FINAL REPORT

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Title: Microbially-Promoted Solubilization of Steel Corrosion Products and Fate of Associated Actinides

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Abstract

Microorganisms have the capacity to modify iron oxides during anaerobic respiration. When the dissimilatory sulfate-reducing bacterium *Desulfovibrio desulfuricans* G20 respires soluble sulfate during colonization of the solid-phase iron oxide hematite, the sulfide product reacts with the iron to produce the insoluble iron sulfide, pyrrhotite. When soluble uranium is present as uranyl ion, these microorganisms reduce the U(VI) to U(IV) as insoluble uraninite on the hematite surface. There is also evidence that a stable form of U is produced under these conditions that displays an oxidation state between U(VI) and U(IV). The dissimilatory iron reducing bacterium, *Shewanella oneidensis* MR1 can utilize insoluble hematite as the sole electron acceptor for anaerobic respiration during growth and biofilm development on the mineral. The growth rate, maximum cell density and detachment rate for this bacterium are significantly greater on hematite than on magnetite (111) and (100). The difference could not be attributed to iron site density in the iron oxide. A gene (*ferA*) encoding a c-type cytochrome involved in dissimulatory iron reduction in the bacterium *Geobacter sulfurreducens* was completed sequenced and characterized. The sequence information was used to develop an in-situ reverse transcriptase polymerase chain reaction assay that could detect expression of the gene during growth and biofilm development on ferrihydrite at the single cell and microcolony level. X-ray photoelectron spectroscopic analysis revealed that the ferrihydrite was reduced during expression of this gene. The assay was extended to detect expression of genes involved in sulfate reduction and hydrogen reduction in sulfate-reducing bacteria.

Purpose of Research:

The goal of the research is to provide the scientific underpinnings for the development of biologically-based approaches for the removal of contaminants from corroding steel surfaces. Specifically, the research will:

- ?? Determine the role of mineral oxide structure, topology, and composition on bacterial attachment and reduction of iron oxide products that form on stainless steels and mild steels.
- ?? Determine how soluble electron shuttles facilitate reductive dissolution of iron oxide corrosion products
- ?? Determine the distribution of radionuclides released during reductive dissolution of iron oxide films

Accomplishments:

- ?? Demonstration of the precipitation of the iron sulfide pyrrhotite during biofilm formation by the dissimilatory sulfate reducing bacterium *Desulfovibrio desulfuricans* G20 on the surface of the iron oxide hematite. *(Neal, A.L., S. Techkarnjanaruk, A. Dohnalkova, D. McCready, B.M. Peyton, and G.G. Geesey. 2001. Iron sulfides and sulfur species produced at hematite surfaces in the presence of sulfate-reducing bacteria. Geochim. Cosmochim. Acta 65:223-235)*

- ?? Demonstration of the ability of *Desulfovibrio desulfuricans* G20 biofilms growing on iron oxide surfaces to reduce soluble uranyl ion to insoluble uraninite and formation of novel complexes yet to be characterized. *(Neal, A.L., D. Brew, B.M. Peyton, and G.G Geesey. Mixed valence U-complexes formed at hematite surfaces by attached sulfate-reducing bacteria. Environ. Sci. Technol., submitted)*

- ?? Development of an approach involving a channel-flow flat plate reactor and a reporter gene to quantify the accumulation rate and detachment rate of iron reducing bacteria growing on mineral surfaces that serve as the sole electron acceptor for energy production and growth *(Manuscript in preparation)*

- ?? Quantification of the accumulation rate, growth rate, maximum surface-associated cell densities, and detachment rate of the dissimilatory iron reducing bacterium *Shewanella oneidensis* MR1 during anaerobic respiration on different solid phase iron oxides, and their dependence on iron site density in the iron oxides. The

?? Determination of the complete base sequence of the *ferA* gene encoding a *c*-type cytochrome involved in dissimilatory iron reduction by the dissimilatory iron reducing bacterium *Geobacter sulfurreducens*. The sequence information provides the opportunity to develop assays to follow the expression of this iron reduction gene during growth on iron oxides. *(Magnuson, T.S., N. Isoyama, A.L. Hodges-Myerson, G. Davidson, M.J. Maroney, G.G. Geesey, and D.R. Lovley. 2001. Isolation, characterization, and gene sequence analysis of a membrane associated 89 kDa Fe(III) reducing cytochrome c from Geobacter sulfurreducens. Biochem. J. 359:147-152.)*

?? Development of an in-situ reverse transcriptase polymerase chain reaction (RT-PCR) to detect expression of genes encoding functions of metal reduction, sulfate reduction and hydrogen reduction in individual cells attached to iron oxide surfaces. *(Magnuson, T.S., N. Isoyama, A.L. Neal, and G.G. Geesey. Surface-associated growth, mineral transformations, and gene expression by Geobacter sulfurreducens on solid-phase mineral surfaces, Appl. Environ. Microbiol., submitted; Magnuson, T.S., A.L. Neal, and G.G. Geesey. Development and use of RT-PCR and in-situ RT-PCR for characterization of functional gene expression in sulfate-reducing bacteria. In revision)*

?? Demonstration of iron reduction and precipitation on iron oxide surfaces during expression of *ferA* by iron oxide associated cells of *Geobacter sulfurreducens*. *(Magnuson, T.S., A.L. Neal, B. Little and G.G. Geesey. Surface-associated growth, mineral transformations, and gene expression by Geobacter sulfurreducens on solid-phase mineral surfaces, Appl. Environ. Microbiol., submitted)*

?? Determination of advantages and disadvantages of different spectroscopic methods available for characterization of secondary mineral phases deposited on mineral surfaces colonized by bacteria. *(Geesey, G.G., A.L. Neal, P.A. Suci, and B.M. Peyton. A review of spectroscopic methods for characterizing microbial transformations of minerals. J. Microbiol. Meth. 51:125-139)*