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Project Title: **Advanced Experimental Analysis of Controls on Microbial Fe(III) Oxide Reduction**

Lead Principal Investigator:

Dr. Eric E. Roden
Associate Professor of Biological Sciences
Department of Biological Sciences
University of Alabama
Box 870206
Tuscaloosa, Alabama 35487--0206
Telephone: 205-348-0556
e-mail: eroden@biology.as.ua.edu

Co Principal Investigators:

Matilde M. Urrutia
Assistant Research Scientist
Biological Sciences
University of Alabama
Box 870206
Tuscaloosa AL 35487 0206
Telephone: 205-348-5191
e-mail: murrutia@biology.as.ua.edu

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Lead Principal Investigator: Eric E. Roden
The University of Alabama
Department of Biological Sciences
Tuscaloosa, AL 35487-0206
(205) 348-0556 (205)
eroden@biology.as.ua.edu

Co-Principal Investigator: Matilde M. Urrutia
The University of Alabama
Department of Biological Sciences
Tuscaloosa, AL 35487-0206
(205) 348-5191
murrutia@biology.as.ua.edu

Research Objectives and Significance:

Understanding factors which control the long-term survival and activity of Fe(III)-reducing bacteria (FeRB) in subsurface sedimentary environments is important for predicting the ability of these organisms to serve as agents for bioremediation of organic and inorganic contaminants. This project seeks to refine our quantitative understanding of microbiological and geochemical controls on bacterial Fe(III) oxide reduction and growth of FeRB, using laboratory reactor systems which mimic to varying degrees the physical and chemical conditions of subsurface sedimentary environments. Methods for studying microbial Fe(III) oxide reduction and FeRB growth in experimental systems which incorporate advective aqueous phase flux are being developed for this purpose. These methodologies, together with an accumulating database on the kinetics of Fe(III) reduction and bacterial growth with various synthetic and natural Fe(III) oxide minerals, will be applicable to experimental and modeling studies of subsurface contaminant transformations directly coupled to or influenced by bacterial Fe(III) oxide reduction activity.

Research Progress and Implications:

This report summarizes research accomplished after approximately 2.5 yr of a 3-yr project. A central hypothesis of the research is that advective elimination of the primary end-product of Fe(III) oxide reduction, Fe(II), will enhance the rate and extent of microbial Fe(III) oxide reduction in open experimental systems. This hypothesis is based on previous studies in our laboratory which demonstrated that association of evolved Fe(II) with oxide and FeRB cell surfaces (via adsorption or surface precipitation) is a primary cause for cessation of Fe(III) oxide reduction activity in batch culture experiments [6].

Semicontinuous culturing was adopted as a first approach to test the above hypothesis [4]. Synthetic goethite or natural Fe(III) oxide-rich subsoils were used as Fe(III) sources, with the Fe(III)-reducing bacterium *Shewanella alga* as the test organism. Average residence times of 18

or 9 d were established by replacing a portion of the aqueous phase of the cultures with fresh sterile, anaerobic culture medium every 3-4 d. Consistent with our basic hypothesis, medium replacement resulted in a 2-4 fold increase in the cumulative amount of Fe(II) produced in the cultures over a 2-month incubation period, relative to that occurring in parallel batch cultures. In addition, Fe(III) reduction in the semicontinuous cultures showed little or no sign of slow-down at the end of the experiment, whereas reduction had virtually ceased in the batch cultures. In all cases the enhanced reduction was accounted for by generation of dissolved Fe(II) which was periodically removed from the cultures during aqueous phase replacement. The Fe(II) content of the solid-phase (sorbed or precipitated) was lower in the semicontinuous, which suggested that aqueous phase flux led to significant Fe(II) desorption or reduced solid-phase association. Together these results indicated that the primary basis for the stimulation of Fe(III) oxide reduction was advective removal of Fe(II). Measurements of bacterial protein in the cultures indicated that advective removal of Fe(II) stimulated bacterial growth, which is consistent with the observed persistence of Fe(III) oxide reduction activity. A simulation model in which Fe(II) sorption to the solid-phase resulted in blockage of surface reduction sites captured the contrasting behavior of the batch vs. semicontinuous Gt reduction systems.

A long-term (5 months) flow-through column experiment was conducted to test the hypothesis that advective Fe(II) removal could allow for complete microbial reductive dissolution of a crystalline Fe(III) oxide [5]. Such complete removal of Fe(III) oxide content has been documented in landfill leachate-contaminated aquifer sediments in Denmark [1]. The results showed that greater than 90% of the Fe content of synthetic goethite-coated sand was reduced and mobilized from the column as aqueous Fe(II) over the course of experiment. In contrast, only ca. 15% of the goethite-coated sand was reduced in parallel batch cultures. The near-complete removal of the Fe(III) oxide content of the goethite-coated sand was verified by total Fe determinations conducted on the contents of the columns at the conclusion of the experiment. Because of the very short residence time in the column reactors (6 hr), this experiment must be viewed as a 'proof of concept' rather than a simulation of in situ subsurface environments. Nevertheless, the findings are very important in that they show for the first time that crystalline Fe(III) oxides can, under the appropriate conditions, be completely reduced and solubilized through bacterial activity. This is in strong contrast to previous studies of crystalline Fe(III) oxide reduction, which typically revealed only relative minor degrees of reduction/solubilization.

In an attempt to more realistically model subsurface sedimentary environments, we conducted another dynamic flow experiment in which larger (3.8 cm diameter, 120 cm^3 internal volume) columns were packed with natural Fe(III) oxide-coated sand (from Abbott's Pit in VA) and pulse-inoculated (8 hour loading period) with FeRB cells to total volume-averaged concentration of 10^6 per mL. The reactors were designed after those developed recently by Roychoudhury et al. [7] for studies of aquatic sediment metabolism. The end-pieces of the reactors were fitted with a diffuser plate to encourage even distribution of fluid/solute flow through the reactor. The residence time in the column reactor was approximately 10 days, still relatively short but much more realistic in terms of natural groundwater aquifer systems. In this experiment, Fe(III) oxide reduction activity (as measured by the cumulative production of aqueous Fe(II) which exited the column) persisted throughout the 2.5-month incubation period. In strong contrast, reduction activity ceased after ca. 2 weeks in parallel batch culture experiments conducted with the same material. These results affirm those from the

semicontinuous culture experiments which suggest that advective aqueous phase flux which leads to residence times on the order of weeks to months could play an important role in sustaining microbial Fe(III) oxide reduction in subsurface environments. In addition, our successful deployment of this experimental methodology gives us confidence that the approach can be used effectively in future studies of contaminant metal/radionuclide transformations coupled to bacterial Fe(III) oxide reduction. We have recently proposed to use this methodology for studies of Sr immobilization coupled to carbonate mineral formation during Fe(III) oxide reduction in subsurface sediment materials from Hanford and Oak Ridge National Laboratory [3]. This proposal contains a summary of important findings from this project as well as the Ferris/Roden EMSP 96-10 project on Sr immobilization under Fe(III) oxide-reducing conditions.

Our findings to date demonstrate that aqueous phase transport and attendant elimination of reaction end-products could play an important role in governing the *in situ* rate and persistence of microbial Fe(III) oxide reduction activity in anaerobic subsurface sediments. This in turn may have an important impact on the survival and activity of FeRB to be exploited for bioremediation purposes (e.g. the reductive immobilization of uranium, chromium, cobalt, and other metal/radionuclide contaminants; oxidation of hydrocarbon contaminants; reductive dechlorination of chlorinated solvents). An important implication of these findings is that models describing bacterial Fe(III) oxide reduction in subsurface environments will have to account for the fate of evolved Fe(II), and relate this in some manner to the abundance of available Fe(III) reduction sites and the viability of Fe(III)-reducing bacteria. A provisional model with these characteristics has been developed [4].

Several additional significant research accomplishments have been supported by this project, including:

(1) Two previous lines of research on the geochemical and microbiological controls on bacterial Fe(III) oxide reduction in batch culture, which were originally supported by a subcontract through Battelle PNNL, have been brought to completion during the course of the EMSP 96-10 project. This has resulted in publication of one paper [9] and submission of another [10].

(2) We have conducted a detailed analysis of the growth rate and biomass yield (derived from measurements of cell protein production) of two representative FeRB (*S. alga* and *Geobacter metallireducens*) growing with different forms of synthetic Fe(III) (Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-NTA, aorphous $Fe(OH)_3$, α-FeOOH) as an electron acceptor [8]. Results indicate that despite considerable differences in the rate and pattern of Fe(III) reduction and FeRB growth, an approximately consistent long-term biomass yield of 5 to 15 mg protein per mmol Fe(III) reduced was observed with the different forms of Fe(III). These results provide a useful point of departure for quantitative modeling of FeRB growth and metabolism in various types of experimental and *in situ* anaerobic sedimentary systems. They also point to an unusual temporal offset between cell division and protein biosynthesis during growth of FeRB on solid-phase Fe(III), which we are currently examining in detail.

(3) We have tested the use of ^{3}H-leucine incorporation as a means of determining short-term (1-2 hr time frame) FeRB (*G. metallireducens* and *S. alga*) growth rates during reduction of soluble and solid-phase Fe(III). Results suggest that *G. metallireducens*, unlike *S. alga*, lacks the ability

to express a high rate of exogenous leucine incorporation (probably due to the lack of an "active" cell membrane leucine uptake system). An important implication of this finding is that ^{3}H-leucine incorporation may not be an appropriate means of estimating FeRB growth rates in natural sediments in which *Geobacter* dominates Fe(III) reduction activity. Another important implication of this study is that rates of *S. alga* biomass production estimated from ^{3}H-leucine incorporation with 10-1000 nM total leucine concentration were much lower than those inferred from independent measurements of biomass yield during Fe(III) reduction, probably because of isotope dilution by *de novo* leucine biosynthesis. A subsequent experiment indicated that additions of at least 1 mM total leucine are required to repress *de novo* biosynthesis during short-term (0.5-2 hr) ^{3}H-leucine incorporation experiments in culture medium. Rates of protein production during growth of *S. alga* on ferric citrate estimated from ^{3}H-leucine incorporation with 1 mM total leucine agreed within a factor of 2 with independent estimates of bacterial protein production. This result indicates that ^{3}H-leucine incorporation can provide a quantitatively valid method for tracking instantaneous bacterial growth rates in experimental Fe(III) reduction systems.

(4) We are conducting a detailed comparison of the heterogeneity in reactivity of synthetic vs. natural Fe(III) oxides toward microbial reduction activity and abiotic reductive dissolution (by ascorbate at acidic pH; [2]). Results indicate that both chemical and biological Fe(III) oxide reduction can be described by first-order kinetics, and therefore data generated in our experiments can be analyzed in terms of the reactive continuum model described by Postma [2]. Initial results indicate that the apparent degree of heterogeneity in oxide reactivity is greater in relation to chemical vs. microbiological reduction mechanisms. These findings have important implications for development of quantitative kinetic models of Fe(III) oxide reduction activity in subsurface environments.

Planned Research and Publication Activities:

Manuscripts on the column Fe(III) oxide reduction experiment and the FeRB growth/biomass production study are in preparation and will be submitted for publication by September 1999. Completion of two papers dealing with ^{3}H-leucine incorporation by FeRB and the use of ^{3}H-leucine incorporation to measure FeRB growth rates is anticipated by the end of the year. A broad-ranging study of the reduction kinetics of natural and synthetic Fe(III) oxide reduction, as influenced by heterogeneity of Fe(III) oxide reactivity (see (4) above) is underway and will be completed in 1999. We also plan to conduct one additional long-term (6 months) experiment on Fe(III) oxide reduction and FeRB growth rates (determined using ^{3}H-Leu) in flow-through column reactors. This experiment is intended to verify the influence of aqueous phase Fe(II) flux on the long-term persistence of Fe(III) oxide reduction activity and FeRB viability.

References:

1. Heron, G., C. Crouzet, A. C. M. Bourg, and T. H. Christensen. 1994. Speciation of Fe(II) and Fe(III) in contaminated aquifer sediments using chemical extraction techniques. Environ Sci Technol. 28:1698-1705.

2. Postma, D. 1993. The reactivity of iron oxides in sediments: a kinetic approach. Geochim. Cosmochim. Acta. 57:5027-5034.

3. Roden, E. E., F. G. Ferris, M. R. Leonardo, C. Zheng, and D. S. Smith. 1999. Geochemical retardation of subsurface strontium migration via carbonate mineral formation coupled to microbial Fe(III) oxide reduction.

4. Roden, E. E., and M. M. Urrutia. 1999. Ferrous iron removal promotes microbial reduction of crystalline iron(III) oxides. Environ. Sci. Technol. 33:1847-1853.

5. Roden, E. E., M. M. Urrutia, and C. J. Mann. 1999. Near-complete bacterial reductive dissolution of crystalline Fe(III) oxide under conditions of advective aqueous phase flux. Manuscript in preparation.

6. Roden, E. E., and J. M. Zachara. 1996. Microbial reduction of crystalline Fe(III) oxides: role of oxide surface area and potential for cell growth. Environ. Sci. Technol. 30:1618-1628.

7. Roychoudhury, A. N., E. Viollier, and P. VanCappellen. 1998. A plug flow-through reactor for studying biogeochemical reactions in undistrubed aquatic sediments. Appl. Geochem. 13:269-280.

8. Urrutia, M. M., and E. E. Roden. 1998. Growth rates and biomass production of dissimilatory Fe(III)-reducing bacteria with soluble and solid-phase Fe(III). Manuscript in preparation.

9. Urrutia, M. M., E. E. Roden, J. K. Fredrickson, and J. M. Zachara. 1998. Microbial and geochemical controls on synthetic Fe(III) oxide reduction by Shewanella alga strain BrY. Geomicrobiol. J. 15:269-291.

10. Urrutia, M. M., E. E. Roden, and J. M. Zachara. 1999. Influence of aqueous and solid-phase Fe(II) sinks on microbial reduction of crystalline Fe(III) oxides. Environ. Sci. Technol. Submitted for publication.

Information Access:

Roden, E.E. and M.M. Urrutia. 1999. Ferrous iron removal stimulates microbial iron(III) oxide reduction in semicontinuous cultures. *Environ. Sci. Technol*. 33:1847-1853.

Urrutia, M.M., E.E. Roden, J.K. Fredrickson, and J.M. Zachara. 1998. Microbial and surface chemistry controls on reduction of synthetic Fe(III) oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga. Geomicrobiol. J.* 15:269-291.

Urrutia, M.M., E.E. Roden, and J.M. Zachara. Influence of aqueous and solid-phase Fe(II) complexants on microbial reduction of crystalline Fe(III) oxides. *Environ. Sci. Technol.* April 1999.

Roden, E. E., F. G. Ferris, M. R. Leonardo, C. Zheng, and D. S. Smith. 1999. Geochemical retardation of subsurface strontium migration via carbonate mineral formation coupled to microbial Fe(III) oxide reduction. Proposal submitted to U.S. Department of Energy, Office of

Science, Notice 99-06, Environmental Management Science Program, Research Related to Subsurface Contamination/Vadose Zone Issues.

Urrutia, M.M. and E.E. Roden. 1997. Growth rates and biomass production of dissimilatory Fe(III)-reducing bacteria with soluble and solid-phase Fe(III). American Society for Microbiology, 97th Annual Meeting, Abstract Volume, p. 337.

Roden, E.E. and M.M. Urrutia. 1997. Microbial Fe(III) oxide reduction in aquatic sediments: new insights into controls and quantitative significance in biogeochemical fluxes. XIII International Symposium on Environmental Biogeochemistry, Abstract Volume, p. 211.

Roden, E.E. and M.M. Urrutia. 1998. Microbial Fe(III) oxide reduction in open experimental systems. American Society for Microbiology, 98th Annual Meeting, Abstract Volume, p. 409.

May, T. and E.E. Roden. ^{3}H-leucine incorporation by Fe(III)-reducing bacteria. American Society for Microbiology, 98th Annual Meeting, Abstract Volume, p. 409.