

## Introduction

DOE sites are contaminated by radionuclides and toxic metals, which are in contact with organic contaminants, reactive minerals, and diverse populations of microorganisms. Actinide species be stabilized or mobilized *in situ* via direct and indirect chemical, biological, and geochemical processes. Actinide contamination tends to be broadly dispersed and present at low concentrations and therefore prohibitively costly to remove using conventional methods. Pu contamination is particularly challenging because of personnel exposure concerns and a lack of disposal sites. Bacterial bioremediation is a preferable treatment approach.

Given that the radionuclides of most concern to the NABIR program are generally more mobile in their oxidized forms (e.g., Pu(VI), Pu(V), U(VI), Tc(VII), Cr(VI)), proposed biostabilization strategies are generally based upon either *in situ* sequestration of the oxidized form (e.g. actinide biosorption and bioaccumulation within exopolymers and biofilms) or biomineralization of the reduced form (e.g., direct or indirect production of insoluble hydroxides by DMRB). The feasibility of these approaches is affected by the speciation of actinides under environmental conditions. For example, actinides can form complexes with co-contaminants (e.g. EDTA) or natural chelators like siderophores and biopolymers. Resulting complexes can interact with bacteria in several ways to yield biostabilized products or more mobile species that could persist. We are investigating how organic chelators 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 DMRB.



## Pu-EDTA and Siderophore Speciation

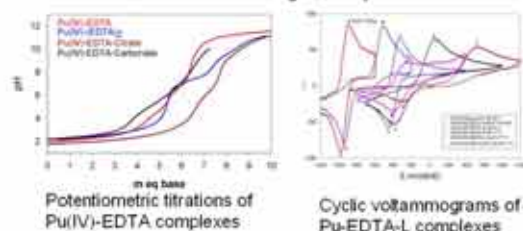
### Effect of EDTA on Pu speciation.

Plutonium ions are strongly complexed by the hexadentate amino carboxylate ligand EDTA, because of their "hard" character.

- In acidic solution (pH < 4), Pu<sup>IV</sup>-EDTA is the predominant species.
- At higher pH, species Pu<sup>IV</sup>EDTA(OH) and Pu<sup>IV</sup>EDTA(OH)<sub>2</sub> form.
- In excess EDTA, Pu<sup>IV</sup>(EDTA)<sub>2</sub> forms, and in the presence of ancillary ligands, mixed-ligand complexes such as Pu<sup>IV</sup>EDTA(citrate) and Pu<sup>IV</sup>EDTA(carbonate) form.
- 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<sup>IV</sup>EDTA at pH = 2.3, E<sub>1/2</sub> = +342 mV (vs. NHE).
- The reduction wave is shifted negative by excess EDTA.



Thermodynamic constants for Pu(IV) complexation

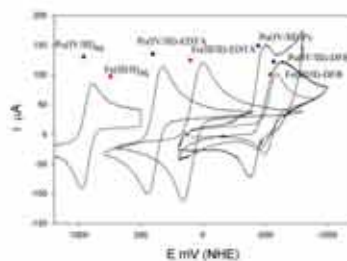
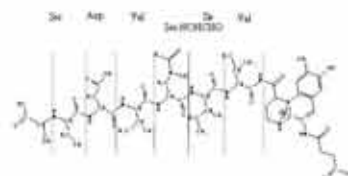
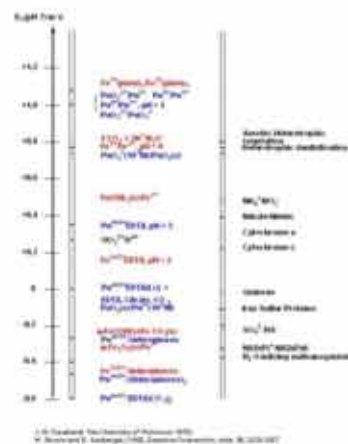
|  |         |
|--|---------|
| Log K <sub>PuEDTA</sub>                        | = 27.19 |
| Log K <sub>PuEDTA(OH)</sub>                    | = 4.36  |
| Log K <sub>PuEDTA(OH)<sub>2</sub></sub>        | = 6.71  |
| Log K <sub>PuEDTA(citrate)</sub>               | = 35.43 |
| Log K <sub>PuEDTA(carbonate)</sub>             | = 34.26 |
| Log K <sub>PuEDTA(carbonate)<sub>2</sub></sub> | = 51.54 |

### Effect of natural hydroxamate and mixed-functional siderophores on Pu(IV) speciation.

Due to their hard oxygen donor groups siderophores can solubilize plutonium, change its oxidation state and significantly increase its bioavailability and mobility.

Pyoverdine, a siderophore produced by *P. putida* ATCC 33015, was purified and its structure characterized by MS and NMR.

- Pyoverdine contains catecholate, hydroxamate and a carboxylate binding groups and is highly fluorescent.
- The redox potentials for Pu(IV/III)-siderophore complexes range from -200 to -370 mV.
- Under conditions where 1:2 Pu:siderophore complexes form, the redox potentials of Pu(IV/III)-siderophore complexes shift to -440 to -550 mV.



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

| Plutonium Complex            | E <sub>1/2</sub> V vs NHE | Iron Complex   | E <sub>1/2</sub> V vs NHE |
|------------------------------|---------------------------|----------------|---------------------------|
| Pu(IV/III)-DFB               | -0.269*                   | Fe(III/II)-DFB | -0.482                    |
| Pu(IV/III)(DFB) <sub>2</sub> | -0.509 ± 25               |                |                           |
| Pu(IV/III)-DFE               | -0.268*                   | Fe(III/II)-DFE | -0.481                    |
| Pu(IV/III)(DFE) <sub>2</sub> | -0.550 ± 25               |                |                           |
| Pu(IV/III)-Rh                | -0.209*                   | Fe(III/II)-Rh  | -0.422                    |
| Pu(IV/III)(Rh) <sub>2</sub>  | -0.455 ± 25               |                |                           |
| Pu(IV/III)-Py                | -0.154*                   | Fe(III/II)-Py  | -0.367                    |
| Pu(IV/III)(Py) <sub>2</sub>  | -0.440 ± 25               |                |                           |

\* Irreversible behavior, values estimated relative to Fe(III/II)-siderophores.

## Reduction by DMRB

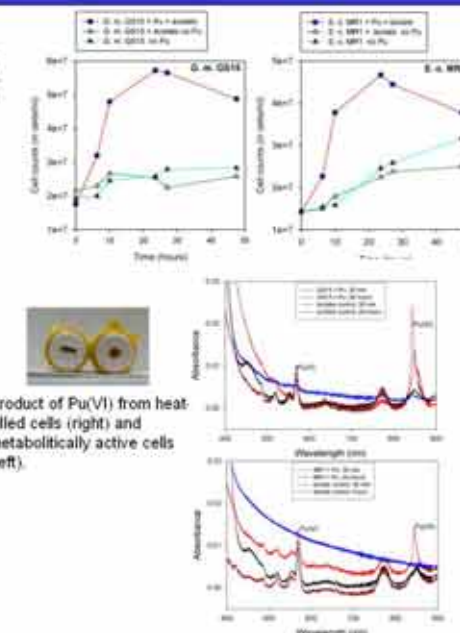
### Dissimilatory

Metal-reducing bacteria (DMRB) utilize Fe(III), Mn(IV) and other species as terminal electron acceptors to support their growth. DMRB have been shown to reduce UO<sub>2</sub><sup>2+</sup> to UO<sub>2</sub> and TcO<sub>4</sub><sup>-</sup> to TcO<sub>2</sub>.

Because the reduction potentials of Pu ions are similar to those of Fe(III) we hypothesized that these bacteria can also reduce PuO<sub>2</sub><sup>2+</sup> and PuO<sub>2</sub><sup>+</sup>.

- In cell suspensions both *G. metallireducens* st. GS15, and *S. oneidensis* st. MR1 reduce Pu(VI) and Pu(V).
- Growth experiments show that *G. metallireducens* st. GS15, and *S. oneidensis* st. MR1 can utilize Pu(V) as an electron acceptor to support their growth.
- Solution spectra indicate the removal of PuO<sub>2</sub><sup>2+</sup> and PuO<sub>2</sub><sup>+</sup> from solution. Preliminary characterization of the products suggests that they are cell associated Pu(IV) (hydr)oxide. Pu(IV) may also be reduced.
- Fe(III/II) redox couples range from +0.77 to -0.6 V.
- Pu(IV/III) redox couples range from +1.0 to -0.5 V.

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



Product of Pu(VI) from heat killed cells (right) and metabolically active cells (left).

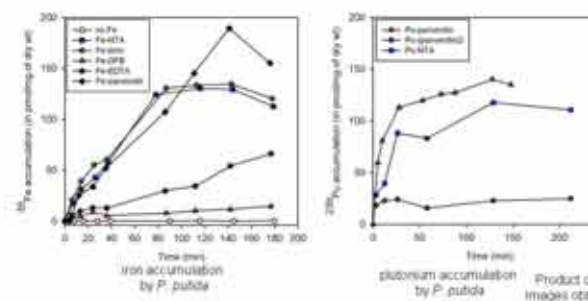
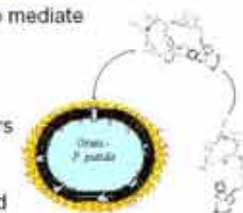
Spectral characterization of Pu(VI) reduction by *G. metallireducens* GS15, and *S. oneidensis* MR1 cell suspension

## Siderophore Facilitated Bioaccumulation

We examined the capacity of pyoverdine, desferrioxamine B, and several synthetic chelators (NTA, tiron and EDTA) to mediate 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 of 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.



Product of Pu(IV)-NTA uptake by *P. putida* images obtained by Dolekova, Alice at PDBL

| Metal Complex                                  | Structure similar to Fe-pyoverdine | Redox accessible | Exchange rate | Uptake by <i>P. putida</i> |
|--|------------------------------------|------------------|---------------|----------------------------|
| Fe <sup>III</sup> -Pyoverdine                  | same                               | Yes              | fast          | Yes                        |
| Fe <sup>III</sup> -L(L= NTA, tiron, EDTA, DFB) | No                                 | Yes              | fast          | Yes                        |
| Al <sup>III</sup> -Pyoverdine                  | Yes                                | No               | fast          | No                         |
| Ga <sup>III</sup> -Pyoverdine                  | Yes                                | No               | fast          | No                         |
| Cr <sup>III</sup> -Pyoverdine                  | Yes                                | Yes              | slow          | No                         |
| Pu <sup>IV</sup> -Pyoverdine                   | Yes                                | Yes              | Fast          | Yes                        |
| Pu <sup>IV</sup> -NTA                          | No                                 | Yes              | Fast          | Yes                        |

## 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/IV) species as a terminal electron acceptor to support their growth.
- Certain Pu(IV)-chelator complexes may not be reduced by DMRB.

## Acknowledgement

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