Project #1021435

Title: Immobilization of Radionuclides Through Anaerobic Bio-oxidation of Fe(ll)

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Results To Date: Anaerobic, Nitrate-Dependent Fe(II) Bio-Oxidation: A Column Study Report FY 2005/2006

Previous studies have demonstrated that nitrate-dependent bio-oxidation of Fe(II) by Azospira suillium strain PS results in the formation of crystalline mixed Fe(II)/Fe(III) mineral phases which results in the subsequent immobilization of heavy metals and radionuclides

1. Greater than 80% of the U(VI) was sequestered by the most dense, crystalline Fe(II)/Fe(III) mineral phases, which are not readily reduced by Fe(III)-reducing bacteria. Given that microbially-catalyzed nitrate-dependent Fe(II) oxidation has been identified as a ubiquitous biogeochemical process contributing to anaerobic iron redox cycling in sedimentary environments. Most probable number enumeration revealed nitrate-dependent Fe(II) oxidizing microbial communities in groundwater and subsurface sediments in the order of 0 - 2.04 x 103 cells mL-1 and 2.39 x 102 - 1.17 x 103 cells (g wet sediment)-1, respectively. The microbial community estimate is consistent with previously reported results including freshwater lake sediment (2.4 x 103-1.47 x 104 cells g-1 sediment)

2. The isolation of a nitrate-dependent Fe(II) oxidizing isolate, strain TPSY, from the MPN enumeration series initiated from groundwater collected from the FRC, Area 2, further supports the potential for an active nitrate-dependent Fe(II) oxidizing microbial community in DOE ERS FRC groundwater and sediments. Strain TPSY is a facultative anaerobe, capable of mixotrophic growth on Fe(II) and nitrate with 0.1mM acetate over a pH range of 4.5 to 9.0. Comparative analysis of the entire 16S rDNA sequence indicated that strain TPSY is a member of the beta subclass of the Proteobacteria. Strain TPSY is closely related to Diaphorobacter nitroreducens. As nitrate is often a co-contaminant found in these environments, these results indicate the potential for an active nitrate-dependent Fe(II) oxidizing microbial population in situ. The efficacy of nitrate-dependent Fe(II) oxidation in subsurface sediments under advective flow was evaluated in a meso-scale column reactor packed with sterile low iron sand amended with subsurface sediments collected from the DOE ERS FRC background field site (10% mass/mass). Continuous flow of minimal medium mimicked the natural groundwater (ddI water containing vitamins and minerals) at the ambient pH, 5.7. Flow velocity was maintained at 2.5 cm d-1. The columns were incubated at room temperature in an anoxic glove bag under a 100% N2 atmosphere. Periodic FeCl2 and nitrate injections over a period of 49 days resulted in the retention of 95% of the iron (~20.3 mmol). Extraction of solid-phase Fe revealed a net increase in Fe(III) of 13.2 mmol above background Fe(III) content indicating that 65% of the injected Fe(II) was oxidized. Differential solubility analysis of 0.5 M HCl-extractable Fe and 3 M HCl-extractable Fe indicated that the oxidation product was crystalline in nature as only 20% was soluble in 0.5 M HCl. This formation of crystalline biogenic Fe(III) oxides is consistent with previous studies. Periodic injections of nitrate and acetate did not result in significant changes in Fe(II)

or Fe(III) throughout a control column. Enumeration of the nitrate-dependent Fe(II) oxidizing microbial community indicated that the Fe(II) and nitrate injection stimulated an anaerobic, nitrate-dependent Fe(II) oxidizing community (7.41 x 105 cells mL-1) just above the injection point (12.5-15 cm depth) . This microbial community was ~40% of the heterotrophic nitrate reducing community and ~350% of the heterotrophic Fe(III) reducing community. The abundance of the nitrate-dependent Fe(II) oxidizing microbial community enumerated in the column injected with nitrate and acetate was less than 0.0001% of the abundance of the heterotrophic nitrate reducing microorganisms suggesting that heterotrophic nitrate reducing microorganisms were not responsible for Fe(II) oxidation. This result was confirmed by small-subunit 16S rDNA clone libraries and a 16S rDNA microarray. At the point of injection ~47% of the microbial community was represented by the Acidobacteria and Actinobacteria in the column injected with Fe(II) and nitrate. Whereas, the injection of acetate and nitrate stimulated the Betaproteobacteria (86%) and was dominated by Azoarcus sp. (66%). The frequency of clones identified as Actinobacteria in the column injected with Fe(II) and nitrate represented the background abundance. However Acidobacteria clones were only observed at the point of injection and represented ~21% of the identified clones. These results suggest that Acidobacteria play a role in anaerobic, nitrate-dependent Fe(II) oxidation in these subsurface sediments. Bidirectional heuristical clustering analysis of hybridization signal intensity of the 16S rDNA supported experimental design revealing similarities in community structure between column treatments and location (lower or upper portion) as well as differences between portions of the column exhibiting geochemical evidence for Fe(II) oxidation and nitrate reduction (middle portion). Comparison of signal intensity from samples collected within each column revealed a variety of bacteria potentially stimulated by the Fe(II) injection. The most significant increase in the hybridization signal was associated with an unclassified member of Xanthomonadaceae. Although this microorganism was identified in the microarray, libraries of small-subunit 16S rDNA clones from the 12-15 cm depth did not identify a member of Xanthomonadaceae, suggesting low abundance relative to other microorganisms identified in the clone library. Nitrate-dependent Fe(II) oxidizing microorganisms are not only prevalent in surface environments but are also present in subsurface environments as demonstrated in the MPN enumeration series initiated from groundwater and sediment samples collected from the DOE ERS FRC. Thus demonstrating the potential for an active nitrate-dependent Fe(II) oxidizing microbial community in situ in subsurface environments such as the DOE ERS FRC. Together these results demonstrate that native subsurface sediments harbor microbial communities capable of nitrate-dependent Fe(II) oxidation under advective flow. The biogenic formation of reactive Fe(III) oxide minerals capable of immobilizing heavy metals and radionuclides presents a plausible bioremediative strategy for contaminated subsurface environments.

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Deliverables: Publications 1. Weber, K.A., Pollock, J., Cole, K.A., O'Connor, S.M., Achenbach, L.A., and Coates, J.D. (2005). Anaerobic nitrate-dependent iron(II) bio-oxidation by a novel, lithoautotrophic, betaproteobacterium, strain 2002. Applied and Environmental Microbiology 72: 686-694. 2. Coates, J.D. and Lovley, D.R. (2005).

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Abstracts and Presentations 1. K. A. Weber, E. J. Miller, B. Wintle, D. Saidou, E. Brodie, L. A. Achenbach, G. Anderson, J. D. Coates. Anaerobic, Nitrate-Dependent Fe(II) Oxidation: A Column Study. DOE-ERSP PI Workshop, Warrenton, VA. April 2-5, 2006.

2. K. A. Weber, B. Wintle, L. A. Achenbach, J. D. Coates. Anaerobic Fe(II) Bio-Oxidation under Advective Flow. American Geophysical Union Fall Meeting, San Francisco, CA. December 5-9, 2005.

3. K. A. Weber, P. Larese-Casanova, M. Scherer, J. Thieme, L. A. Achenbach, J. D. Coates. Biogenic Green Rust Formation by a Nitrate-Dependent Fe(II) Oxidizing Bacterium. 105th General Meeting American Society for Microbiology, Atlanta, GA. June 5-9, 2005.

4. J. D. Coates, K. A. Weber, P. Larese-Casanova, M. Scherer, J. Thieme, L. A. Achenbach. Anaerobic Metal Nitrate-Dependent Metal Bio-Oxidation. Department of Energy, NABIR PI Workshop, Warrenton, VA. April 18-21, 2005.

5. J.D. Coates, K.A. Weber, and L.A. Achenbach. Implications and application of nitrate-dependent Fe(II)-biooxidation. Telluride Workshop: Iron Redox Chemistry at Environmentally Relevant Surfaces", July 25-28, 2006, Telluride, CO

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7. K.A. Weber, E.J. Miller, B.E. Wintle, D. Saidou, L.A. Achenbach, and J.D. Coates. Anaerobic Fe(II) bio-oxidation in sedimentary environments. 232nd ACS National Meeting, San Francisco, CA, September 10-14, 2006