Biotransformations of Plutonium and Uranium by Naturally-Occurring Bacteria

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Introduction

DCE sites are contaminated by radionuclides and toxic metals, which are in contact with organic contaminants, reactive materials, and diverse populations of microorganisms. Actinide species stabilized or mobilized in situ via indirect and direct chemical, biological, and geologic processes are transported. Actinide contamination tends to be poorly dispersed and present at low concentrations and therefore potentially costly to remove using conventional methods. Pu contamination is particularly challenging because of its high-energy exposure concerns and a lack of disposal sites. Bacterial bioremediation is a preferred treatment approach.

Given that the radiocurrieologies of most concern to the NABR program are generally more mobile in their oxidized forms (e.g., Pu(VI), Pu(V), U(VI), U(V)) proposed bioremediation strategies are generally based upon either in situ biodegradation of the oxidized form (e.g. actinide bioreduction and bioaccumulation within epoxymers and biofilms) or bioremediation of the reduced form (e.g., direct or indirect production of insoluble hydroxides by DMRR). The feasibility of these approaches is affected by the sorption of actinides under environmental conditions. For example, actinides can form complexes with co-contaminants (e.g., EDTA) or natural chelators like siderophores and biopolymers. Reducing complexes can interact with bacteria in several ways to yield biotransformed products or more mobile species that could desorb. We are investigating how siderophores affect the speciation and biotransformation of U and Pu. Previously, we reported how these siderophores bind, desorb and solubilize radionuclides. Here we present new results on EDTA complexation, siderophore-mediated Pu accumulation by aerobic bacteria, and initial studies of Pu reduction by DMRR.

Pu-EDTA and Siderophore Speciation

Effect of EDTA on Pu speciation.

Plutonium ions are strongly complexed by the bisamino-ethane carbonate ligand EDTA because of their "hard" character.

- In acidic solution (pH < 4), Pu-EDTA is the predominant species.
- At pH > 8, species Pu(EDTA)2, Pu(EDTA)3, and Pu(EDTA)4 are formed.
- In excess EDTA, Pu(EDTA)5, form, and in the presence of anionic ligands, mixed-ligand complexes such as CDPu(EDTA) can be formed.
- Pu environmental behavior and past complexation and solubilization studies involving Pu-EDTA can be reevaluated based on these new species and their corresponding constants.
- The redox potentials of Pu species cover a wide range.
- Pu(EDTA) at pH = 2, E° = 1.32 eV (vs. NHE).
- The reduction of Pu is shifted negatively by excess EDTA.

Effect of natural oxynitrate and mixed-functional siderophores on Pu(VI) speciation.

Due to their hard oxygen donor groups siderophores can solubilize plutonium. This observation, the reduction state, and significantly increase its bioavailability and mobility.

Pyoverdine, a siderophore produced by P. putida ATCC 33005, was purified and its structure characterized by MS and NMR.

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- Pyoverdine contains catecholate, hydroxamate and a carboxylate binding groups and is highly fluorescent.
- This redox potentials for Pu(VI)-siderophore complexes range from -200 to -370 mV.
- Under conditions where 
- Pu/siderophore complexes form, the redox potentials of 
- Pu(VI)-siderophore complexes shift to -400 to -500 mV.

Redox potentials of Pu(VI) and Fe(III)-siderophore complexes

Reduction by DMRR

Disinfectants

Metal-reducing bacteria (DMRR) utilize Fe(III), Mn(III), and other species as terminal electron acceptors. Growth and DMRR have been shown to reduce NO3, U(VI), and Tc(VII) to TOC.

Because the reduction potentials of Pu are similar to those of Fe(III) we hypothesized that these bacteria can also reduce Pu(VI) and Pu(IV).

- In cell suspensions both G. metallireducens strain G515 and S. oneidensis strain MR1 reduce Pu(VI) and Pu(IV).
- Growth experiments show that G. metallireducens strain G515 and S. oneidensis strain MR1 utilize Pu(VI) as an electron acceptor to support their growth.
- Solution spectra indicate the removal of Pu(IV) and Pu(VI) from solution. Preliminary characterization of the products suggests that they are cell associated Pu(VI) (hydrated) or Pu(VI) or Pu(IV) (hydrated).
- Fe(III) redox couples range from -0.77 to -0.46 eV.
- Pu(IV) redox couples range from -1.0 to -0.65 eV.

The redox for comparable Fe(III) and Pu(IV) species is generally more positive than Pu(IV) species. Pu(IV) can accommodate more donor groups and form additional species, which have more negative reduction potentials. Thus, certain Pu(VI) species may be beyond the reducing range of DMRR, and perhaps even some of their reduction products (e.g. Fe(III)). We have begun to investigate the reduction of Pu(VI) by DMRR.

Siderophore Facilitated Bioaccumulation

We examined the capacity of pyoverdine, desferrioxamine B, and several synthetic chelators (NTA, Fe(II), and EDTA) to facilitate Fe(III) and Pu(IV) transport in P. putida.

We found that:

- P. putida is able to acquire Fe and Pu from all the chelate forms tested.
- The dependence on iron accumulation by the cells on the ligand affinity for iron suggests that the chelators release iron to pyoverdine (exchange reaction) which mediates its transport inside the cell.
- Our results suggest that the metal-siderophore complex undergoes a reduction during the uptake process.
- From competition experiments performed by simultaneous addition of Pu-NTA and Fe-NTA we show that Fe and Pu are taken up efficiently and simultaneously by the cells.

Conclusions

- Synthetic and natural ligands like siderophores can form stable and highly soluble Pu species, making them more bioavailable.
- The redox potential of most complexes formed is accessible to natural reducers.
- Aerobic bacteria can take up Pu(IV) via hydroxamate and pyoverdine mediated Fe(III) uptake process, suggesting that siderophores facilitated transport of Pu is a general phenomenon.
- Microorganisms can use Pu(VI) species as a terminal electron acceptor to support their growth.
- Certain Pu(IV)-chelator complexes may not be reduced by DMRR.

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