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Title: Reaction-Based Reactive Transport Modeling of Iron Reduction and Uranium Immobilization at Area 2 of the NABIR Field Research Center – 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 “Reaction-Based Reactive Transport Modeling of Iron Reduction and Uranium Immobilization at Area 2 of the NABIR Field Research Center”, 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. William Burgos (The Pennsylvania State University) was the overall PI/PD for the project, which included Brian Dempsey (Penn State), Gour-Tsyh (George) Yeh (Central Florida University), and Eric Roden (formerly at The University of Alabama, now at the University of Wisconsin) as separately-funded co-PIs. The project focused on development of a mechanistic understanding and quantitative models of coupled Fe(III)/U(VI) reduction in FRC Area 2 sediments. The work builds on our previous studies of microbial Fe(III) and U(VI) reduction, and was directly aligned with the Scheibe et al. ORNL FRC Field Project at Area 2.

Area 2 is a shallow pathway for migration of contaminated groundwater to seeps in the upper reach of Bear Creek at ORNL, mainly through a ca. 1 m thick layer of gravel located 4-5 m below the ground surface. The gravel layer is sandwiched between an overlying layer of disturbed fill material, and 2-3 m of undisturbed shale saprolite derived from the underlying Nolichucky Shale bedrock. The fill was put in place when contaminated soils were excavated and replaced by native saprolite from an uncontaminated area within Bear Creek Valley; the gravel layer was presumably installed prior to addition of the fill in order to provide a stable surface for the operation of heavy machinery. The undisturbed saprolite is highly weathered bedrock that has unconsolidated character but retains much of the bedding and fracture structure of the parent rock (shale with interbedded limestone). Hydrological tracer studies conducted during the Scheibe et al. field project indicate that the gravel layer receives input of uranium from both upstream sources and from diffusive mass transfer out of highly contaminated fill and saprolite materials above and below the gravel layer. This research sought to examine biogeochemical processes likely to take place in the less conductive materials above and below the gravel during the in situ ethanol biostimulation experiment conducted at Area 2 during 2005-2006. The in situ experiment in turn examined the hypothesis that injection of electron donor into this layer would induce formation of a redox barrier in the less conductive materials, resulting in decreased mass transfer of uranium out these materials and attendant declines in groundwater U(VI) concentration. Our research was directed toward the following three major objectives relevant to formation of this redox barrier: (1) elucidate the kinetics and mechanisms of reduction of solid-phase Fe(III) and U(VI) in Area 2 sediments; (2) evaluate the potential for long-term sustained U(IV) reductive immobilization in Area 2 sediments; (3) numerically simulate the suite of hydrobiogeochemical processes occurring in experimental systems so as to facilitate modeling of in situ U(IV) immobilization at the field-scale. This report summarizes research activities conducted at The University of Alabama (2004-2005) and the University of Wisconsin (2005-2007) in pursuit of these objectives.

B. Kinetic and Thermodynamic Controls on Microbial Fe(III) Oxide Reduction

Acquisition of a quantitative (predictive) understanding of the controls on microbial Fe(III) oxide reduction is a key ingredient to development of accurate models of U(VI) reductive immobilization, since dissimilatory iron-reducing microorganisms (DIRMs) are likely to be responsible for much of the U(VI) reduction activity in biostimulated sediments. The results of extensive DOE-funded studies on this basic issue were synthesized to gain insight into what is likely to be the rate-limiting step during the initial stages of microbial Fe(III) oxide reduction in soils and sediments containing excess electron donors for microbial respiration. Ten different oxide phases with a range of surface areas and thermodynamic properties were employed in these experiments. The short-term kinetics of Fe(III) oxide reduction by *Geobacter sulfurreducens* were examined previously under nongrowth conditions with excess H_2 as the electron donor. Analogous experiments were conducted (as part of this project) in the presence of 0.1 mM of the soluble electron shuttling compound AQDS. In addition, a set of experiments examined both the short- and long-term kinetics of abiotic Fe(III) oxide reduction by excess (10 mM) AH_2DS . The long-term abiotic reduction experiments were used to estimate the reduction potential (E_h^0) of the different oxide phases. There was a significant correlation between oxide E_h^0 and short-term surface-area normalized rates of abiotic reduction by 10 mM AH_2DS . In contrast, there was no correlation between oxide E_h^0 and initial surface area-normalized microbial reduction rates, either the presence or absence of AQDS. Separate experiments conducted with amorphous Fe(III) oxide showed that 0.001 mM AQDS was sufficient to saturate rates of oxide reduction at the levels of oxide surface loading used in other experiments. Thus, the abundance of AQDS did not limit initial rates of reduction in the reaction systems with 0.1 mM AQDS. Together these results suggest that the overall rate of electron flow from intracellular metabolism to the outside of the cell was the rate-limiting step in oxide reduction in the presence of AQDS. These findings support the conclusion based on previous studies that surface area-normalized rates of microbial Fe(III) oxide reduction fail to correlate with oxide E_h^0 because the rate of electron transfer from the cell to the oxide is the rate-limiting step in microbial Fe(III) oxide reduction, as opposed to Fe(II) detachment which is the acknowledged rate-limiting step during abiotic reductive dissolution.

C. Enzymatic Reduction of Solid-Phase Fe(III) and U(VI) in FRC Area 2 Sediments

Pure culture experiments with *G. sulfurreducens* were conducted with FRC Area 2 sediments analogous (or identical) to those used in the long-term SCR experiments. An initial experiment demonstrated that both *G. sulfurreducens*-amended and unamended slurries showed complete reduction of aqueous U(VI) and ca. 60% reduction of solid-associated U(VI) after ca. 1 month of incubation in the presence of 10 mM ethanol. The presence of 0.1 mM AQDS as an electron shuttling compound stimulated Fe(III) reduction, but had no impact on the rate or extent of U(VI) reduction. A second experiment showed similar results, with only ca. 50% of total U(VI) undergoing reduction over a ca. 50-day incubation. Detailed studies of controls on enzymatic and chemical reduction of solid-associated U(VI) in Area 2 sediments and representative pure-phase mineral specimens present in Area 2 sediments were conducted at PSU. Additional experiments with *G. sulfurreducens* are underway to examine the kinetics of Fe(III) oxide vs. phyllosilicate Fe(III) reduction in Fe(III)-rich FRC Area 2 sediments, and to assess the impact of particle size, surface area, and porosity on coupled Fe(III) and U(VI) reduction. The results of

these experiments will be incorporated into the batch microbial physiology-based reaction model described below.

D. Long-term Area 2 Sediment Semicontinuous Culture Experiments

Experimental methods and sampling/analytical techniques for long-term semicontinuous culture reactor (SCR) studies of Fe(III)/U(VI) reduction and other terminal electron-accepting processes (TEAPs) were developed during the initial stages of this project. The SCR systems were designed to support relatively long hydraulic residence times (10-20 days) comparable to those used in the column reactor experiments conducted at PSU, and similar to those that are likely to be in force pore within pore domains in the fill and saprolite materials above and below the gravel layer at Area 2. The SCRs 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. As such, they provide ideal datasets for reaction-based modeling of the biogeochemistry of ethanol-stimulated Area 2 sediments. Two major experiments were conducted with Fe(III)/U(VI)-rich materials from below the gravel layer at Area 2. A final round of experiments to be conducted in the coming months will be conducted with Fe(III)-depleted, Fe(II)-rich materials from the gravel layer.

The composition of the fluid used in the SCR experiments (0.5-1 mM nitrate, 1 mM sulfate, 5 μ M U(VI), pH 6.8) was designed to match the geochemistry of the groundwater at Area 2. Duplicate reactors received throughput of this medium, at a residence time of 10 d, with or without 1 mM ethanol. Ethanol was consistently consumed in the ethanol-amended (+EtOH) reactors, with little or no accumulation of acetate. Nitrate was completely consumed in the +EtOH reactors, in contrast to the ethanol-free (-EtOH) systems in which consumption of only ca. 50% of the incoming nitrate took place. The lack of significant ammonium production suggested that denitrification was the major pathway for nitrate reduction. Significant accumulation of dissolved Mn(II) took place in the +EtOH reactors during the first two weeks of the experiment, after which Fe(III) oxide reduction commenced, and took over as the predominant TEAP. A minor amount of sulfate reduction began to take place after about 40 days in the +EtOH reactors, as indicated by the accumulation of solid-phase acid volatile (iron) sulfide (AVS) and μ M amounts of dissolved HS⁻. No methane production was observed in either set of reactors. Concentrations of dissolved hydrogen were an order of magnitude higher (1-10 ppm) in the +EtOH compared to the -EtOH (0.1-1 ppm) reactors. Dissolved and sodium bicarbonate-extractable U(VI) concentrations were consistently lower in the +EtOH reactors. However, U(VI) was not completely reduced, and in general it was not possible to interpret the aqueous/solid-phase and redox speciation of uranium in terms of standard conceptual models of uranium behavior in sediments (Langmuir, 1997). These results were consistent with a batch slurry experiment conducted as part of the Scheibe et al. FRC Area 2 field project.

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 provided independent information on the abundance of microbial groups responsible for U(VI) reduction and other TEAPs in the bioreactors. 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 septicum*. 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. A set of RT-PCR analyses were conducted on samples from the first SCR experiment by Brett Baldwin and Aaron Peakcock at the University of Tennessee through support of the Istok et al. ERSP project. The primers used for RT-PCR genes included a broad-spectrum *nirS* to target NO_3^- reducers; 16S rRNA genes for two major DMRB groups (*Geobacter* and *Anaeromyxobacter*; the *dsr* gene for SRB, and a general δ -proteobacterial probe that targets both SRB and DMRB. The results of these analyses were consistent with the culture-based enumerations. Analogous measurements are being conducted on archived samples from the second SCR experiment, and will also be applied to the upcoming experiment with gravel materials. Additional 16S rRNA gene-based microbial community fingerprinting analyses (terminal restriction length polymorphism) are underway, the results of which will be compared to those from gel element microarrays analyses to be conducted by Darrell Chandler and Aaron Peacock starting in 2008.

E. Microbial Physiology-Based Terminal Electron Accepting Process Model

A reaction-based model (coded in Fortran 95) called TEAPREVU, which stands for Terminal Electron Accepting Processes in a hypothetical Representative Elementary Volume of Uranium contaminated subsurface sediment. The model was developed to simulate the results of the batch slurry experiment with FRC Area 2 sediment conducted through the Scheibe et al. FRC Area 2 field project, with the idea that the developed framework would eventually be incorporated into a field-scale reactive transport simulation of in situ biostimulation at Area 2. A detailed description of the design and implementation of the model for simulation of redox processes in sediments is available on the ORNL FRC web-site (<http://public.ornl.gov/nabirfrc/frcdoc12.cfm>). The model envisions flow of ethanol-containing fluid through a single reactor cell (the fluid flow rate is set equal to zero to model the batch slurry experiment). The incoming fluid contains soluble electron acceptors (O_2 , NO_3^- , U(VI), SO_4^{2-}) whose abundance, together with the abundance of solid-phase electron acceptors (MnO_2 , FeOOH , S^0) in the sediment, control the relative rates of various terminal electron accepting processes (TEAP) and other biogeochemical reactions over time in the reactor. The model accounts for complete (to HCO_3^-) or incomplete (to acetate) oxidation of ethanol, as well as oxidation of acetate to HCO_3^- and/or CH_4 , via 18 different TEAP pathways. Each of the TEAP reactions are dependent on the biomass of one or more distinct microbial populations (8 total) chosen based on current knowledge of the kinds of organisms likely to proliferate in response to biostimulation of subsurface sediments. Growth of these populations is described using the bioenergetics-based approach developed by Rittman and McCarty (2001) for simulation of

wastewater (i.e. sewage) treatment, in which the partitioning of organic carbon flow between energy generation and cell biomass production is dependent on the free energy of the corresponding TEAP, which is computed dynamically during the simulation as a function of the abundance (concentration and/or activity) of the reactants and products involved in the process. This approach alleviates the need for making a priori assumptions about the biomass yield for the different physiological functional populations. Kinetic constants for uptake of electron donors, electron acceptors, and inorganic nitrogen compounds, as well for the inhibition of specific TEAP reactions (37 total) by the presence of more favorable electron acceptors, were either chosen arbitrarily or constrained by the physiological properties of pure culture representatives and/or by values required to reproduce the results of the batch slurry experiment. Each of the TEAP reactions results in production of various inorganic compounds, which either accumulate in solution or undergo reactions (sorption and/or mineral precipitation) with the solid-phase. The model also accounts for a wide variety of secondary redox reactions that may potentially occur in sedimentary environments (e.g. oxidation of reduced species such as Mn(II), Fe(II), U(IV), S(-II), S^0 , and CH_4 by aqueous or solid-phase electron acceptors such as O_2 , NO_3^- , MnO_2 , and $FeOOH$), as well as for precipitation/dissolution of mineral phases that may be associated with microbial activity in sediments. In this way the model is capable of simulating time-dependent changes in the abundance of various oxidized and reduced species and mineral phases as a function of the input of external electron acceptors/donors and other aqueous species. This capacity is critical for field-scale simulation of biogeochemical processes in subsurface sediments.

The model reproduced the basic patterns of organic substrate metabolism, consumption of electron acceptors, and accumulation of reduced end-products of anaerobic respiration in the batch sediment slurry experiment. Although the timing and magnitude of the predicted accumulation of acetate resulting from partial oxidation of ethanol (and the subsequent utilization of acetate) did not exactly match the experiment results, the general agreement between the simulation and the data suggests that the developed reaction network provides a reasonable explanation for this pattern of substrate metabolism. The strategy for simulating the interaction between the different TEAPs also seems generally valid, given the close resemblance of the predicted and observed patterns of electron acceptor (NO_3^- , Fe(III), SO_4^{2-}) consumption and reduced end-product accumulation (Fe(II) and CH_4 ; note that the abundance of reduced sulfur compounds (e.g. HS^- , FeS) was not determined). However, the predicted aqueous/solid speciation of uranium did not match the experimental data. A significant fraction (ca. 50%) of solid-associated U(VI) failed to desorb during biostimulation and therefore (as discussed above) remained unreduced at the end of the incubation.

A slightly modified version of TEAPREVU was incorporated (by Yilin Fang at PNNL) into a general biogeochemical reaction simulation framework (Fang et al., 2003; Fang et al., 2005), which in turn was linked with a three-dimensional field-scale reactive transport model (HYDROGEOCHEM) (Yeh et al., 2004) of the Area 2 experimental site. The model was used to help design the in situ ethanol biostimulation experiment described above, and successfully simulated the early-time aquifer response to ethanol amendment.

Recently, a collaboration was initiated with Dr. Qusheng Jin at the University of Oregon, as a result of Jin's desire to create a model of our Area 2 slurry experiment en route to

development of a field-scale reactive transport simulation of the results of biostimulation push-pull tests with ethanol at Area 2. Jin's model was implemented in the geochemical modeling software package Geochemist's Work Bench (GWB) (Bethke, 2002), which has been used previously to simulate microbial redox metabolism in both experimental systems and natural sedimentary environments (Jin and Bethke, 2003, 2005; Jin, 2007). The general structure of the GWB model is similar to that of TEAPREVU in that (i) a predetermined set of microbial functional groups is assumed to catalyze the various redox reactions; (ii) thermodynamic calculations are used to constrain the favorability of various reaction pathways. A key aspect of the new model is that, unlike TEAPREVU, the production and consumption of molecular hydrogen (H_2) is included within the reaction network. Although H_2 concentrations were not measured in the slurry incubation or the in situ biostimulation experiment, such measurements were made during the SCR experiments with Area 2 sediments. The model accurately reproduced all of the results of the slurry experiment, including the kinetics of ethanol consumption and transient acetate accumulation as a function of the biomass and metabolism of various functional metabolic groups. These results clearly illustrate how physiologically-based reaction models can capture the basic patterns of redox metabolism that are typically observed in sedimentary environments. We plan to transfer the structure of the new model to Yilin Fang at PNNL so that it can be incorporated (in place of the microbial physiology components of TEAPREVU) into the field-scale reactive transport for Area 2 and used to simulate the overall results of the in situ biostimulation experiment.

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Research Products

A. Referred publications

- 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.
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B. Submitted for publication

- Istok, J. D., M. Park, M. Michalsen, A. M. Spain, L. R. Krumholz, C. Liu et al. 2007. 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, E.E. Sustained enzymatic reduction of U(VI) in flow-through reactor systems that mimic natural subsurface environments. *Environ. Sci. Technol.*

Roden, E.E and Q. Jin. The rate-limiting step of microbial Fe(III) oxide reduction in the presence and absence of a soluble electron shuttle. *Environ. Sci. Technol.*

Jin, Q., S. R. Mohanty, and E. E. Roden. 2007. A physiologically-based microbial reaction model for ethanol-amended uranium-contaminated subsurface sediments. *Environ. Sci. Technol.*

D. Unpublished reports

Roden, E. E., Y. Fang, T. D. Scheibe, S. C. Brooks, and W. D. Burgos. 2005. TEAPREVU: A numerical simulation model of Terminal Electron-Accepting Processes in a Representative Elementary Volume of Uranium-contaminated subsurface sediment. Available at: <http://public.ornl.gov/nabirfrc/frcdoc12.cfm>.

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

Roden, E.E. "The rate-limiting step of microbial Fe(III) oxide reduction in the presence and absence of a soluble electron shuttle", Abstract N-107, American Society for Microbiology General Meeting, 2007.

Roden, E., Mohanty, S., B. Kollah, T. Scheibe, Y. Fang, and S. Brooks, "Numerical simulation of terminal electron-accepting processes in ethanol-stimulated subsurface sediments", Abstract Q-394, American Society for Microbiology General Meeting, 2005.

Roden, E. E., "TEAPREV: A numerical tool for assessing rates of terminal electron-accepting processes in a representative elementary volume of subsurface sediment," Geological Society of America *Abstracts with Programs*, 37(7): 535, 2005.

Fang, Y., T. D. Scheibe, E. E. Roden, and S. C. Brooks, "Field-scale reactive transport simulations of multiple terminal electron accepting processes," Geological Society of America *Abstracts with Programs*, 37(7): 535, 2005.

F. Workshop presentations (Roden group)

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., "Kinetic and thermodynamic controls on microbial Fe(III) oxide reduction", Telluride Summer Research Workshop on Iron Redox Chemistry, 2006.
- Roden, E.E., "TEAPREV: A numerical simulation model of Terminal Electron-Accepting Processes in a Representative Elementary Volume of subsurface sediment", U.S. Department of Energy, Natural and Enhanced Bioremediation Program ORNL Field Research Center Meeting, 2005. (Poster presentation)
- Jeon, B.H., E.E. Roden, B.A. Dempsey, W.D. Burgos, M.O. Barnett, "Biological versus chemical reduction of U(VI) at the solid-water interface: implications for in situ uranium bioremediation, , U.S. Department of Energy, Natural and Enhanced Bioremediation Program Annual PI Meeting, 2005. (Poster presentation)