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

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Research Objectives

This project will probe fundamental scientific issues regarding a microbial process with potential for decontaminating corroding metal surfaces. We hypothesize that dissimilatory iron-reducing bacteria (DIRB), via anaerobic respiration, can quantitatively dissolve amorphous and crystalline iron oxides and thereby release oxide-associated radionuclide contaminants. Associated actinides will be sorbed by cell surfaces or precipitated within biofilms that can be removed and recovered by enzymatic digestion of microbial attachment factors. This environmentally benign, enzymatic process avoids the use of hazardous or toxic chemicals, minimizes the volume and toxicity of secondary wastes, and could be applied in situ. Although an increasing body of scientific literature supports this working hypothesis, a basic understanding is needed of the biological and chemical processes that impact 1) attachment and detachment of iron-reducing bacteria to oxide surfaces; 2) the rate, extent, and products of iron reduction; and 3) the fate of radionuclides following enzymatic reduction of corroding steel (which is needed to evaluate and develop effective biological approaches for decontamination of aging metallic structures and piping). The goal of this project is to provide the scientific underpinnings for the development of biologically based approaches for the removal of contaminants from corroding steel surfaces. Specifically, this research will accomplish the following:

- determine the role of oxide structure, topology, and composition on bacterial attachment and subsequent reductive dissolution of Fe(III) oxide corrosion products that form on stainless and mild steels

- identify how soluble electron "shuttles" can facilitate the rate and extent of microbial reductive dissolution of iron oxide corrosion products, including surface features and pores inaccessible to bacteria

- determine the distributions of radionuclides released during reductive dissolution of oxide films on metal surfaces as a function of aqueous geochemical composition.

This project uniquely couples PNNL's expertise in microbial metal reduction and biogeochemistry with Montana State University's Center for Biofilm Engineering's expertise and capabilities in biofilm analysis, engineering, and corrosion. In addition, this research project will take advantage of the capabilities and expertise at PNNL in actinide chemistry and advanced instrumentation for probing the distribution and chemical nature of surface-associated radionuclides and metals associated with the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL).

Problem Statement

Contaminated surfaces of various metals, including stainless steel, copper, nickel, iron, and carbon steel, pose significant problems to the ongoing efforts of the U.S. Department of Energy (DOE). Contamination consisting of nuclear fuel components contaminated with plutonium, uranium, etc., constitutes the most significant problems at DOE facilities and is commonly associated with the surfaces of metal piping, tanks, gloveboxes, and other structures. Current technologies for removing the contaminants are costly and produce large volumes of secondary waste; hence, the bulk of contaminated metal

is commonly disposed of as radioactive waste. Also, interior surfaces of the piping are inaccessible to many standard decontamination methods. DOE recognizes that new or significantly improved techniques are needed for decontaminating stainless steel, iron, copper, and other metals with surface-associated radionuclides and metals.

Processing and disposal of radionuclides has contaminated metallic surfaces throughout the DOE complex. Currently, DOE estimates that nearly 180,000 metric tons of contaminated stainless and mild steel structures and piping will be disposed of as radioactive solid waste because current technologies for dealing with these wastes are inadequate and costly. DOE is faced with meeting decommissioning and decontamination 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 in 1993. Thus, if effective and economic approaches were developed for decontaminating metal surfaces, they could result in considerable savings by reducing the volume of radioactive waste requiring disposal and by rendering the metals useful and 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 make up the scales; and the post-reduction solubility and distribution of the scale-associated contaminants.

This research project will investigate the microbial reductive dissolution of mild and stainless steel corrosion products and the fate of associated radionuclide and metal contaminants. The general goals are as follows:

- 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 and/or precipitation within biofilms as a function of solution chemistry.

To this end, the proposed research will address fundamental scientific questions regarding the microbial dissolution of corrosion products and associated radionuclide and metal contaminants. These questions include the following:

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

Research Progress and Implications

The research is structured into four primary tasks. Task 1 will investigate the factors controlling the attachment to and release from oxide scale that forms on corroding mild and stainless steel by metal-reducing bacteria to Fe(III). Task 2 will probe the effects of iron oxide composition and surface properties on cell attachment and biofilm formation. Task 3 will examine and quantify the reductive dissolution of synthetic iron oxide thin films deposited on internal reflective elements 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. Oxygen concentration gradients throughout the developing biofilm will be measured and the impact of aerobic conditions external to the biofilm on the rate and extent of iron reduction will be evaluated. Task 4 will determine the distribution of actinides released from Fe 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.

Work has begun on all four tasks with contributions from each of the three collaborating laboratories. Frank Caccavo at the University of New Hampshire has been addressing Task 1 by investigating the attachment of iron-reducing bacteria to a variety of iron oxide minerals that are typical components of corroding steels. Dr. Caccavo found that the attachment of cells to minerals is influenced by the mineral form and by the ionic strength of the aqueous medium. For brevity, only selected data are presented. Figure 1 shows the impact of ionic strength on the attachment of the iron-reducing bacterium *Shewanella alga* strain BrY to a synthetic hydrous ferric oxide (HFO). The data illustrate that bacterial attachment is reduced as the ionic strength of a synthetic groundwater medium increases.

These results have important implications for the application and attachment of bacteria to contaminated corrosion of mild and stainless steels. First, they define the operational conditions that optimize the attachment of cells to contaminated, corroded steel. Second, they provide insight into potential approaches for removing and recovering bacterial cells once they have extracted radionuclides from the contaminated surfaces.

Dr. Gill Geesey at Montana State University has begun work on Tasks 2 and 3. Dr. Geesey and his coworkers have purchased and recently installed a Raman microscopic imaging system. When fully functional, this Raman microscope will offer a powerful alternative to high vacuum surface chemical analysis where reaction product stability is strongly influenced by the presence of water or where

nondestructive sampling of hydrated surfaces is necessary for the characterization of surface chemical reaction kinetics. Dr. Geesey and his coworkers will use Raman microscopy to spatially resolve

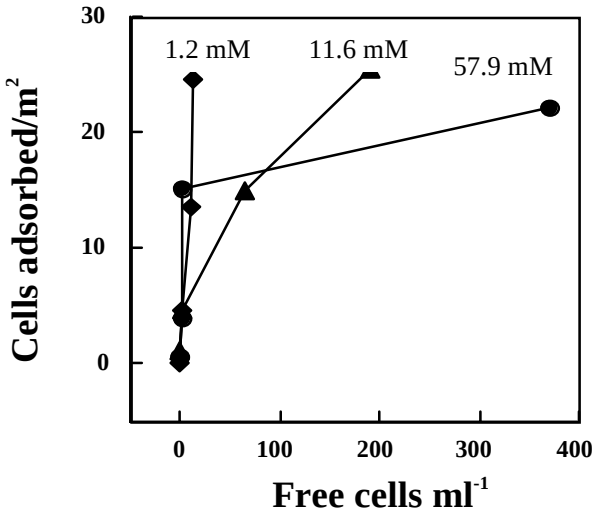


Figure 1. Effect of Ionic Strength on the Attachment of Iron Reducing Bacteria to HFO

chemical transformations in fully hydrated iron oxide films during colonization by DIRB in a nondestructive, real-time mode. They will 1) establish the role of the film-associated DIRB in iron transformation and 2) determine the influence of such transformations on the stability of sorbed actinide ions, which can also be detected by Raman scattering. Because the spatial resolution of this imaging spectroscopic technique is on the order of a few micrometers, localized reactions at the individual bacterial cell or microcolony level will be resolved. This will allow discrimination of biotically and abiotically mediated iron transformations that occur over the duration of bacterial attachment and biofilm formation on the iron oxide film.

Researchers at PNNL are addressing Task 4 by evaluating the effect of soluble electron shuttle compounds on the rate and extent of reduction of iron oxides typically associated with corroding steels as well as the effects of these compounds on the fate of actinides entrained or sorbed to the corrosion products. The majority of the work has focused on the chemistry of the redox-reactive compound anthroquinone disulfonate. This chemical can serve as an electron shuttle between iron-reducing bacteria and solid-phase iron oxides that the organisms reduce as a mode of respiration.

Dr. Fredrickson, co-principal investigator on this project, has demonstrated that microbially reduced AQDS can increase the rate and extent of iron oxides reduction, such as HFO. Figure 2 illustrates that reduction of HFO proceeds more rapidly and to a greater extent in the presence of AQDS than in its absence.

We have begun to evaluate the effect of microbially reduced AQDS on the valence transformation and solubility of plutonium. Pu(IV) is exceedingly insoluble at pH values above 2 and reaches levels of detection (10 – 8.5) at around pH 3. In contrast, Figure 3 illustrates that plutonium, initially added as Pu(IV), remained dissolved at pH values between 0.5 and 5.5 in the presence of microbially reduced AQDS. The marked increase in dissolved plutonium is attributed to reduction of Pu(IV) to Pu(III), which is soluble to pH values approaching neutrality. This conclusion will soon be confirmed by spectroscopic analyses that discriminate between the various valence states of plutonium. The results have important implications for evaluating the potential of AQDS as part of a biological strategy for removing plutonium from corroded steel while avoiding acidic conditions necessary for dissolving Pu(IV) solids.

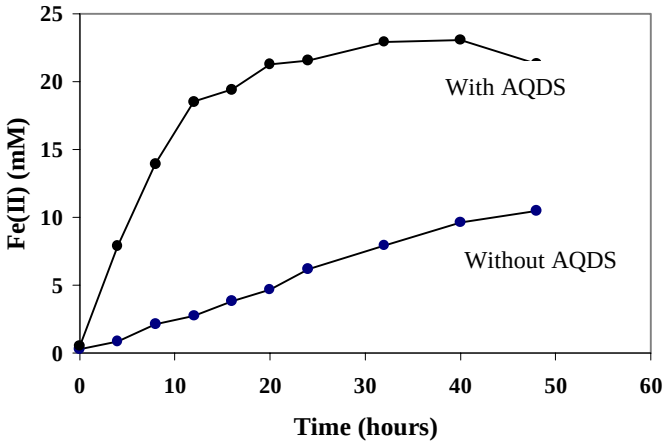


Figure 2. Effect of Dissolved AQDS on the Rate and Extent of HFO Reduction by Iron-Reducing Bacteria

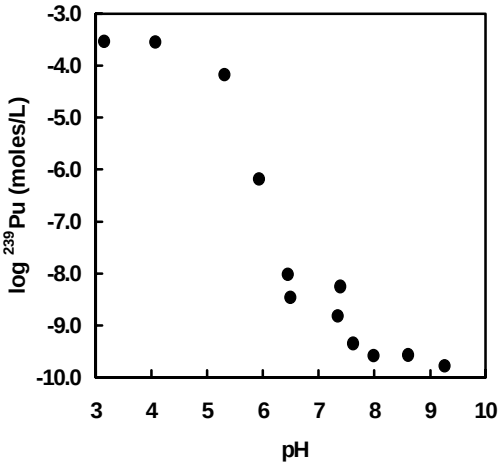


Figure 3. Solubility of Plutonium in the Presence of Microbially Reduced AQDS

Planned Activities

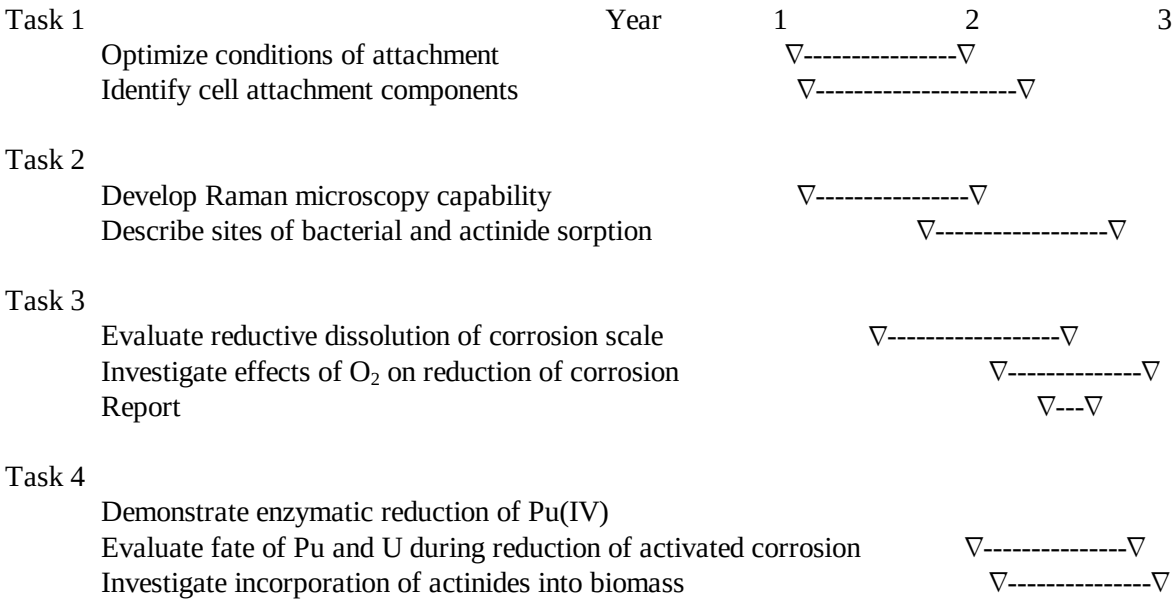
Progress will continue on all aspects of the proposed research. Collaborators at the University of New Hampshire will continue to investigate conditions that promote the attachment and detachment of bacterial cells on iron oxides associated with corrosion scales. Researchers at the University of Montana will monitor and describe the bacterial colonization of oxide minerals using infrared spectroscopy by following the intensity of the amide I and amide II bands. Dissolution or sloughing of the oxide film due to bacterial colonization and enzymatic reduction will be evaluated by monitoring increases in the water adsorption bands compared with that obtained from sterile controls run in parallel. Bacterial colonization of the oxide film will be simultaneously characterized by reflectance differential interference contrast light microscopy or scanning confocal laser microscopy via a specially designed shuttle that translocates the flow channel reactor between the microscope stage and the optical bench of the spectrometer without disrupting flow or

disturbing the biofilm. Through this approach, we will be able to relate structural and chemical changes in the oxide film by attenuated total reflectance/Fourier Transform infrared to the microbiological properties, such as cell density and biofilm structure on the film. The infrared technique averages spectral information gathered from the entire surface of the oxide film. Specific biofilm features will be investigated using the newly installed Raman imaging capability. Because Raman is sensitive to chemical changes in the inorganics located on the surface and insensitive to the solvent, the complementary visual and chemical imaging techniques of the coupled system will provide vital information on the inorganic chemistry occurring in the biofilm, visual information of the biofilm, and changes in the oxide film beneath the biofilm. Raman spectroscopy is very valuable for distinguishing between bonding environments of the partially oxidized iron species. Spectra will be collected with integration times of about 30 seconds, which makes the process ideal for the identification of kinetic products and intermediates on the time scale of iron oxidation.

PNNL researchers will continue to investigate the fate of actinides during reductive dissolution of contaminated corrosion scale. Total alpha activity to evaluate the amount of plutonium and uranium that is released into solution, partitioned into reduced secondary mineral phases, or incorporated into microbial biomass in the presence and absence of the soluble electron shuttle AQDS. Oxidation states of the actinides will be determined by x-ray adsorption near-edge spectroscopy by requesting beam time at Stanford Synchrotron Research Laboratory. When applicable (i.e., under low pe conditions imposed by the bacteria), Nd(III) will be used as a non-radioactive analog for Pu(III).

Because the phosphoryl substituents of lipopolysaccharides on the outer membrane of gram-negative bacteria exhibit a high affinity for polyvalent cations, experiments will be conducted to determine what role these components may play in accumulation of uranium and plutonium by DIRB under aerobic and iron-reducing conditions. Similar bioaccumulation experiments will be conducted using cells in which the production of exopolysaccharide has been induced. Exopolysaccharide, an extracellular component implicated in the long-term attachment of DIRB to iron oxides, may be an important sink for actinides during reduction of oxide scale and should therefore receive experimental attention. Together, topics addressed in Task 4 will provide information vital for evaluating the fate of actinides during and following the reduction of contaminated corrosion products.

Schedule and Milestones



Publications and Presentations

To date no publications or presentations have resulted from this work. Publications are expected within the second and third years of the project's duration.