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

Hakim Boukhalfa *, Gary A. Icopini, Sean D. Reilly, Mary P. Neu *

*Chemistry and Bioscience Divisions, LANL, mneu@lanl.gov, hakim@lanl.gov

Introduction

Plutonium has a long half-life (2.4 x 10^4 years) and is of concern because of its chemical and radiological toxicity, high-energy alpha radioactive decay. A full understanding of its speciation and interactions with environmental processes is required in order to predict, contain, or remediate contaminated sites. Under aerobic conditions Pu is sparingly soluble, existing primarily in its tetravalent oxidation state. To the extent that pentavalent and hexavalent complexes and small colloidal species form they will increase the solubility and resultant mobility from contamination sources. There is evidence that in both marine environments and brines substantial fractions of the plutonium in solution is present as hexavalent plutonyl, PuO_2^{2+}.

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 hydoxides 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. The goal of this work is to understand and optimize mechanisms for *in situ* immobilization of Pu species by naturally-occurring bacteria. We examined the ability of metal-reducing bacteria *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 to reduce soluble Pu(VI) and Pu(V) species under cell suspension conditions and examined the ability of these organisms to utilize Pu(VI) and Pu(V) as the sole electron acceptor to support their growth. We also examined the ability of these organisms to to enzymatically reduce freshly precipitated Pu(IV)$(OH)_{4(am)}$ and soluble Pu(IV)(EDTA).

Experimental conditions

Bacterial Strain and Culture Conditions.
G. metallireducens and *S. oneidensis* were obtained from the American Type Culture Collection.
Growth media per liter is: 3.4 g NaOH; 12.25 g Fe-citrate anhydrous; 0.25 g NH_4Cl; 1.08 g Glycerol-2-PO_4; 0.025 g KCl; 10.46 g MOPS; 10.0 mL Wolfe's minerals; 10 mL Thaver's vitamins; and 10 mM acetate (*G. metallireducens*) or 10 mM lactate (*S. oneidensis*).

Cell suspensions with cell densities of 5 $\times 10^8$ cells/mL at pH 7.0 were used for all cell suspension experiments and an initial cell density of 2 $\times$ 10^7 cells/mL for growth cultures.

Pu(VI)/Pu(V) Reduction by DMRB

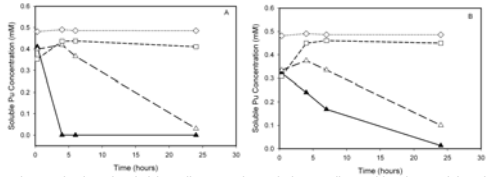
The ability of *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 to reduce Pu(VI), Pu(V), Pu(IV)$(OH)_{4(am)}$ and Pu(IV)(EDTA) was assessed by incubating 0.5 mM of Pu with a cell suspensions of approximately 5 $\times$ 10^8 cells/mL in 100 mM MOPS at pH 7.0 with 10 mM acetate (*Geobacter*) or lactate (*Shewanella*). Each cell-suspension experiment had **controls** consisting of **no cells**, **no electron donor**, and **heat killed cells**.

All control and experimental conditions were conducted in **triplicate** and incubated at 30°C in sealed anaerobic serum vials.

Pu(VI) concentrations in the cultures was monitored by liquid scintillation counting (LSC) analysis and by vis-NIR spectroscopy. Samples collected for LSC were collected from each triplicate and one culture of each triplicate was sampled for vis-NIR spectroscopy for each sampling interval.

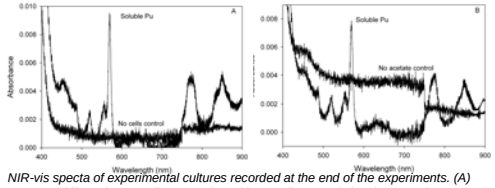
Samples were collected using sterile syringes with metal needles that were purged with sterile Ar and immediately filtered through a 0.2 µm PTFE filter (Millex® SLLG013SL) into a vessel containing 15 µL of concentrated HCl.

Manipulations of cultures containing Pu were done within a radiological fume hood, which was open to the atmosphere.



Direct reduction of Pu(VI) by cell suspensions of Shewanella Oneidensis MR1(A) and Geobacter metallireducens GS15 (B). Conditions: (▲) Live cells with the electron donor, (△) live cells with no electron donor, (■) no cells control, (◆) heat killed cells control.

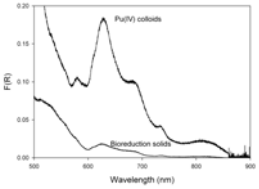
•After 24 hours incubation time, the cultures of live *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 with the appropriate electron donor precipitated all the soluble Pu(VI) initially added to the cell suspensions.
•The most rapid decrease in soluble Pu concentrations is observed for cultures with live cells and in the presence of an electron donnor.
•The concentration of Pu in cultures without added electron donor also decreased to nearly the same magnitude as the experimental cultures.
•There were no significant changes in Pu concentrations in the no-cell and heat killed cells controls.
•Under our experimental conditions Pu(V) is favored over Pu(VI) and partial reduction of Pu(VI) to Pu(V) was observed in all cultures initially.



NIR-vis specta of experimental cultures recorded at the end of the experiments. (A) Spectra of filtered MR1 cell suspension with no cells control showing a peak characterizing Pu(V) at 570 nm and a flat line for the experimental culture with live cells and electron donor, (B) Spectra of filtered GS15 cell suspension with cultures at the end of the experiment showing the spectra of the solution with live cells and acetate (flat line) and the control with no acetate showing the presence of Pu(V/VI).

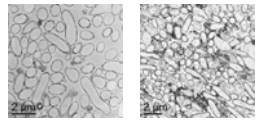
A yellow green colloidal precipitate was visible at the bottom of the serum vials at the end of the experiment. We characterized the solids formed using defuse reflectance and transmission electron microscopy imaging (TEM).

The defuse reflectance spectra of the bio-precipitates are flat for most wavelengths with an absorbance wall at around 400 nm. This is typical for Pu(IV) hydroxides. The defuse reflectance spectra of colloidal Pu(IV) obtained by Pu(IV) precipitation at high pH and the defuse reflectance spectrum of the bio-reduction products are presented here. The spectra in the figure are very similar and strongly suggest that the reduction product from our experiments is indeed colloidal Pu(IV).



Defuse reflectance spectra of colloidal Pu(IV) obtained by precipitation of Pu(IV) at near neutral pH and bioreduction solids obtained by incubation of 0.5 mM Pu(IV) with a cell suspension of S. oneidensis.

TEM images showing Pu(VI) (A) and U(VI) (B) solids precipitated along with bacterial cells of *S. Oneidensis*. Both images show actinide deposition as clusters outside the cells and on the cells surface. Figure A with plutonium seems to show less deposition of solids in the periplasmic space relative to the uranium solids on Figure B.



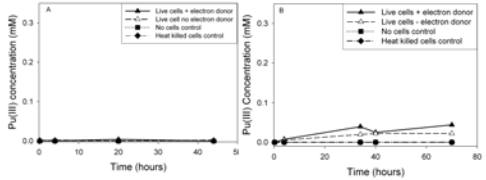
(A) Pu(VI) bioreduction (B) U(VI) bioreduction

$Pu(IV)(OH)_{4(am)}$ Reduction by DMRB

•Fresh plutonium hydroxide Pu$(OH)_{4(am)}$ was prepared by hydrolysis of a pure Pu(IV) by addition of sodium hydroxide.

•Pu(IV)(EDTA) was prepared by slowly adding one equivalent of acidic Pu(IV) to an aqueous solution containing one equivalent of EDTA.

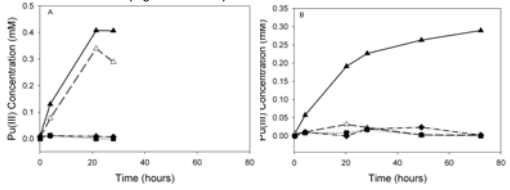
The ability of *G. metallireducens* and *S. oneidensis* cell suspensions to enzymatically reduce Pu$(IV)(OH)_{4(am)}$ (with and without EDTA present) was assessed by following changes in Pu(III) concentration in the cultures over time.



Direct reduction of Pu$(OH)_{4(am)}$ by cell suspensions of G. metallireducens (A) and S. oneidensis (B). (▲) Live cells with the electron donor, (△) live cells with no electron donor, (■) no cells control, (◆) heat killed cells control.

Traces of Pu(III) were observed in the cell suspensions of *S. oneidensis* and *G. metallireducens* incubated with Pu$(IV)(OH)_{4(am)}$ after the first time point. However, at longer incubation times (~ 44 hours) no Pu(III) could be detected in the cultures. The amounts of Pu(III) observed in the cell suspensions of *S. oneidensis* (FigureB) was significantly higher than that observed with *G. metallireducens*. The amount of Pu$(IV)(OH)_{4(am)}$ reduced by the cell suspensions of *S. oneidensis* was less than 8 % of the total Pu present in the cultures.

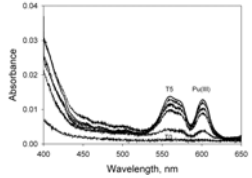
Cell suspensions of both *G. metallireducens* and *S. oneidensis* reduced most (approximately 80% and 60%, respectively) of the available Pu$(IV)(OH)_{4(am)}$ in the presence of 0.5 mM EDTA, which was added at just prior to addition of cells to the cultures (Figures A and B).



Direct reduction of Pu$(OH)_{4(am)}$ by cell suspension of G. metallireducens (A) and S. oneidensis (B) with 0.5 mM EDTA. (▲) Live cells with the electron donor, (△) live cells with no electron donor, (■) no cells c

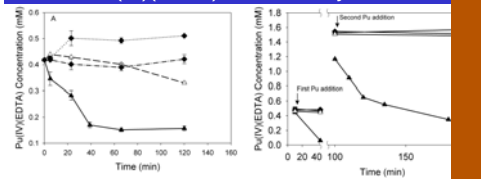
•The UV-visible spectra of the acidified filtrates from *S. oneidensis* cell suspension experiment are shown in the figure , which shows an increase over time in the absorbace peak for Pu(III) at 600 nm.

• Aqueous Pu(III) produced in the cell suspensions of *S. oneidensis* and *G. metallireducens* in the presence of EDTA remained stable under reducing conditions.



Variation of the optical absorbance spectra of acidified solutions containing Pu$(OH)_{4(am)}$ in the presence of a cell suspension of S. oneidensis and 0.5 mM of EDTA. T0 represents a time point taken at 15 min and T5 at 72 hours.

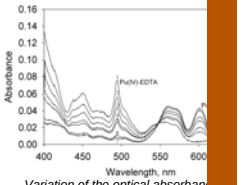
Pu(IV)(EDTA) Reduction by DMRB



Direct reduction of Pu(IV)(EDTA) by cell suspension of G. metallireducens (A) S. oneidensis (B). (B) Pu(IV) EDTA was added in two steps, the first addition mM was at t0 and the second addition of 1.0 mM was at t_1 = 47min. Total Pu(IV)(EDTA) added is 1.50 mM. (▲) Live cells with the electron donor, (△) li cells with no electron donor, (■) no cells control, (◆) heat killed cells control.

•In cultures with initial concentrations of 0.5 mM Pu(IV)(EDTA) almost all of the Pu(IV)(EDTA) was reduced to Pu(III)(EDTA) in less than 40 minutes.
•Production of Pu(III) was not observed in controls with either no cells or heat l cells.

The evolution of the Pu(III)/(IV) species concentrations during the cell-suspension experiments are shown by the UV-visible spectra in the figure. The data show a gradual decrease of the peak corresponding to Pu(IV)(EDTA) at 495 nm with the concurrent increase in the absorption band corresponding to Pu(III) at 600 nm. Controls with live cells and no electron donor show the production of less Pu(III), where as the controls with no cells or dead cells show no development of Pu(III).



Variation of the optical absorban spectra of solutions containing P EDTA complex with a cell suspe S. oneidensis.

Pu Accessibility to Bacterial Reduction

Reduction potentials of predominant Pu species calculated at pH 7.

Redox system	Species, pH 7	Reduction reaction	E_0	E (V NHE
Pu(VI)/Pu(IV)	$PuO_2(OH)_2$	$PuO_2(OH)_2 + 2H^+ + 2e^- = PuO_2(s) + 2H_2O$	1.387	0.79
	$(PuO_2)_2(OH)_4^{2+}$	$(PuO_2)_2(OH)_2^{2+} + 4H^+ 4e^- = 2PuO_2(s) + 4H_2O$	1.328	0.82
	PuO_2CO_3	$PuO_2CO_3 + H^+ + 2e^- = PuO_2(s) + HCO_3^-$	0.762	0.67
	$PuO_2(CO_3)_2^{2-}$	$PuO_2(CO_3)_2^{2-} + 2H^+ + 2e^- = PuO_2(s) + 2HCO_3^-$	0.609	0.60
Pu(IV)/Pu(III)	$PuOH_4$	$Pu(OH)_4(s) + 4H^+ + e^- = Pu^{3+} + 4H_2O$	0.922	-0.41
	Pu(EDTA)	$Pu(EDTA) + e^- = Pu(3+)(EDTA)^-$	0.334	0.21

The reduction of Pu(VI) and Pu(V) to Pu(IV) is predicted by analysis of the redo potentials of the species of these oxidation states that are all within the range accessible to bacterial reduction (Table). The reduction of Pu(IV) to Pu(III) is m difficult to predict because the redox potential of Pu(IV/III) couple at pH 7 estima at –0.42 V is situated near the lower limit accessible to bacterial reduction

Conclusions

We have demonstrated that Pu(VI), Pu(V) can be reduced to insoluble hydroxides by metal-reducing bacteria. We also found that Pu(IV) can be reduced to Pu(III) by direct enzymatic reduction when EDTA is present.

Much work remains to be done to fully understand the consequence hydrous-oxide reduction on the fate and transport of Pu in the environment. Th remedial approach consisting of biological reduction and subsequent precipi actinides that has been recommended as a remedial strategy for U conta subsurface environments may be more complicated to implement for Pu conta sites. Our results indicate that strongly anaerobic conditions may increase the s of Pu. In many areas Pu contamination is primarily present as insoluble Pu(IV) oxides or associated to colloids. If these Pu(IV) solids are subjected to a conditions where metal-reducing bacteria dominate, the concentration of sol could increase, especially if chelating ligands are present.

Acknowledgements and Funding

This research was supported by the Environmental Remediation sciences Pro (ERSP), Office of Biological and Environmental Research, Office of Science, o U.S. Department of Energy.