Bacterial Biotransformations for the *In situ* Stabilization of Plutonium

Mary Neu, Hakim Boukhalfa, Gary Icopini, Larry Hersman, Joe Lack, John Priester, Scott Olson, Patricia Holden

Chemistry & Biology Divisions, Los Alamos National Laboratory
Bren School of Environmental Science and Management, UCSB
Overview

Plutonium contamination in the environment is generally low-level and may be present and transported in a range of forms (IV, V, VI).

Current remediation strategies are costly, financially and in terms of increased exposure risk to people and the environment. In situ bacterial biostabilization is a promising alternative.

Aqueous Speciation Related to Environmental Conditions

- Plutonium(VI) vs Uranium(VI) Hydrolysis
- Plutonium(IV/III) EDTA Speciation and Stability
- Siderophore Stabilization of Plutonium(IV)
- New Reduction Potentials

Bacterial Biotransformations

- Siderophore-mediated Accumulation by Aerobic Bacteria
- EPS and Cell Adsorption by Aerobic Bacteria
- Reduction by DMRB
Pu (VI) Hydrolysis

Why biostabilization methods being developed for U, e.g. reduction by DMRB, may or may not work for Pu

~600 papers on U(VI) hydrolysis
5 papers on Pu(VI) hydrolysis
2 papers on Pu(V) hydrolysis
Pu(IV)EDTA Aqueous Speciation

Cyclic Voltammetry

New species are identified including Pu(EDTA)$_2$, log $\beta = 35.43$

Stability of Pu(IV) is enhanced at environmentally relevant pH by the formation Pu(IV)-EDTA-hydroxo and Pu(IV)-EDTA-L mixed complexes.

Unusual speciation due to high charge and large coordination sphere

Pu Speciation Under Environmental Conditions

$[\text{Pu}]_{\text{tot}} = 10^{-5} \text{ M}$
$[\text{CO}_3]_{\text{tot}} = 10^{-2.6} \text{ M}$
$T = 25^\circ \text{C}, \ P = 1 \text{ atm}$

Pu Speciation:

PuO$_2^{2+}$  $\log K_{sp} = 5.5$
PuO$_2^+$$\log K_{sp} = 5.0$
Pu$^{4+}$  $\log K_{sp} = -2.0$
Pu$^{3+}$  $\log K_{sp} = 15.8$

E$_{\text{red}}$ (mV) vs NHE, pH = 7

Stability Fields of Solids:
- Pu(OH)CO$_3$(s)
- Pu(OH)$_4$(s)
- PuO$_2$(OH)$_2$(s)

Solubility of primary (hydr)oxide phases

PuO$_2^{2+}$  $\log K_{sp} = 5.5$
PuO$_2^+$$\log K_{sp} = 5.0$
Pu$^{4+}$  $\log K_{sp} = -2.0$
Pu$^{3+}$  $\log K_{sp} = 15.8$

e.g., Pu$^{4+}$/PuO$_2$(hyd) $[H^+]^4$
Pu(VI) as a Terminal Electron Acceptor for DMRB

**Shewanella oneidensis MR1**
- 10 mM Lactate
- 5 x 10^8 Cells/mL

**Geobacter metallireducens GS15**
- 10 mM Acetate
- 5 x 10^8 Cells/mL

DMRB reduce Pu(VI) and Pu(V)
Product appears to be PuO₂ (hyd)
Characterization and dissolution studies in progress
Can Pu be accumulated by other bacteria via other types of siderophores?
Uptake inversely proportional to Fe-L complexes stability.

Fe uptake profile suggests that exogenous ligands release iron to the siderophore either in solution or at the membrane surface.
Requirements for Metal–Siderophore Uptake

Siderophore binding, membrane protein recognition, metal shuttle, intra-cellular release thought to require specific physico-chemical characteristics.

\[
\begin{align*}
\text{Pu accumulation} & \quad \text{Fe-NTA uptake} \\
\text{Time (min)} & \quad 0 50 100 150 200 250 \\
\end{align*}
\]

\[
\begin{align*}
P. \text{Putida} \text{ cells take up NTA and pyoverdin complexes, both in the presence and absence of Fe.}
\end{align*}
\]

\[
\begin{align*}
P. \text{Putida} \text{ cells pre-incubated with 2 } \mu\text{M of pyoverdin complexes of Cr(III), Ga(III) and Al(III)} \text{ unable to acquire Fe from NTA, EDTA or pyoverdin complexes.}
\end{align*}
\]

Characteristics required for complete translocation?
- specific radii, trivalent charge, specific molecular conformation, neutral molecular charge, metal reduction, ligand exchange (solution or membrane)…..

Pu(IV) and Th(IV) uptake reveal combination of ligand exchange and reduction is key
Metal Binding of Microbial Extracellular Polymers

**PGA of B. licheniformis**

~800 kDa, forms soluble metal complexes, generally >10:1 glu to M

~0.12 mmol metal bound per mg PGA (alone)

Pu(IV) remains associated with PGA during repeated pH cycling 2-12

Whole cells (in culture media) take up more Pu per mass than does polyglutamate

Siderophores and EPS associate Pu with cells
**Effect of U(VI) on *P. putida* Biofilms**

*P. Putida* grown on membrane discs on U-containing agar with minimal nutrients

\[ [U] = 10^{-4} \text{ M} \]

or 50 µg/g

72 hr growth

Fractionation by cent.

EPS, but not cell growth, affected by U.

U(VI) adsorbed onto Fe(III) minerals increased EPS produced by *P. putida*
Distribution of U(VI) within *P. putida* Biofilms

Less U removed from substrate by *P. putida* in the presence of EDTA

U preferentially associated with cell fraction
Advances in Pu aqueous geochemistry

Solubilization, biosorption, bioaccumulation, mineralization biotransformation mechanisms all affect Pu

Stability of products, rates of combined processes,…?
Acknowledgements

Dr. Hakim Boukhalfa
Dr. Gary Icopini
Mr. Sean Reilly

Dr. Larry Hersman
Prof. Patricia Holden
Dr. Cheryl Kuske

U.S. DOE, OSc, OBER, ERSD, NABIR