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

Yuri Gorby[1], Frank Caccavo[2], Jr., Gill Geesey[3], Andy Neal[3], Dahnpat Rai[1], Amitabha Das[2],
Alice Dohnalkova[1], Matt Gray[1], Jeff McLean[1], and Jim Fredrickson[1]
[1]Pacific Northwest National Laboratory, [2]University of New Hampshire, [3]Montana State University

PROBLEM STATEMENT

Contamination consisting of nuclear fuel components (e.g., Pu and U) constitute the most significant problems at DOE facilities and are commonly associated with corroding surfaces of pipes, tanks, and other structure (Fig. 1). The Environmental Management Science Program (EMSP) request for D&D applications emphasized the need for fundamental research directed toward developing "new or significantly improved decontamination techniques for stainless steel….and for in situ decontamination of contaminated pipes". To this end, this research project focuses on elucidating principles involved in microbially-promoted dissolution of corroded metal surfaces and recovery of associated actinide contaminants.

Figure 1. Iron oxide corrosion products on mild steel piping and on the weld seem of a stainless steel storage tank form the 200 West Area, Hanford.

CONCEPT

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 costeffective strategy for in situ decontamination of structural surfaces and piping. Figure 2 illustrates a conceptual model of a potential biological decontamination application. In such an application, corroding metal surfaces accumulate actinides and other cationic contaminants (A). Iron reducing bacteria attach to and colonize the iron oxide corrosion (B). The bacteria enzymatically reduce and dissolve the oxide layer and reduce actinides that become incorporated into microbial biomass (C). The bacteria with attendant actinides are selectively released by nutrient limitation, enzymatic digest, or mild chemical treatment, from the surface and subsequently collected for disposal.

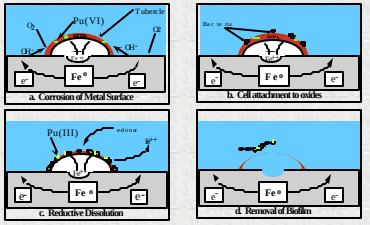

Figure 2. Conceptual model of biological decontamination of corroding steels

Office of Science and Office of Environmental Management
Environmental Management Sciences Program

RESULTS AND DISCUSSION

Plutonium Reduction and Cationic Sorption

Enzymatic reduction of Pu(IV) to Pu(III) was evaluated in anaerobic suspensions containing the iron reducing bacterium *Shewanella alga* strain BrY with H_2 as the electron donor. The pH of the medium was buffered to 6.0 with 10 mM PIPES buffer. The results demonstrated that:

- The iron reducing bacterium *S. alga* strain BrY reduced Pu(IV), provided as PuO_2, to Pu(III) with H_2 as the electron donor.
- Pu(III) partitioned between the aqueous and solid phases, apparently through cationic sorption to cell biomass
- Studies using neodymium, Nd(III), as a non-radioactive analogue for Pu(III) demonstrated a large sorbtive capacity of cells for trivalent cations.

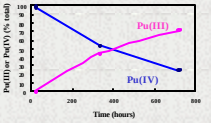

Figure 3. Enzymatic reduction of Pu(IV) to Pu(III) by S. alga strain BrY in an anaerobic medium with H2 as the electron donor.

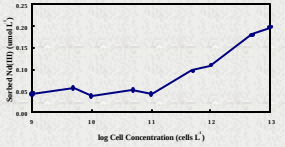

Figure 4. Sorption of Nd (III) onto cells of *S. alga* strain BrY. Initial concentration of dissolved Nd (III) was 3 uM at pH 6.5. Cell concentration ranged from 10^9 to 10^{12} cells L^{-1}.

Bacterial 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) was correlated with ionic strength, and thus was accurately described by the DLVO theory.
- Rate of solid phase iron reduction was directly correlated with adhesion of cells with surfaces and, hence, with ionic strength of the medium.

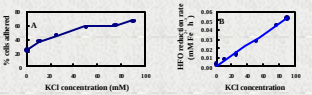

Figure 5. Effect of ionic strength on (A) cell adhesion and (B) reduction with by *S. alga* strain BrY with hydrous ferric oxide (HFO) the sole terminal electron acceptor.

Bacterial Attachment (cont.)

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. Hence, cells were imaged by confocal laser microscopy as they entered an anaerobic flow chamber and attached to the surfaces of specular hematite. Figure 6 illustrates the distribution of cells on the hematite surfaces.

- 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.

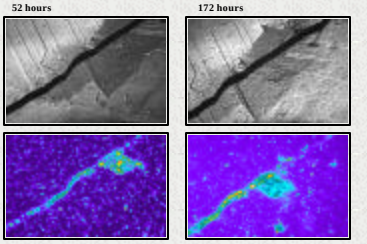

Figure 6. Confocal images of S. putrefaciens strain MR1 taken on a (001) hematite surface after 52 h and 172 hours. Fluorescence results from excitation of a green fluorescence protein (GFP) that was inserted and expressed within the bacterium. Bright cells are particularly associated with the step edge, suggesting that these cells are actively metabolizing and reducing Fe(III) available 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 7, 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.

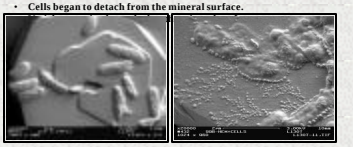

Figure 7. SEM image of bacterial cells on crystals of synthetic tabular hematite after 1 day (left) and 5 days (right) of electron donor limitation. Membrane vesicles appear as 100 nm blebs around the perimeter of cells.

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

Publications:

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

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

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