

2006 ERSD Annual Report

DOE-BER Environmental Remediation Sciences Project # 1010283

Biotransformation of PuEDTA: Implications to Pu Immobilization

PI: Harvey Bolton Jr.¹

Co-PIs: Dhanpat Rai¹ and Luying Xun²

Pacific Northwest National Laboratory, Richland, WA¹

Washington State University, Pullman, WA²

Research Objectives:

This project integrates three distinct goals to develop a fundamental understanding of the potential fate and disposition of plutonium in sediments that are co-contaminated with EDTA. The three objectives are:

1. Develop thermodynamic data for Pu-EDTA species and determine the dominant mobile form of Pu under anaerobic conditions.
2. Elucidate the mechanism and rates of Pu(IV) and Pu(IV)-EDTA reduction by metal-reducing bacteria and determine where the Pu is located (in solution, biosorbed, bioaccumulated).
3. Enrich and isolate anaerobic EDTA-degrading microorganisms to investigate the anaerobic biodegradation of Pu-EDTA

The research related to objectives 1 and 2 above is being carried out at PNNL, while that related to objective 3 is at WSU.

Research Progress and Implications:

This report presents research conducted during the first 2 years of a 3 year project. Extensive studies are being carried out to obtain values for thermodynamic equilibrium constants for the formation of Pu(III)-EDTA complexes. Our approach consists of developing extensive data 1) to determine the solubility product for PuPO₄(s), currently unavailable, through studies of PuPO₄(s) as a function of pH at fixed phosphate concentrations, and as a function of phosphate concentrations at a fixed pH value and to use this information to accurately define Pu³⁺ activities required for determining Pu(III)-EDTA complexes. The data on Pu(III)-EDTA complexes will be obtained through extensive PuPO₄(s) solubility studies 1) as a function of pH (1.5 - 14), and at fixed phosphate concentration of 10^{-3.5} M and EDTA concentrations of 10^{-3.4} and 10^{-2.7}, and 2) as a function of EDTA concentrations ranging from 0.0001 M to 0.1 M, and at fixed pH values (4, 11, 13) and at fixed phosphate concentrations of 10^{-3.5} M. Reducing agents are used in all of the studies to maintain Pu in the trivalent state. The Pitzer model will be used to interpret the data and will yield thermodynamic equilibrium constants necessary to determine Pu behavior in anaerobic geologic environments. Experimental work on these various aspects under this task is nearly complete, and emphasis will now shift to interpretation of data and drafting manuscripts for publication in the open literature. This work [specifically the extraction techniques developed as a side line to quantify the Pu oxidation states, especially the Pu(III)] has already led to techniques to quantitatively

assess the distribution of Pu oxidation states in the microbial cultures used in goal 2 activities.

Efforts toward objective 2 have thus far used *Shewanella oneidensis* as the model organism for establishing incubation parameters and the previously mentioned Pu oxidation state fractionation protocol. *S. oneidensis* strain MR-1 was used to test the hypothesis that it can reduce Pu(IV)O₂(am) under anaerobic conditions. The ability of *S. oneidensis* MR-1 to reduce Pu(IV) with and without AQDS (an electron shuttle), and with and without EDTA (a likely co-contaminant) was determined in pure culture at near-neutral conditions during incubations of less than one week. The electron shuttle AQDS increased the rate and magnitude of Pu reduction. EDTA also enhanced rates and magnitudes of biological Pu solubilization and reduction by increasing the solubilization of Pu(IV) and Pu(III). *S. oneidensis* MR-1 reduced Pu(IV) to Pu(III) within 2 days of exposure. AQDS, an electron shuttle, significantly increased reduction (solubilization) of Pu(IV). In the biological system, EDTA significantly increased Pu(IV) reduction.

The progress toward the third objective is provided in a separate report, prepared by Luying Xun.

The overall project outcomes may be briefly summarized,

1. Pu(III) solubility data was collected and used to develop a model that demonstrated that PuEDTA⁻ is the dominant species occurring within the range of environmental conditions modeled.
2. *S. oneidensis* MR-1 rapidly reduced Pu(IV) in dilute aqueous systems. This reduction was enhanced by the addition of electron donor (H₂) and electron shuttle (AQDS).
3. Degradability of metal-EDTA is related to the formation of free EDTA to bind to EppA for transport into the cell

Planned Activities:

As mentioned above, the experimental work to obtain thermodynamic equilibrium constants for Pu(III)-EDTA complexes is nearly complete. The main emphasis under this task for FY 2007 will be the thermodynamic interpretations of the data and drafting of manuscripts for publication in the open literature. Three different manuscripts (effect of Fe(III) on Pu(IV)-EDTA complexes important in Pu mobility in geologic environments, solubility product of PuPO₄(s), and Pu(III)-EDTA complexes) are planned.

Objective 2: By the end of December 2006 complete an assessment of the ability of *Geobacter* to reduce Pu, and prepare a manuscript comparing this with the observed *S. oneidensis* reduction in early 2007. We will also conduct further incubations with *S. oneidensis* to determine the scope of its reductive potential in sediments by using biogenic Fe as reducing agent in January/February 2007, and conduct incubations with a range of terminal electron acceptors in the months following. We are planning to determine Pu localization relative to cell physiology using electron microscopy; however, current technical limitations are requiring us to archive samples while an appropriate lab is sought.

Information Access:

Rai, Dhanpat, A. R. Felmy, D. A. Moore, and H. Bolton, Jr. 2005. Environmental Mobility of Pu(IV) in the Presence of EDTA: Myth or Reality. Presented at Migration 2005. September, 2005, Avignon, France.

Plymale AE, VL Bailey, D Rai, DA Moore, and H Bolton, Jr. 2006. "Reduction of amorphous Pu(IV)O₂ by *Shewanella oneidensis* MR-1." Presented at the 106th American Society for Microbiology General Meeting, Orlando, FL.

Bailey VL, AE Plymale, SJ Fansler, DA Moore, D Rai, and H Bolton, Jr. 2006. "Reduction of Pu by Dissimulatory Metal-Reducing Microorganisms." Abstract submitted to 2006 AGU Fall Meeting, San Francisco, CA.

Optional Additional Information: None

Optional Proprietary Information: None