

# **Microbially Promoted Solubilization of Steel Corrosion Products and Fate of Associated Actinides**

**(Project Number: 64931)**

## **Principal Investigator**

Yuri A. Gorby  
Pacific Northwest National Laboratory  
P.O. Box 999, MSIN P7-50  
Richland, WA 99352  
509-373-6177 (phone)  
yuri.gorby@pnl.gov

## **Co-Investigators**

Gill G. Geesey  
Montana State University  
Center for Biofilm Engineering  
409 Cobleigh Hall  
Bozeman MT 59717  
406-994-4770 (phone)  
gill\_g@erc.montana.edu.

Frank Caccavo, Jr.  
University of New Hampshire  
Department of Microbiology  
Durham, NH 03824  
603-862-2443 (phone)  
fcj@hopper.unh.edu.

James K. Fredrickson  
Pacific Northwest National Laboratory  
P.O. Box 999, MSIN P7-50  
Richland, WA 99352  
509-373-6177 (phone)  
jim.fredrickson@pnl.gov

## Research Objective

The research is designed to evaluate the impact of metal-reducing bacteria on the release of radionuclides, specifically uranium and plutonium, from iron hydroxide minerals formed on the surfaces of corroding mild and stainless steels. The ultimate goal is to develop a safe and effective biological approach for decontaminating mild and stainless steels that were used in the production, transport, and storage of radioactive materials.

## Problem Addressed

DOE needs statements call for “biological and physical chemical parameters for effective decontamination of metal surfaces using environmentally benign, aqueous-based biopolymer solutions and microbial processes with potential for decontaminating corroding metal surfaces.” Improved understanding of the fundamental processes of microbial reductive dissolution of iron oxide scale on corroding carbon steel will support assessment and potential application of an environmentally benign and cost-effective strategy for in situ decontamination of structural metal surfaces and piping. The research also addresses issues related to the development of oxide-reducing biofilms on steel surfaces under hydrated but nonsaturating conditions.

## Research Progress and Implications

This report summarizes accomplishments after one and a half years of a three-year project. Significant advances have been realized in each aspect of the proposed research.

The research is structured into four primary tasks that are being addressed by members of the collaborative team.

- Task 1 investigates the factors controlling the attachment to and release from oxide scale that forms on corroding mild and stainless steel by metal-reducing bacteria.
- Task 2 probes the effects of iron oxide composition and surface properties on cell attachment and biofilm formation.
- Task 3 examines and quantifies the reductive dissolution of synthetic iron oxide thin films as well as the reductive dissolution of iron oxide scale on corroding steel in the presence and absence of soluble electron shuttles that can enhance the rate and extent of enzymatic iron reduction.
- Task 4 determines the distribution of actinides released from iron oxides during reductive dissolution of scales that are colonized by metal-reducing bacteria. Particular attention will be given to the processes that direct the incorporation of actinides into the biomass.

Due to the brief format of this report, results and major implications are summarized to provide a generalized summary of salient points. This research provides information needed to

develop optimal approaches for 1) delivering and distributing cells to contaminated corrosion films, 2) determining the fate of contaminants affected by attached bacteria, and 3) recovering contaminants for further processing.

*Reduction and fate of Pu(IV) by metal-reducing bacteria.* Iron-reducing bacteria were tested for their ability to reduce insoluble Pu(IV), provided as PuO<sub>2</sub>, to soluble Pu(III). The fate of Pu(III) was determined using neodymium, Nd(III), as a nonradioactive analogue. The significant results demonstrated that

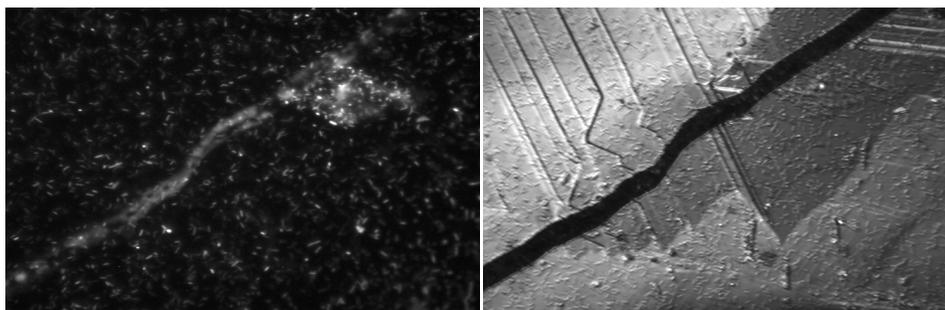
- Iron-reducing bacteria enzymatically reduced insoluble Pu(IV) to Pu(III).
- At pH values approaching 6, Pu(III) sorbed to cell surfaces.
- Cell biomass provides a significant sink for accumulation of actinides

Considering the sorption of Pu(III) with microbial biomass, mechanisms of attachment and detachment to solid substrata (e.g., crystalline and poorly crystalline iron oxides typically associated with corrosion films) were examined in detail.

*Cell attachment.* Adhesion of the iron reducing bacterium *Shewanella alga* strain BrY to hydrous ferric oxide, goethite, and hematite was examined. The results demonstrated that

- The bacteria readily adhere to both crystalline and amorphous Fe(III) oxide surfaces.
- Adhesion of *S. alga* strain BrY to hydrous ferric oxide (HFO) correlates with ionic strength, and thus is accurately described by the DLVO theory.
- The rate of solid phase iron reduction was directly correlated with adhesion of cells with surfaces and hence with the ionic strength of the medium.

Attachment of cells to the crystalline iron oxide hematite was also examined. Genes encoding for green fluorescence protein (GFP) were inserted into iron-reducing bacteria. Thus cells were imaged by confocal laser microscopy as they entered an anaerobic flow chamber and attached to the surfaces of specular hematite. Microscopy studies were carried out at the EMSL facility at PNNL. Figure 1 illustrates the distribution of cells on the hematite surfaces.



**Figure 1.** Images Taken on a (001) Hematite Surface of Strain *S. Putrefaciens* Strain MR1 (p519nGFP) After 52 Hours. Bright cells are particularly associated with the step edge. DIC images (left) were captured using an exposure of 32 msec, epifluorescence images (right) with an exposure of 7 sec. Gain for all images was 4 dB.

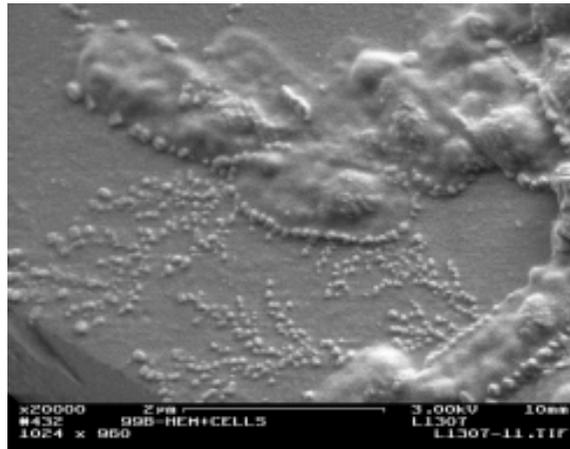
Results from this work demonstrated that:

- Primary cells adhesion occurred through flagellar attachment.
- Cells were heterogeneously distributed on hematite surfaces, with preferential attachment observed at cracks, steps, and crystal defects.
- Luminescence was greater with cells attached at the cracks, steps, and defects, suggesting that cells were metabolically active at these sites.

*Cell detachment.* Considering the ability for metal reducing bacteria to reduce and accumulate plutonium, approaches for removing or detaching cells from oxide surfaces were investigated. Cells attached to hematite surfaces were starved for electron donor and other nutrients required for growth. As shown in Figure 2, results demonstrated that

- Cells treated in this manner formed small (100 nm) vesicles on the cells' surface.
- Cells began to detach from the mineral surface
- Vesicles remained attached to the mineral surface.

Recent results show that these vesicles can enzymatically reduce metals and, therefore, have implications for the fate of multivalent radionuclides that are subject to enzymatic reduction.



**Figure 2.** SEM Image of Bacterial Cells with Attendant Vesicles on a Hematite Crystal

## Planned Activities

The remainder of the project will focus on

- Describing the role vesicles play in radionuclide reduction and accumulation
- Optimizing conditions for biomass/radionuclide recovery

- Evaluating decontamination of activated corrosion films
- The proposed research is expected to be completed within the timeframe of the project.

## **Information Access**

Das A and F Caccavo. 2000. *Adhesion of the dissimilatory Fe(III)-reducing bacterium Shewanella alga BrY to crystalline Fe(III) oxides* (submitted).

Das A and F Caccavo. 2000. *Dissimilatory Fe(III) Oxide Reduction by Shewanella alga BrY Requires Adhesion* (submitted).

Gorby Y, D Weaver, C Brown, M Romine, and A Neal. 2000. *Expression of Green Fluorescence Protein in Dissimilatory Iron Reducing Bacteria* (submitted).