Stabilization of Plutonium in Subsurface Environments via Microbial Reduction and Biofilm Formation

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Contaminant Pu is present at DOE sites that have complicated hydrogeology, and redox environments are affected by direct and indirect microbially mediated processes. The influence of these processes is particularly difficult to predict, due to the complicated redox behavior and rich chemistry of Pu. Our team aims to understand how bacteria can affect Pu speciation and environmental mobility, with a focus on (1) biotransformation and cell internalization by aerobic bacteria, (2) accumulation and immobilization in biofilms, and (3) immobilization via direct enzymatic and indirect biogeochemical reduction of Pu species by metal-reducing bacteria.

Biotransformation of Pu Species. Plutonium is accumulated by *Microbacterium flavescens* (JG-9) via an active hydroxamate siderophore recognition and uptake process. In order to determine if siderophore-mediated bacterial internalization of Pu is a general phenomenon, the pyoverdin/*Pseudomonas putida* system is being studied. The pyoverdin produced by *P. putida* strain ATCC 33015 contains a chromophore group derived from 2,3-diamino-6,7-dihydroxyquinoline with a catechol linked to a six amino acid peptide chain (determined using mass spectroscopy and multinuclear, multidimensional NMR). Pyoverdin binds Pu(IV) and Th(IV) to form 1:1 or 1:2 metal:pyoverdin complexes, which are being characterized. This siderophore mediates the transport of Pu, Fe, and Th into cells at similar accumulation rates and amounts. These studies indicate that under environmental conditions Pu^{IV} will be accumulated by bacteria via siderophore receptors and encourage us to investigate Pu accumulation by bacteria in biofilms.

Stabilization of Pu within Biofilms. Bacteria in nature grow mostly in biofilms, surface associated cells enveloped by hydrated extracellular polymeric substances (EPS). Plutonium may be sequestered within biofilms. Unsaturated biofilms of *P. putida* are being cultivated on membranes overlaying solid media with the individual addition of soluble metal (Cr, U, or Pu), mineral (hematite), and metal + mineral. Biofilms are characterized by imaging techniques, and then separated into cell and EPS fractions for subsequent inorganic speciation and biomolecule analyses. Initial results confirm that biofilm morphology varies by treatment and show how Cr is distributed between cells and EPS.

Bacterial reduction of Pu(VI/V). Pu(V) and Pu(VI) are the most soluble Pu species found under environmental conditions. Their reduction by metal-reducing bacteria to Pu(IV) solids could stabilize Pu in situ with respect to migration. Cell suspensions of *Shewanella oneidensis* MR1 and *Geobacter metallireducens* GS15 rapidly reduce Pu(V) and Pu(VI) to form a solid that has spectroscopic features consistent with Pu(IV) (hydr)oxides. For example, with an approximate cell density of 5×10^9 cells/mL, *S. oneidensis* removed from solution ~99% of the added Pu within 4 hr, and *G. metallireducens* removed from solution ~97% of the added Pu within 24 h. Preliminary experiments indicate plutonyl may also support growth of these bacteria.