which are likely to approximate reality for biostimulated pore domains above and below the gravel layer at the Area 2 field site (Brooks et al., 2009). In addition, they allow for determination (over time) of virtually all aqueous-, solid-, and gas-phase reactants and products of microbial metabolism, as well as changes in microbial community structure. Work supported by this project focused on comparison of cultivation-based vs. molecular analysis of respiratory microbial groups associated with bulk TEAPs and U(VI) reduction activity in the SCR3s.

A roll-tube based (Hungate, 1969) dilution-to-extinction method was used to enumerate and isolate representatives of various respiratory organisms in samples from the SCR experiments. These studies provide independent information on the abundance of microbial groups responsible for U(VI) reduction and other TEAPs in the bioreactors. In addition, the 16S rRNA gene sequences of isolated organisms is being used to help constrain the accuracy of 16S rRNA-based microbial community fingerprinting analyses. Initial culturable cell densities of ethanol- and acetate-utilizing nitrate-reducers were ca. 100 times higher than Fe(III) and sulfate reducers. All three respiratory groups increased in abundance by 3-6 order of magnitude in response to ethanol amendment. Tubes from the last positive dilutions were used for isolation of the representatives of specific microbial physiological groups of microorganisms. Nitrate-reducing isolates were representatives of Beta- and Gamma Proteobacteria (*Ralstonia metallidurans*, *Pseudomonas rhodesiae*, *Acidovorax delafieldii*, and a bacterium 92% similar to *Propionivibrio limicola*). Sulfate-reducing isolates are multiple *Desulfovibrio* strains (Deltaproteobacteria) and Gram positive *Desulfosporosinus* strains. Fe(III)-reducing isolates are *Geobacter* sp. (Deltaproteobacteria) and a strain 88% similar to a coryneform *Cellulomonas septica*. Detailed studies of the physiology (including U(VI) reduction potential) of these and other isolates from ethanol-stimulated FRC Area 2 sediments are underway.

Portions of sediment slurry were collected during each semi-weekly sampling and archived for molecular microbial community analysis in conjunction with this project. A set of RT-PCR analyses were conducted on samples from SCR1 by Brett Baldwin and Aaron Peacock at the University of Tennessee through financial support of the Istok et al. ERSP project. The primers used for RT-PCR genes included a broad-spectrum *nir S* to target NO₃⁻ reducers; 16S rRNA genes for two major DMRB groups (*Geobacter* and *Anaeromyxobacter*; the *dsr* gene for SRB, and a general Deltaproteobacterial probe that targets both SRB and DMRB. The results of these analyses were consistent with the culture-based enumerations. Ongoing work at UW is focused on analysis of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene amplicons from the archived SCR1 and SCR2 samples. Note here that our prior

results with the bead microarray system illustrated the importance of conducting independent molecular fingerprinting to constrain the accuracy of microarray data.

D. Gel element microarray analyses

Gel element 16S rDNA microarray analyses were conducted on samples from SCR2 and SCR3. The original 16S rRNA microbead array (Chandler et al., 2006) was translated to a gel element format (Guschin et al., 1997), adapted for use of PCR-amplified 16S rDNA, and expanded to include additional DOE-relevant organisms. DNA extracts from duplicate reactors with and without ethanol addition were shipped to Akonni Biosystems where the array analyses were conducted. Each DNA sample was processed in triplicate, i.e. three independent amplifications (each on 3 uL of template DNA) were conducted, and the products of each amplification were applied to a single array. Hybridization was overnight at 37C as per prior work with this gel element system. The arrays had 3 replicate probes per target. A signal-to-noise threshold of three was applied after averaging over all replicates. The results of these analyses are still being analyzed. Preliminary results indicate that organisms (e.g. Fe(III)- and sulfate-reducing bacteria) expected to be stimulated by ethanol addition were in fact detected in significant numbers in the +ethanol reactors. However, the arrays did not appear to provide quantitative information directly comparable with other measurements of biomass (e.g. roll-tube enumerations).

E. References

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- Brooks, S. C., E. E. Roden, S. R. Mohanty, W. Kamolpornwijit, D. Watson, and T. D. Scheibe. 2009. Field-scale biostimulation of uranium(VI) reduction in heterogenous subsurface sediments. Manuscript in prep.
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- Mohanty, S., B. Kollah, D. Hedrick, A. Peacock, R. K. Kukkadapu, and E. E. Roden. 2008. Biogeochemical processes in ethanol stimulated uranium-contaminated subsurface sediments. *Environ. Sci. Technol.* 12:4384-4390.
- Mohanty, S. R., E. L. Brodie, B. Kollah, T. C. Hazen, and E. Roden. 2009. Comparative analysis of microbial communities in ethanol-amended subsurface sediment by 16S rRNA clone libraries and a high density phylogenetic DNA microarray. Manuscript in prep.

Wilson, K. H., W. J. Wilson, J. L. Radosevich, T. Z. DeSantis, V. S. Viswanathan, T. A. Kuczmarski, and G. L. Andersen. 2002. High-density microarray of small-subunit ribosomal DNA probes. *Appl. Environ. Microbiol.* 68:2535-2541.

Research Products

A. Referred publications

Mohanty, S., B. Kollah, D. Hedrick, A. Peacock, R. K. Kukkadapu, and E. E. Roden. 2008. Biogeochemical processes in ethanol stimulated uranium-contaminated subsurface sediments. *Environ. Sci. Technol.* 42:4384-4390.

Roden, E. E. 2008a. Microbiological controls on geochemical kinetics 1: Fundamentals and case study on microbial Fe(III) reduction. In S. L. Brantley, J. Kubicki, and A. F. White (eds.). *Kinetics of Water-Rock Interactions*. Springer, New York.

Roden, E. E. 2008b. Microbiological controls on geochemical kinetics 2: Case study on microbial oxidation of metal sulfide minerals and future prospects. In S. L. Brantley, J. Kubicki, and A. F. White (eds.). *Kinetics of Water-Rock Interactions*. Springer, New York.

Roden, E. E., and D. Emerson. 2007. Microbial metal cycling in aquatic environments. *Manual of Environmental Microbiology*, 3rd Edition. American Society for Microbiology, Washington, DC.

Chandler D.P., Jarrell A.E., Roden E.R., Golova J., Chernov B., Schipma M.J., Peacock A.D., and Long P.E. 2006. Suspension array analysis of 16S rRNA from Fe- and SO_4^{2-} -reducing bacteria in uranium-contaminated sediments undergoing bioremediation. *Appl. Environ. Microbiol.* 72: 4672-4687.

B. Submitted for publication

Shelobolina, E.S., H. Konishi, H. Xu, and E.E. Roden. U(VI) sequestration in calcium-phosphate minerals produced by mMicrobial glycerol 3-phosphate metabolism. *Appl. Environ. Microbiol.* Submitted for publication.

Istok, J. D., M. Park, M. Michalsen, A. M. Spain, L. R. Krumholz, C. Liu et al. A thermodynamically-based model for predicting microbial growth and community composition coupled to system geochemistry: application to uranium and technetium bioreduction. *Geochim. Cosmochim. Acta*. Submitted for publication.

C. Publications in preparation (Roden group)

Mohanty, S. R., E. L. Brodie, B. Kollah, T. C. Hazen, and E.E. Roden. Comparative analysis of microbial communities in ethanol-amended subsurface sediment by 16S rRNA clone libraries and a high density phylogenetic DNA microarray. Manuscript in prep.

Jin, Q. and E.E. Roden. Biogeochemical reaction modeling of ethanol metabolism in subsurface sediments. Manuscript in prep.

Mohanty, S. R., B. Kollah, B. Baldwin, A. Peacock, and E.E. Roden. Experimental reactor study of microbial redox metabolism in biostimulated uranium-contaminated subsurface sediments. Manuscript in prep.

Roden, E.E, S.R. Mohanty, B. Kollah, B. Baldwin, A. Peacock. Reactor studies and physiology-based modeling of of microbial metabolism in biostimulated subsurface sediments. Manuscript in prep.

D. Presentations at national/international scientific conferences (Roden group)

Roden, E.E., S. Mohanty, B. Kollah, E. Shelobolina, A. Peacock, D. Hedrick, and Q. Jin, “Microbial physiology-based reaction model of ethanol metabolism in subsurface sediments”, Abstract Q-042, American Society for Microbiology General Meeting, 2009.

Mohanty, S., B. Kollah, and E. Roden, “Bioreduction of uranium and microbial dynamics in the FRC sediment amended with ethanol during flow through process”, Abstract Q-533, American Society for Microbiology General Meeting, 2008.

Mohanty, S., E.L Brodie, B. Kollah, T.C. Hazen, E. Roden, “Comparative analysis of microbial communities associated with U(VI) bioreduction in ethanol-amended subsurface sediment by 16S rRNA clone libraries and a high density phylogenetic DNA microarray”, Abstract Q-182, American Society for Microbiology General Meeting, 2007.

Mohanty, S., B. Kollah, A.E. Jarrell, D.P. Chandler, D. Hedrick, A. Peacock, E. Roden, “Microbial community dynamics in ethanol-stimulated subsurface sediments”, Abstract Q-160, American Society for Microbiology General Meeting, 2006.

E. Workshop presentations (Roden group)

Roden, E.E., S. Mohanty, B. Kollah, E. Shelobolina, A. Peacock, D. Hedrick, and Q. Jin, “Experimental reactor studies and physiology-based modeling of microbial redox metabolism in biostimulated uranium-contaminated subsurface sediments”, U.S. Department of Energy, Environmental Remediation Sciences Program Annual PI Meeting, 2009. (Poster presentation)

Roden, E.E., S. Mohanty, and B. Kollah, “Flow-through reactor experiments with ethanol-amended FRC Area 2 sediments”, U.S. Department of Energy, Natural and Enhanced Bioremediation Program ORNL Field Research Center Meeting, 2006. (Poster presentation)

Roden, E.E., S. Mohanty, and B. Kollah, “Biogeochemical processes and microbial community structure in ethanol-amended FRC Area 2 sediments”, U.S. Department of Energy,

Natural and Enhanced Bioremediation Program ORNL Field Research Center Meeting, 2005. (Oral presentation)

Roden, E.E., S. Mohanty, and B. Kollah, "Biogeochemical processes and microbial community structure in ethanol-amended FRC Area 2 sediments", U.S. Department of Energy, Natural and Enhanced Bioremediation Program ORNL Field Research Center Meeting, 2005. (Poster presentation)