

Entry for “most recent results to date”

Our research was funded by a grant to The Pennsylvania State University, University of Central Florida, and The University of Alabama in the Biotransformation Element of the NABIR Program (DE-FG02-01ER63180). Our research focused on (1) microbial reduction of Fe(III) and U(VI) individually, and concomitantly in natural sediments, (2) Fe(III) oxide surface chemistry, specifically with respect to reactions with Fe(II) and U(VI), (3) the influence of humic substances on Fe(III) and U(VI) bioreduction, and on U(VI) complexation, and (4) the development of reaction-based reactive transport biogeochemical models to numerically simulate our experimental results. In addition, Eric Roden has been working with Tim Scheibe (PNNL) and Scott Brooks (ORNL) on a NABIR FRC Field Project at Area 2.

We have made considerable progress in recent years on several fronts and in several areas. We have divided our progress report into scientific accomplishments, and research products. Scientific accomplishments refer to our most significant findings and advancements in basic sciences. Research products refer not only to peer-reviewed publications but also to the scientists we have trained. Arguably, the major function of research universities is to produce the next generation of scientists and academicians.

#### Microbial Fe(III) Reduction

We have continued our investigations on microbial reduction of Fe(III) oxides. Modeling of our earlier experimental results required the assumption of a hydrated surface for hematite, more reactive than predicted based on theoretical solubility (Burgos et al., 2002). Subsequent studies with *Shewanella putrefaciens* and *Geobacter sulfurreducens* confirmed that the rates of Fe(III) bioreduction depend on oxide surface area rather than oxide thermodynamic properties (Roden, 2003a, b; 2004; Burgos et al., 2003). We have conducted a theoretical analysis of the effect of Fe(II) accumulation on the long-term kinetics of oxide bioreduction (Roden, 2004), the results of which are consistent with studies where variations in mixing intensity and pre-loading of Fe(II) vs. Mn(II) showed that the rate of bioreduction by *Shewanella putrefaciens* decreased due to accumulation of Fe(II) at the oxide interface (Royer et al., 2004). These experiments also demonstrated the significance of mixing or flow conditions and of surface hydration for bioreduction modeling. We investigated the effects of cell density on reduction rate (Roden, 2003a, Roden and Sedo, 2003), and provided information required for simulation of oxide reduction kinetics in nonsteady-state systems in which DMRB cell density varies over time. A phospholipid fatty acid (PLFA)-based approach (Findlay et al., 1989) for quantifying changes in DMRB abundance in relatively low biomass (ca.  $10^5$  to  $10^8$  cells mL<sup>-1</sup>), mineral-rich reaction systems is underway. The developed framework accurately reproduces the results of batch and column studies of synthetic and natural Fe(III) oxide reduction (Roden and Sedo, 2003), and has been used to assess the impact of field-scale physical/chemical heterogeneity on spatial and temporal patterns of bacterial Fe(III) oxide reduction (Roden, 2003c).

#### Microbial U(VI) Reduction

We have obtained kinetic parameters for reduction of soluble U(VI)-carbonate complexes by *G. sulfurreducens* and *S. putrefaciens* (Roden and Scheibe, 2003; Burgos et al., 2004). We examined the potential for bioreduction of U(VI) by *Geobacter sulfurreducens* in the presence of synthetic Fe(III) oxides and natural Fe(III) oxide-containing solids (Jeon et al., 2004a, b) in which more than 95% of added U(VI) was sorbed to mineral surfaces. The results showed that a significant portion of solid-associated U(VI) was resistant to both enzymatic and abiotic (Fe(II)-driven) reduction, but that the rate and extent of bioreduction of U(VI) was increased due to the addition of anthraquinone-2,6-disulfonate (AQDS). Other work has demonstrated that the rate of bioreduction of U(VI) can be decreased due to the presence of humic substances (Burgos et al., 2004). Overall, these findings indicate that the rate and extent of U(VI)

bioreduction is strongly influenced by both aqueous and solid-phase geochemical conditions, and that the effects of certain components of natural systems and of amendments such as humic substances need to be studied in greater detail.

We have conducted long-term semicontinuous culture (cf. (Roden and Urrutia, 1999)) and column experiments on coupled Fe(III) oxide/U(VI) reduction. These experiments were conducted with natural subsurface sediment from the Oyster site in Virginia, whose Fe content and microbial reducibility are comparable to ORNL FRC sediments (Jeon et al., 2004b). The results have conclusively demonstrated the potential for sustained removal of U(VI) from solution via DMRB activity in excess of the U(VI) sorption capacity of the natural mineral assemblages. Development of reaction-based numerical simulations of the experimental results are underway, and will include information on the U(VI) sorption characteristics of the Oyster sediment materials (Jeon et al., 2004a).

#### Iron Oxide Surface Chemistry

We have continued our abiotic investigations of the Fe(III) oxide-water interface. Several of our studies have demonstrated changes in the bulk phase due to Fe(II) and other sorbates (e.g., Jang et al., 2003; Jeon et al., 2003). Jang (2004) demonstrated that the hydrated surface of nano-particles of hematite (prepared according to well-cited recipes and confirmed to be 100% hematite by Mossbauer spectroscopy and XRD) exhibited the solubility of hydrous ferric oxide (HFO). Jang (2004) also demonstrated that the sorptive reactivity of hematite and HFO were identical except for different specific surface area and pH<sub>zpc</sub>, and that the reduction of U(VI) by sorbed Fe(II) in the presence of the two phases was also similar in spite of theoretical predictions of large differences in Nernst potential. These results are consistent with the modeling of hematite bioreduction experiments where the thermodynamic potential of hematite had to be adjusted to represent a more disordered “surface” phase in order to accurately model bioreduction kinetics (Burgos et al., 2002, 2003). In other words, we now have a series of convergent observations that suggest that the reactivity of thermodynamically distinct ferric oxides are all similar once hydrated. Our investigations have also revealed that phases formed during exposure to low concentrations of oxidizing agents at neutral pH are extremely redox active (Dempsey and Park, in preparation). These results present opportunities for simplifications in modeling and could provide significant mechanistic insights into the effects of oxidizing agents such as AQDS, nitrate or U(VI) on abiotic and biotic systems.

#### Humic Chemistry

We have demonstrated that humic substances enhance solid-phase Fe(III) bioreduction via both electron shuttling and Fe(II) complexation (Royer et al., 2002a, b). While humic substances can enhance Fe(III) reduction, we also discovered that, with *S. putrefaciens* CN32, humic substances increase divalent metal toxicity during solid-phase Fe(III) bioreduction (Stone et al., 2004). Another surprising finding was that humic substances were shown to inhibit the bioreduction of dissolved U(VI) and that soluble humic-U(IV) complexes were likely formed (Burgos et al., 2004). We have also measured abiotic reactions between humic substances and U(VI). Kirkham (2004) measured and modeled complexation of U(VI) by humic substances as a function of pH, PCO<sub>2</sub>, U(VI) concentration, and humic concentration, and demonstrated that humic substances can complex U(VI) even at neutral pH values and in the presence of high (ca. 30 mM) carbonate concentrations. Jang (2004) measured the abiotic reduction of U(VI) by Fe(II) sorbed to Fe(III) oxides in the presence/absence of humic substances and demonstrated that humic substances inhibited the heterogeneous reduction of U(VI). All of these findings highlight the additional complexity that humic substances add to the system.

#### Reaction-Based Reactive Transport Modeling

We have recently developed, validated, and documented a series of diagonalized reaction-based reactive transport computer models (HYDROGEOCHEM; Yeh et al., 2004a, b). We demonstrated that parallel kinetic reactions could be modeled if separate experiments are used to independently measure each

contributing kinetic reaction (Burgos et al., 2003). The handling of parallel kinetic reactions in Fe(III)/U(VI) reaction systems will be critical to the success of any modeling effort. We have demonstrated the use of a reaction-based reactive transport model (HYDROGEOCHEM) for the simulation of biological iron reduction in natural sediment columns (Burgos and Yeh, unpublished results). Finally, we have developed a preliminary reaction-based model of coupled Fe(III) oxide/U(VI) reduction that has been employed in numerical simulations of U(VI) bioreduction in bench-scale (Roden, 2003d) and field-scale (Roden and Scheibe, 2003; Roden, 2003c) systems. This progress gives us confidence that these models can be successfully applied to field conditions that required large reaction networks and physical heterogeneity.

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### Entry for “papers and other products”

Only manuscripts published, accepted or submitted to peer-reviewed journals and peer-reviewed conference proceedings have been included in the list below. We have attempted to publish our results only in the top journals in our fields. In addition, we currently have over 10 manuscripts in preparation.

Summary of all peer-reviewed publications supported through Grants DE-FG02-98ER62691 and DE-FG02-01ER63180:

1. Burgos, W.D, J.J. Stone, Z. Shi, R. Kirkham, R.A. Royer, B.A. Dempsey, E.E. Roden, K.M. Kemner, S.D. Kelly and B. Gu. 2004. Humic Materials Inhibit Biological Uranium Reduction by *Shewanella putrefaciens* CN32. *Environmental Science & Technology*. Submitted.
2. Jeon, B.H., S.D. Kelly, K.M. Kemner, M.O. Barnett, W.D. Burgos, B.A. Dempsey and E.E. Roden. 2004. Microbial reduction of U(VI) at the solid-water interface. *Environmental Science & Technology*. Submitted.
3. Jeon, B.H., S.D. Kelly, K.M. Kemner, M.O. Barnett, W.D. Burgos, B.A. Dempsey and E.E. Roden. 2004. Abiotic, Fe(II)-catalyzed reduction of U(VI) at the solid-water interface. *Environmental Science & Technology*. Submitted.
4. Stone J.J., W.D. Burgos, R.A. Royer and B.A. Dempsey. 2004. Impact of Zinc on Biological Fe(III) and NO<sub>3</sub><sup>-</sup> Reduction by *Shewanella putrefaciens* CN32. *Environmental Science & Technology*. Re-submitted with revisions.
5. Stone J.J., W.D. Burgos, R.A. Royer and B.A. Dempsey. 2004. Zinc Inhibition of the Biological Reduction of Hematite by *Shewanella putrefaciens* CN32. *Environmental Science & Technology*. Re-submitted with revisions.
6. Suk, H., and G.T. Yeh. 2004. Multiphase flow modeling with general boundary conditions and phase configuration changes using fractional flow approaches. *ASCE Hydrologic Engineering*. Submitted.
7. Suk, H., and G.T. Yeh. 2004. Particle tracking algorithm for the Lagrangian finite element method under transient conditions in multi-dimensions. *International J. Numerical Methods in Engineering*. Submitted.
8. Suk, H., and G.T. Yeh. 2004. Three-dimensional three-phase flow simulations using the Lagrangian-Eulerian approach with adaptively zooming and peak/valley capturing scheme (LEZOOMPC). *ASCE Hydrologic Engineering*. Submitted.
9. Roden, E.E., and T.D. Scheibe. 2004. Conceptual and numerical model of uranium(VI) reductive immobilization in structured subsurface media. U.S. Department of Energy, Natural and Enhanced Bioremediation (NABIR) Program, Oak Ridge Field Research Center Web Site, Reports/Presentations by NABIR Investigators. <http://public.ornl.gov/nabirfc/other/Modeling%20Manuscript.pdf>
10. Jeon, B.H., B.A. Dempsey, W.D. Burgos and R.A. Royer. 2004. Modeling the sorption kinetics of divalent metal ions to hematite. *Water Research*. In Press.
11. Jeon, B.H., B.A. Dempsey and W.D. Burgos. 2004. A Low-Temperature Oxygen Trap for Maintenance of Strict Anaerobic Conditions. *ASCE Journal of Environmental Engineering*. Accepted.

12. Royer R.A., B.A. Dempsey, B.H. Jeon and W.D. Burgos. 2004. Inhibition of Biological Reductive Dissolution of Hematite by Ferrous Iron. *Environmental Science & Technology*. 38:187-193.
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14. Jeon, B.H., B.A. Dempsey and W.D. Burgos. 2003. Kinetics and Mechanisms for Reactions of Fe(II) with Iron(III) Oxides. *Environmental Science & Technology*. 37:3309-3315.
15. Roden, E.E. 2003. Fe(III) oxide reactivity toward biological versus chemical reduction. *Environmental Science & Technology*. 37:1319-1324.
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19. Jang, J.H., B.A. Dempsey, G.L. Catchen and W.D. Burgos. 2003. Effects of Zn(II), Cu(II), Mn(II), NO<sub>3</sub><sup>-</sup>, or SO<sub>4</sub><sup>2-</sup> at pH 6.5 and 8.5 on transformation of hydrous ferric oxide (HFO) as evidenced by Mossbauer spectroscopy. *Colloids & Surfaces A*. 221:55-68.
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Over nearly 6 years of NABIR support has led to the completion of five Ph.D. and five M.S. degrees. All of these students completed high quality research and were first-authors or co-authors on many of our publications. Furthermore, two Ph.D. students have begun tenure track faculty positions at strong research institutions, and two other Ph.D. students continue to work on NABIR-funded research through post doc positions at PNNL. Compared to all our publications, we are most proud of these research “products”.

Summary of all graduate students supported through Grants DE-FG02-98ER62691 and DE-FG02-01ER63180 and their current employment:

(student, degree-granting university, degree, date earned, current affiliation)

1. Jiangtao Sun, Univ. Central Florida, M.S. Water Resources, February 2004, Engineering Application and Science, Inc., Tampa, FL
2. Je-Hun Jang, Penn State University, Ph.D. Environmental Engineering, January 2004, Geochemistry, Penn State University, Post Doc with Dr. Sue Brantley
3. Rachel Kirkham, Penn State University, M.S. Environmental Engineering, January 2004, Camp, Dresser & McKee Consulting Engineers, Harrisburg, PA

4. Yilin Fang, Penn State University, Ph.D. Environmental Engineering, May 2003, Pacific Northwest National Laboratory, Post Doc with Dr. Tim Scheibe
5. Hee-Jun Suk, Penn State University, Ph.D. Environmental Engineering, May 2003, Assistant Professor, Civil and Environmental Engineering, Seoul National University, Korea
6. Zhen Shi, Penn State University, M.S. Environmental Engineering, May 2003, BioHydrogen, Inc., State College, PA
7. Yuan Li, Univ. Central Florida, M.S. Water Resources, May 2003, Taylor Engineering, Jacksonville, FL
8. James J. Stone, Penn State University, Ph.D. Environmental Engineering, December 2002, Assistant Professor, Civil and Environmental Engineering, South Dakota School of Mines & Technology
9. Byong-Hun Jeon, Penn State University, Ph.D. Environmental Engineering, December 2001, Pacific Northwest National Laboratory, Post Doc with Dr. Chongxuan Liu
10. Angela Fisher, Penn State University, M.S. Environmental Engineering, December 2000, The Keystone Center, Keystone, CO
11. Richard Royer, Penn State University, Former Post Doc, Environmental Technology Laboratory, General Electric Company