

FINAL REPORT

U.S. Department of Energy

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

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EXECUTIVE SUMMARY

Radionuclides and heavy metals contaminate metal surfaces used in the production, processing, and storage of radioactive material at many DOE sites. Much of the contamination is associated with surface metal oxides formed during the corrosion of the steel surfaces.

Dissimilatory iron reducing bacteria (DIRB) can potentially remove contaminated surface corrosion through an enzymatic process known as reductive dissolution, where iron oxide solids present on the corroding are reduced to soluble forms that are either released into solution or accumulate on the surface bacterial surface. The contaminants, particularly plutonium and technetium, are also amenable to enzymatic reduction by metal reducing bacteria. The reduced forms of Pu(III) are more soluble

The ultimate goal of this project was to demonstrate that metal-reducing bacteria could be used to remove heavy metal and radionuclide contaminants from the surfaces of corroding steel surfaces. Toward this end, fundamental scientific issues regarding (1) factors influencing the adhesion and colonization of DIRB on mineral surfaces, (2) the enzymatic activity of cells once they have adhered to mineral surfaces, (3) and (4) methods for recovering bacteria and attendant radionuclides following release from mineral surfaces were addressed. the fate of radionuclides (plutonium) contaminants following reduction by DIRB,

Research results demonstrate that dissimilatory iron reducing bacteria attach to mineral surfaces primarily, but not exclusively, by means of their flagella. Once initial attachment is complete, the bacteria more firmly adhere to the mineral surface apparently through the use of hydrophobic proteins on the bacterial cell surface. Attached bacteria enzymatically reduce ferric iron, Fe(III), to ferrous iron, Fe(II), by terminal metal reductases (proteins) located on their external surface. The bacteria remain firmly attached to the mineral surface, withstanding physical and chemical

treatments designed to remove them. Although DIRB strongly adsorb trivalent cations, such as Pu(III), our results suggest that recovery of firmly attached DIRB and associated radionuclides would be impractical as a means for decontaminating corroding stainless steel. Hence, we investigated the ability of soluble, reduced metabolic products to transform radionuclides into forms that could be recovered. We demonstrated that Fe(II) and reduced quinone-like compounds, which are both products of anaerobic respiration and can be generated by DIRB not attached to mineral surfaces, can rapidly reduce solid Pu(IV) to soluble Pu(III). We demonstrated that Fe(II) and reduced quinone-like compounds, which are both products of anaerobic respiration and can be generated by DIRB not attached to mineral surfaces, can rapidly reduce solid Pu(IV) to soluble Pu(III). Given these results, we conceptualized and partially tested a microbial bead-based method in which bacteria are encased in porous alginate beads. The beads, which prevent attachment of bacteria to mineral surfaces, can be easily manipulated and recovered as part of a treatment for removing corrosion scale from contaminated metal surfaces.

The fundamental research embodied by this project supports the objectives of the DOE EMSP by investigating the potential utility of an environmentally benign approach for removing heavy metals and radionuclides from corroded steel. Although the research apparently has no immediate impact on the decontamination and decommissioning of DOE facilities, it serves to advance our understanding of the biogeochemical processes related to bacterial-mineral interactions; processes that control not only the release of contaminants from corroding surfaces, but also fate and transport of metal contaminants from oxide minerals typically found in subsurface sediments.

1.0 RESEARCH OBJECTIVES

Processing and disposal of radionuclides has contaminated metallic surfaces throughout the DOE complex. Currently, the DOE Decontamination and Decommissioning Program (D&D) estimates that nearly 180,000 metric tons of contaminated stainless and mild steel structures and piping will be disposed of as radioactive solid waste, since current technologies for dealing with these wastes, are inadequate and costly. DOE is faced with meeting D&D obligations at many of its facilities by the year 2019. If decontamination to levels required for free release could be achieved, some of this material could be released as scrap into the commercial sector for reuse or recycling. The estimated value of scrap metal across the DOE complex was estimated to be greater than \$1 billion dollars in 1993. Thus, if effective and economic approaches for decontaminating metal surfaces were developed they could result in considerable savings by reducing the volume of radioactive waste requiring disposal and by rendering the metals as a potentially valuable commodity. Microbial enzymatic dissolution of post-corrosional oxide scales has considerable potential for environmentally benign and economic treatment of contaminated metallic surfaces. However, fundamental scientific information is lacking in key areas that prevent the development of such approaches. These areas include the mechanisms involved in the attachment, colonization, and detachment of iron-reducing bacteria from corrosion scales, the quantitative reductive dissolution of mineralogically, morphologically, and compositionally diverse metal oxides that comprise the scales, and the post-reduction solubility and distribution of the scale-associated contaminants.

This research project investigated processes related to the microbial reductive dissolution of mild and stainless steel corrosion products and the fate of associated radionuclide and metal contaminants. The general goals were to:

- develop an improved understanding of microbial reductive dissolution of iron oxide scales that form on corroding steel and act as highly efficient scavengers of radionuclides, as a function of oxide form and composition
- evaluate approaches for promoting the attachment to and colonization of surfaces by iron-reducing bacteria and the biological and chemical factors that promote the formation of iron-reducing biofilms.
- identify the potential for actinide binding to cells as a function of solution chemistry.

To this effect, the proposed research addressed fundamental scientific questions regarding the attachment and colonization of iron reducing bacteria to corrosion products and the fate of associated radionuclide and metal contaminants. These questions include:

- How do the mineralogy, morphology, and contaminant composition of oxide minerals formed on corroded steel influence the attachment and colonization by iron-reducing bacteria and the subsequent reductive dissolution reactions?
- How does aqueous chemical composition influence the rate and extent of microbial reductive dissolution of corrosion products and the subsequent solubility of associated contaminants?
- What is the fate of radionuclides associated with corrosion products that are dissolved via microbial reductive dissolution? Are the contaminants selectively accumulated or biosorbed by the cells or biofilms, released into solution, or repartitioned to the surface of the metal or into secondary mineral phases?
- Can soluble quinones, such as anthraquinone disulfonate (AQDS), facilitate dissolution of oxides and oxide films, including surface features and pores inaccessible by the

bacteria, via electron ‘shuttling’ from cell to oxide surfaces with subsequent bonded electron transfer, and thereby enhance removal of contaminants from the surface?

This research contributes to our understanding for developing a safe and effective biological approach for decontaminating mild and stainless steels that were used in the production, transport, and storage of radioactive materials. The research complements a variety of fundamental and applied research programs within the DOE Office of Science. Those projects include, but are not limited to:

The Natural and Accelerated Bioremediation Research Program (NABIR) Office of Biological and Environmental Research (BER).

Effect of Microbial Exopolymers on the Spatial Distributions and Transformations of Cr and U at the Bacteria-Geosurface Interface

PRINCIPAL INVESTIGATOR: Dr. Ken Kemner

Fe(II)-Induced Inhibition of Dissimilatory Bacterial Reduction of Metals and Radionuclides: The Role and Reactivity of Cell-Surface Precipitates

PRINCIPAL INVESTIGATOR: Yuri A. Gorby

Microbial Stabilization of Plutonium in the Subsurface Environment

PRINCIPAL INVESTIGATOR: Bruce Honeyman

Biogeochemical Processes Controlling Microbial Reductive Precipitation of Radionuclides

PRINCIPAL INVESTIGATOR: Jim K. Fredrickson

Impacts of Mineralogy and Competing Microbial Respiration Pathways on the Fate of Uranium in Contaminated Groundwater

PRINCIPAL INVESTIGATOR: Joel E. Kostka

2.0 METHODS AND RESULTS

The project has resulted in 6 peer-reviewed publications. An additional 2 publications are in preparation for submission. Published articles appear in Appendix A and should be referenced for details of materials and methods.

Results from our work definitively demonstrates that *metal reducing bacteria can remove Pu from Fe(III) oxides through enzymatic reduction and dissolution*. Consistent with our hypotheses, biogenic Pu(III), formed from the reduction of insoluble Pu(IV), accumulated on the bacterial cells. However, we incorrectly hypothesized that cells could be easily removed and recovered from the metal oxide surfaces. Results are summarized below and clearly show that:

- Metal reducing bacteria enzymatically reduce Pu(IV) solids to soluble Pu(III), which subsequently sorbed to bacterial cells.
- Bacterial cells attach to oxide surfaces, but attached biomass cannot be easily removed or recovered

- Microbially-reduced AQDS can reduce Pu(IV) to Pu(III) and can serve as an electron shuttle between bacterial cells and Pu(IV).
- Cells embedded in alginate beads can facilitate the removal of Pu(IV) from Fe(III) oxides (corrosion products). Pu(III) generated by this process accumulates in the beads and can be easily separated from the bulk aqueous phase.
- Flagella are involved in, but not required for, the attachment of DIRB to mineral surfaces.
- Bacteria sorb and accumulate trivalent cations, such as Pu(III).
- Bacteria attached to oxide surfaces are very difficult to remove and, hence, recovery of bacteria with sorbed Pu(III) is impractical.
- Fe(II) and reduced quinone-like compounds, which are both products of anaerobic respiration, can chemically reduce solid Pu(IV) to dissolved Pu(III).
- Channel-flow flat plate reactor and reporter genes can be used 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.
- Accumulation rate, growth rate, maximum surface-associated cell densities, and detachment rate of the dissimilatory iron reducing bacterium *Shewanella oneidensis* MR1 during anaerobic respiration can be quantified on different solid phase iron oxides. The bacteria colonize hematite surfaces and accumulate to greater cell densities on hematite compared to magnetite (111) and (100) surfaces
- Iron site density alone in iron oxide minerals does not control bacterial cell accumulation rates and maximum surface-associated cell densities during anaerobic iron oxide reduction by *Shewanella oneidensis* MR1.
- Iron sulfide pyrrhotite precipitated during biofilm formation by the dissimilatory sulfate reducing bacterium *Desulfovibrio desulfuricans* G20 on the surface of the iron oxide hematite.
- *Desulfovibrio desulfuricans* G20 biofilms growing on iron oxide surfaces reduce soluble uranyl ion to insoluble uraninite
- 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* was determined. The sequence information provides the opportunity to develop assays to follow the expression of this iron reduction gene during growth on iron oxides.
- In-situ reverse transcriptase polymerase chain reaction (RT-PCR) can be used to detect expression of genes encoding functions of metal reduction, sulfate reduction and hydrogen reduction in individual cells attached to iron oxide surfaces.

2.1 MICROBIAL REDUCTION OF PU(IV).

Plutonium on iron oxide surfaces and corrosion products is expected to be present primarily in the tetravalent state. Pu(IV) readily forms very insoluble hydrous oxide. The solubility product of hydrous oxide ($\text{PuO}_2(\text{am}) + 2 \text{H}_2\text{O} = \text{Pu}^{4+} + 4 \text{OH}^-$) is very low ($\log K^0 = -56.85$, Rai 1984), and thus shows that Pu(IV) would be soluble in appreciable quantities only under very acidic conditions. Microbial activity can cause reducing conditions that can transform Pu(IV) to Pu(III). With the

exception of phosphate compounds, most Pu(III) compounds are highly soluble in acidic to near-neutral conditions. Therefore, we previously hypothesized that metal reducing bacteria could enzymatically reduce insoluble Pu(VI) to soluble Pu(III) and that the Pu(III) would sorb to the surface of the bacteria. As part of our initial EMSP project, we confirmed that cells of *S. putrefaciens* strain BrY reduced Pu(IV) to Pu(III) with H₂ as the electron donor (Fig. 1).

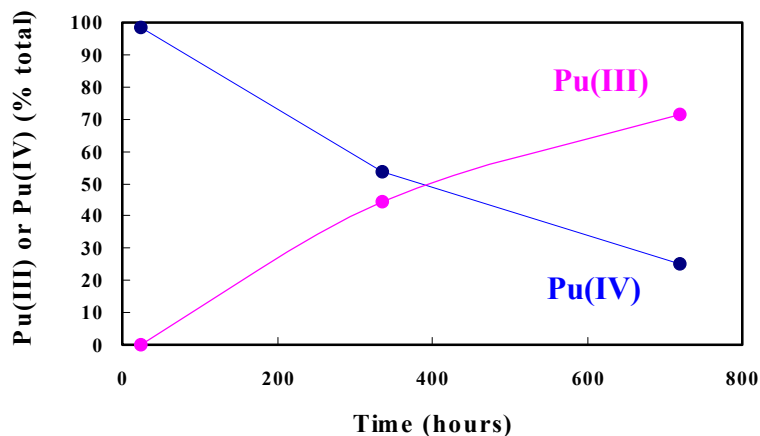


Figure 1. Enzymatic reduction of Pu(IV) to Pu(III) by *S. putrefaciens* strain BrY in an anaerobic medium with H₂ as the electron donor. Oxidation states of Pu were determined by scintillation counting using a modified extraction method (Schramke et al. 1989).

Virtually all of the Pu(III) generated through the direct enzymatic of Pu(IV) solids was associated with a phase (presumably bacterial surfaces) that could be removed from the suspension by passing the solution through 0.2 μm filters. The sorptive capacity of cells of *S. putrefaciens* strain BrY was examined using neodymium, Nd(III), as a non-radioactive analogue for Pu(III). A solution of 3 μM NdCl₃ received washed cells of strain BrY ranging from 10⁶ to 10¹⁰ cells ml⁻¹. Figure 2 demonstrates that aqueous equilibrium concentration and the amount of sorbed Nd(III) per cell increased with decreasing cell concentration. The data suggest that Nd began to precipitate on cell surfaces when the aqueous equilibrium concentration reached about 2.6 μM . *The results support our hypothesis that sorption/precipitation is an important process for removing trivalent cations, such as Pu(III), from solution.* These results underscore the importance of investigating the factors that influence attachment and detachment of metal reducing bacteria to for developing the approaches for recovering cells and cell-associated actinides as part of a remediation strategy.

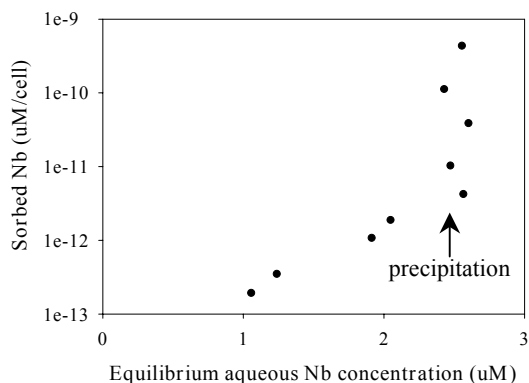


Figure 2. Sorption of Nd(III) onto cells of *S. alga* strain BrY. Initial concentration of dissolved Nd(III) was 3 μM at pH 6.5. Cell concentration ranged from 10⁶ to 10¹⁰ cell ml⁻¹.

2.2 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.

The distribution of attached cells on synthetic hematite crystals was examined to determine if cells preferentially attached to sites on the oxide surface. To facilitate direct observation of cells on oxide surfaces without using fluorescent stains that would compromise cell viability, genes encoding for green fluorescence protein (GFP) were inserted into strain BrY. Hence, cells were imaged by confocal laser microscopy as they entered an anaerobic flow chamber and attached to the surfaces of specular hematite. The results, some of which are illustrated in Figure 3, revealed 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.

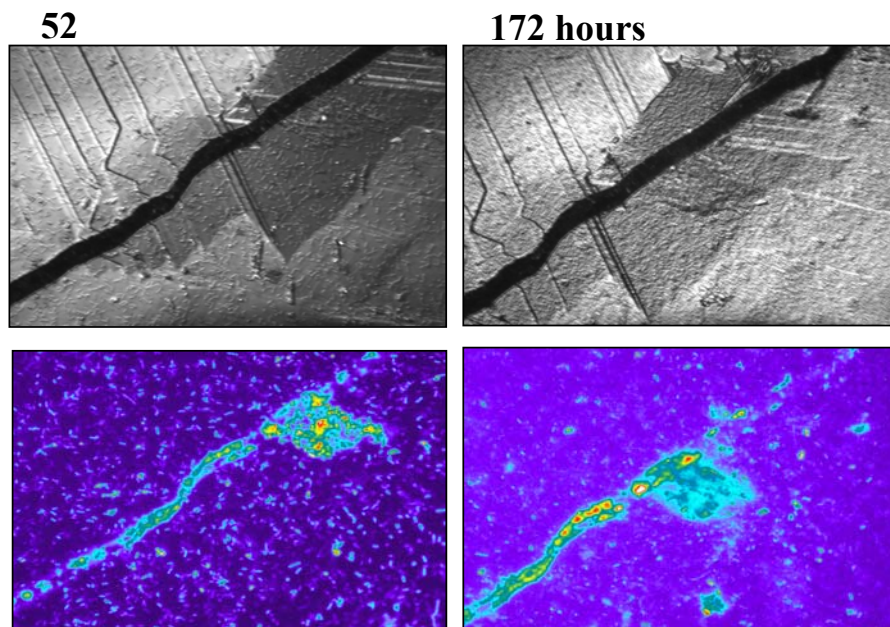


Figure 3. Confocal images of *S. alga* strain BrY taken on a (001) hematite surface after 52 and 172 hours. The top panels were taken using the reflectance channels of the confocal microscope. The bottom panels were obtained using the fluorescence channel. Fluorescence results from excitation of a green fluorescence protein (GFP) that was inserted into the bacterial genome and expressed. Bright cells are particularly associated with the step edge, suggesting that these cells are actively metabolizing and reducing Fe(III) available at these sites.

2.3 BACTERIAL 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 4:

- 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
- Vesicles could not be removed by benign changes in aqueous chemistry (ionic strength, lowering the pH) or by enzymatic digestion with trypsin.

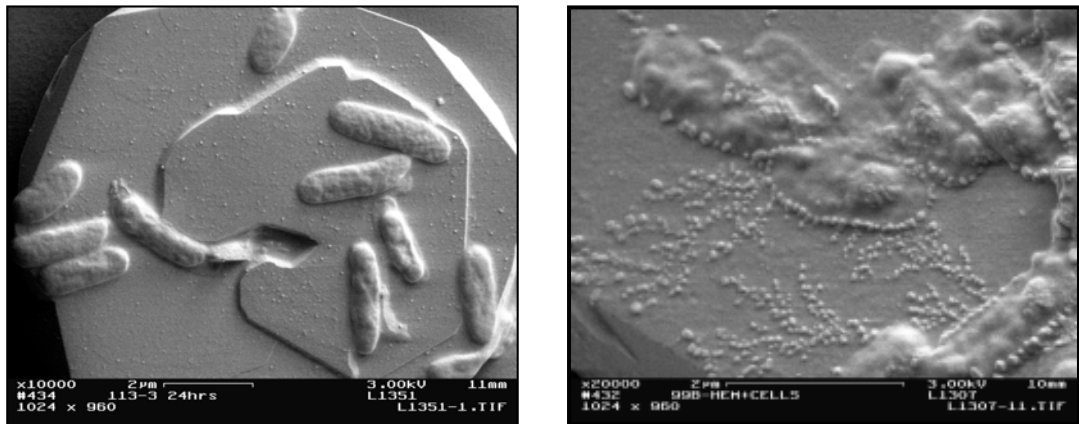


Figure 4. SEM images of *S putrefaciens* strain BrY on crystals of synthetic tabular hematite after 1 day (left) and 5 days (right). Membrane vesicles, which arise from the outer membrane of Gram-negative bacteria, appear as 100 nm blebs around the perimeter of the cells and are distributed over the hematite surfaces. These vesicles are highly negatively charged and effectively bind cations.

Dissimilatory iron-reducing bacteria enzymatically reduce and dissolve iron oxides, which are common components of corrosion films, and release soluble species of plutonium, Pu(III). Consistent with our previous hypothesis, cell surfaces sorb Pu(III) and remove it from the bulk aqueous phase. However, we incorrectly hypothesized that bacteria with sorbed actinides could be easily detached and recovered from the surfaces that they had colonized and enzymatically altered. In fact, we have demonstrated that although cells do naturally detach from oxide surfaces during their growth cycle, they leave behind negatively charged reactive portions of their outer surface that are strong sorbants for cations. Without a means for recovering both intact bacteria, their subcellular products and associated contaminants, the use of iron-reducing bacteria for decontaminating corroded steel surfaces would not be feasible. Hence, we have targeted an approach that avoids direct contact and attachment of cells to the corrosion films but allows for reduction, dissolution, and sorption of corrosion products and associated actinides. The inability to remove cells and membrane vesicles from oxide surfaces justified our investigation of a bead-

based technology for removing Pu from iron oxides that are common corrosion products. The following sections describes recent results obtained from a proof-of-principle experiment.

2.4 DESCRIPTION OF BEAD-BASED TREATMENT

Below is shown the conceptual model of a bead-based system for decontaminating corroded steels. Iron reducing bacteria are encapsulated in small beads of sodium alginate. Encapsulation prevents direct contact between the bacteria and the contaminated oxide surface. Anthraquinone disulfonate (AQDS) is used as a dissolved electron shuttle to carry electrons from the bacteria to Fe(III) and Pu(IV) on the corrosion film. AQDS reduces Fe(III) to Fe(II) and Pu(IV) to Pu(III). The reduced forms of these metals are very soluble and partition to the aqueous phase. The bacterial surface and the sodium alginate sorb and accumulate Fe(II) and Pu(III). The beads, which now contain most of the Pu(III), can be easily separated from the bulk aqueous phase and the uncontaminated steel. The benign process requires no hazardous chemicals or extreme pH conditions.

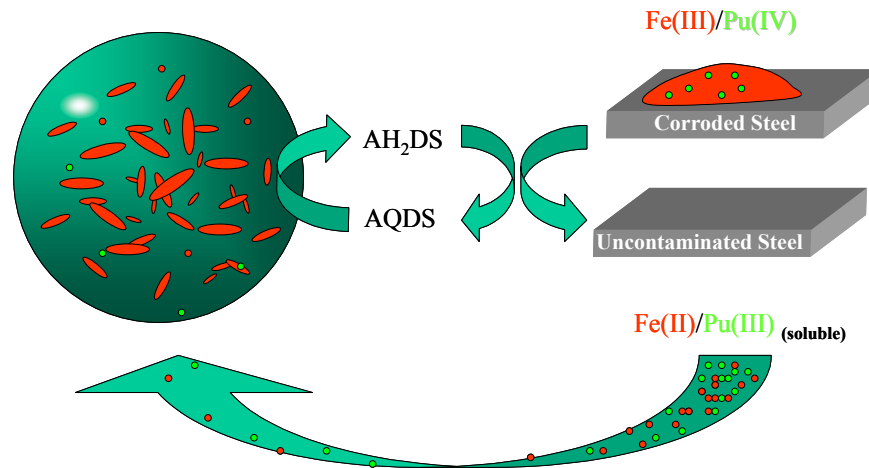


Figure 5 . This illustrates a conceptual model of a bead-based system for decontaminating corroded steels. Metal-reducing bacteria are enrobed in porous alginate beads. Oxidized anthraquinone disulfonate, AQDS, which will serve as a dissolved electron shuttle between immobilized cells and elements in the corrosion film, diffuses into the beads and is enzymatically reduced by the bacteria. The reduced AQDSH diffuses out of the bead and chemically reduces and dissolves Fe(III) and Pu(IV) in the corrosion film. Soluble Fe(II) and Pu(III) sorb to cationic exchange sites within the alginate beads. The beads and accumulated actinides can then be easily separated from the bulk aqueous phase and the uncontaminated steel.

The microbial bead-based technology was tested for its ability to reduce and remove Pu(VI) from Fe(III) oxides. Cells of *S. alga* strain BrY were enrobed in beads of sodium alginate. The beads, each measuring a few millimeters in diameter, were added to a suspension of synthetic HFO (5 mM) that was co-precipitated with approximately 0.5 mM Pu(IV). AQDS (0.1 mM) was added as the soluble electron shuttle. The contents of the tubes were made anaerobic by bubbling with N₂ and the tubes were incubated on their side at room temperature. Control tubes

lacked either cells or the electron donor. Following 24 hour incubation, beads were separated from the aqueous suspension and analyzed for Pu by liquid scintillation counting. The amount of Pu remaining in the aqueous suspension, which included the liquid medium and any undissolved Fe/Pu precipitates, was also evaluated. A portion of this sample was passed through a 0.036 um membrane filter and the filtrate was analyzed for soluble Pu by scintillation counting. Oxidation state of Pu was determined in each fraction by a modification of the solvent extraction method of Schramke et al., 1989. The results tabulated in Table 1 reveal that bacterial cells embedded in alginate reduced and dissolved 25% of the total amount of Pu that was co-precipitated with the HFO. More than 96% of the Pu that partitioned into the beads was present as Pu(III). Pu(IV) was not reduced and less than 1 % of the total Pu partitioned into the beads for the 3 treatments that served as controls. *These preliminary results clearly indicated the potential of the microbial bead-based technology as part of a decontamination strategy for removing Pu from Fe(III) oxide components of corrosion.*

Table 1. Fate of Pu during the reduction of Fe(III)/Pu(IV) oxides.

Treatment	Pu in beads	Pu in unfiltered suspension	Pu in filtered (soluble) fraction
Cells, AQDS, H ₂	25 (96 % as Pu(III))	75	0
Cells, no AQDS, H ₂	0.7	99	0
Cells, AQDS, no H ₂	0.9	99	0
No cells, AQDS, H ₂	0.5	99	0

The bead-based system was also tested for its ability to remove dissolved radionuclides (uranium and technetium) from aqueous media. Figure 2 illustrates that iron reducing bacteria embedded in a porous alginate matrix reduce dissolved Tc(VII) (as pertechnetate ion) to poorly soluble TcO₂. Vials 1 and 2 contain alginate beads that are blackened by TcO₂ precipitates. Vial 3 served as a control and contains beads with no cells. All of the Tc(VII) remained in the aqueous phase. Vials containing dissolved U(VI) in lieu of Tc(VII) yielded similar results. These results clearly demonstrate the potential for removing dissolved radionuclides from solution using an environmentally benign bead-based approach.

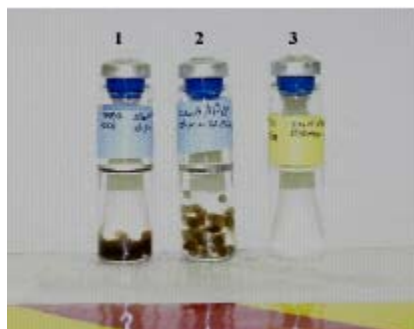


Figure 2. Vials demonstrating the ability of iron-reducing bacteria in alginate beads to remove dissolved Tc(VII) from solution. 100 uM of Tc(VII) were reduced and precipitated within the beads in vials 1 and 2. Vial 3 served as a negative control and lacked bacterial cells. Beads without bacteria are difficult to see because they contain none of the dark Tc(IV) precipitate.

Information Access

3.0 RELEVANCE, IMPACT, AND TECHNOLOGY TRANSFER

This project targeted scientific questions that highlight gaps in our understanding of how iron reducing bacteria catalyze the reductive dissolution of iron oxides typically associated with corrosion films on mild and stainless steel surfaces. These films represent a major sink for radionuclides and heavy metals used during the processing, transport, and storage of radioactive materials and constitute a significant environmental problem facing DOE. In its present form, the scientific knowledge generated by this project is not directly applicable toward the decontamination and decommissioning of DOE facilities. However, the results do advance our understanding of the biological processes associated with reductive dissolution of iron oxides and the interaction of radionuclides and heavy metals with bacterial surfaces. Such information contributes to an increasing wealth of knowledge concerning the role of metal reducing bacteria in controlling the fate and transport of inorganic contaminants in saturated subsurface and sedimentary environments and for their *potential* roles in decontamination metal surfaces. Published results from this research project have already impacted the scientific research community and have stimulated the development of collaborations between microbiologists, geochemists, and physicists. Such interactions are vital for linking fundamental science with needs-driven technologies.

Scientific hurdles still exist before a biologically-based means for decontaminating corroded steels can be realized. First, we have only just begun to understand the environmental and genetic factors that regulate the metabolism and physiology of metal reducing bacteria. An awareness of need for a holistic understanding of microbial systems and the environmental/growth parameters that impact the regulation of their physiological activities is reflected in new programs developing within the DOE office of science. In particular, the Genomes to Life program maintains lofty, yet tractable, goals to understand the activity of bacteria by taking a “systems biology” approach. Notably, iron reducing bacteria (*Shewanella oneidensis* strain MR-1) is a primary target for this program and the researchers it supports. Through a more complete understanding of the biology of this organism, new approaches and insights will certainly be realized concerning the biogeochemical processes important to reductive dissolution of contaminated corrosion scale and, more generally, the fate of radionuclides and heavy metals associated with oxide surfaces..

4.0 PROJECT PRODUCTIVITY

The project demonstrated significant productivity through peer-reviewed publications and advancement of fundamental scientific research questions involving metal reducing bacteria and metal oxide surfaces that they colonize. Many of the objectives related to the interaction of actinides with bacterial surfaces were also addressed. Consistent with our previous hypothesis, cell surfaces sorb Pu(III) and remove it from the bulk aqueous phase. However, we incorrectly hypothesized that bacteria with sorbed actinides could be easily detached and recovered from the surfaces that they had colonized and enzymatically altered. In fact, we demonstrated that although cells do naturally detach from oxide surfaces during their growth cycle, they leave behind negatively charged reactive portions of their outer surface that are strong sorbants for cations. Without a means for recovering both intact bacteria, their subcellular products and

associated contaminants, the use of iron reducing bacteria for decontaminating corroded steel surfaces would not be feasible. Hence, we diverged from some proposed goals and targeted an approach that avoids direct contact and attachment of cells to the corrosion films but allows for reduction, dissolution, and sorption of corrosion products and associated actinides.

Our preliminary results obtained using the bead-based technology demonstrated that anthroquinone disulfonate, AQDS, serves as a soluble electron shuttle between the enzymes on the bacterial cell surface and Fe(III) and Pu(IV) present in the corrosion film. Soluble Pu(III) released during reduction accumulated within the beads, presumably by sorption of the trivalent cations to alginate and the embedded cells. The beads containing sorbed Pu(III) were then easily separated from the bulk aqueous phase. This environmentally-benign microbiological process avoids the use of hazardous or toxic chemicals, minimizes the volume and toxicity of secondary wastes, and could be applied in situ. Although a renewal proposal that targeted the evaluation of the bead technology for removing actinides from contaminated surfaces was denied, we maintain that the concepts are sound and warrant further consideration.

5.0 PERSONNEL SUPPORTED

This project provided full support for 2 post-docs, partial support for 2 university faculty members, and partial support for 3 national lab staff scientists and associated technical staff. The names and institutions of the collaborating teams are listed below.

University of New Hampshire

Dr. Frank Caccavo (faculty)

Dr. Amitabha Das (Post Doc)

Montana State University

Dr. Gill Geesey (faculty)

Dr. Andrew Neal (post-doc)

Pacific Northwest National Lab

Dr. Yuri Gorby (staff scientist)

Dr. Dhanpat Rai (senior research scientist)

Dr. Jim Fredrickson (senior research scientist)

Mathew Gray (post bachelors)

Jeff McLean (post masters)

Dean Moore (science and engineer associate)

Alice Dohnalkova (science and engineer associate)

Andrew Plymale (science and engineer associate)

6.0 PUBLICATIONS

Rai D, YA Gorby, JK Fredrickson, DA Moore, and M Yui. 2002. Reductive Dissolution of PuO₂(am): The Effect of Fe(II) and Hydroquinone. *J. Sol. Chem.* 31(6): 433-453.

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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.

7.0 INTERACTIONS

Yuri Gorby presented *Microbially Promoted Solubilization of Steel Corrosion Products and Fate of Associated Actinides* (04/24/2000 - Invited Speaker) at EMSP National Workshop in Atlanta GA, US with contributions from: YA Gorby, F Caccavo, G Geesey, A Neal, D Rai, A Das, A Dohnalkova, MS Gray, JS Mclean, and JK Fredrickson.

Kenote address: Goldschmidt Geochemistry Conference. May, 2001. Physiological Responses And Environmental Implications Of Fe(II)-Induced Inhibition Of Microbial Iron Reduction. Y. A. Gorby, C. Liu, T. J. Beveridge, F. G. Ferris, and J. M. Zachara.

Invited speaker: International Symposium of Ecology and Biogeochemistry. Sept., 2001. Membrane Vesicles from Iron Reducing Bacteria: a Potential Source of Biogenic Nanofossils. YA Gorby and TJ Beveridge.

8.0 TRANSITIONS

None

9.0 PATENTS

None

10.0 FUTURE WORK

The scientific advances realized during the tenure of this project are expected to proliferate into a variety of research avenues involving metal reducing bacteria and their roles and associations with heavy metals and radionuclides. Indeed, several research projects targeting the physiological controls of metal reducing bacteria growing in biofilms and attached to mineral surfaces are already underway. The use as alginate beads as tools for separating, manipulating, and recovering metal reducing bacteria in solid phase suspensions is expected to benefit those interested in investigating biogeochemical processes in the absence of direct microbe-mineral contact. Hopefully, the potential for using bacteria enrobed in alginate beads as a means for recovering heavy metals and radionuclides from aqueous solutions and contaminated surfaces will also receive appropriate attention.

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12.0 FEEDBACK

13.0 APPENDICES

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