**Anaerobic Biotransformation and Mobility of Pu and Pu-EDTA**


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**Introduction**

EDTA (Figure below) can form strong water-soluble complexes with radionuclides and co-disposed with radionuclides (e.g., 60Co, Pu) and has enhanced their transport in the Shewanella oneidensis. EDTA confers specificity for transport by an ABC transporter.

**Hypotheses**: MgEDTA is the form of EDTA transported into the cell, which has to bind to the periplasmic binding protein before transport can occur. MgEDTA is likely dissociated before binding, while more stable complexes in groundwater at DOE sites.

**Results**: Results have been submitted for publication (Xun et al. 2006).

**Task 1: Pu-EDTA Aqueous Chemistry**

**Research Objectives**: Under anaerobic conditions Pu(III) is most likely the mobile species. Therefore, fundamental data for Pu(III) reactions, expected to be important in geologic environments are needed. We will determine the solubility of a sparingly soluble Pu(III) compound as a function of pH and (EDTA).

**Hypothesis**: EDTA will enhance the solubilization of Pu(III) in abiotic systems.

**Approach**: Solubility of PuPO₄(s) (a stable solid form of Pu(III)) was measured as a function of pH, phosphate, and time to develop/validate thermodynamic models for this system (Figs. 1, 2, 3).

**Conclusion**: A preliminary model was used to interpret solubility data. The results show that EDTA forms very strong complexes with Pu(III) and that PuEDTA is the dominant Pu species in a large range of environmental interest. This study will yield/validate fundamental data for the solubility product of PuPO₄(s), and phosphate and EDTA complexes of Pu(III) produced biotically.

**Task 2: Reduction of Pu by Microorganisms**

**Research Objectives**: To elucidate the mechanism and rates of Pu(IV) and Pu(III)EDTA reduction by metal-reducing bacteria and determine where the Pu is located (in solution, biosorbed, biocumulated).

**Hypotheses (subtask)**: S. oneidensis strain MR-1 can reduce Pu(IV)O₂(am) under anaerobic conditions. The electron shuttle AQDS will enhance the rate and magnitude of Pu reduction. EDTA will also enhance rates and magnitudes of biological Pu solubilization and reduction by enhancing the solubilization of Pu(IV) and Pu(III).

**Approach**: (1) Measure anaerobic Pu reduction by S. oneidensis strain MR-1 with H₂ as the electron donor and 500 μM Pu(IV)O₂(am) as the electron acceptor, in the presence and absence of 100 μM AQDS, pH 7. (2) Measure anaerobic Pu reduction by S. oneidensis strain MR-1 with H₂ as the electron donor, 500 μM Pu(IV)O₂(am) as the electron acceptor, in the combined presence and absence of both 100 μM AQDS and 500 μM EDTA, pH 7.

**Measurements of Pu(IV) and Pu(III) in the aqueous phase at days 1 and 6 (Experiment 1) and day 2 (Experiment 2 – analyses ongoing)**

**Conclusions**: S. oneidensis strain 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.

**Task 3: Anaerobic Biodegradation of EDTA**

**Research Objectives**: Complete past work on the transport of EDTA into an EDTA degrading bacterium using the periplasmic binding protein, which confers specificity for transport by an ABC transporter.

**Hypotheses**: MgEDTA is the form of EDTA transported into the cell, which must bind to the periplasmic binding protein before transport can occur.

**Approach**: Clone, express, and purify the periplasmic binding protein (EppA) from the EDTA degrading bacterium BRC1 using E. coli. Utilize various metal- and free-EDTA complexes to investigate binding of these substrates to EppA using isothermal titration calorimetry and fluorescence spectroscopy.

**Conclusion**: EppA bound free (i.e., no chelate metal) EDTA and NTA. MgEDTA likely dissociated before binding, while more stable complexes (e.g., ZnEDTA) did not bind. Degradability of metal-EDTA is related to the formation of free EDTA to bind to EppA for transport into the cell. Results have been submitted for publication (Xun et al. 2006).

**References**