Final technical report on The exact title as it appears on the cover sheet is: Interaction of Actinide Species with Microorganisms & Microbial Chelators: Cellular Uptake, Toxicity, & Implications for Bioremediation of Soil & Ground Water.

Contributed scientific papers from this project.

Siderophore Mediated Transport of Pu(IV) and Th(IV) into *Pseudomonas Putida* Cells. Boukhalfa Hakim; Neu Mary P., paper submitted to PNAS.

Comparative Study of Fe(III) and Pu(IV) Binding by Natural Siderophores : Thermodynamic and Electrochemical Characterization. Boukhalfa Hakim; Sean D. Reilly; and Neu Mary. P., paper in preparation.

Iron(III) Coordination Properties of a Pyoverdin Siderophore Produced by *Pseudomonas putida* ATCC 33015. Hakim Boukhalfa, Sean D. Reilly, Ryszard Michalczyk; Srinivas Iyer; and Mary P. Neu. Accepted for publication in Inorg. Chem.

Actinide and Metal Toxicity to Prospective Bioremediation Bacteria. Ruggiero Christy, E.; Boukhalfa Hakim; Forsythe Jennifer, H.; Lack Joseph, G.; Hersman Larry, E.; Neu Mary, P. *Environmental Microbiology*, (2005), 7,(1), 88-97.

EDTA and mixed-ligand complexes of tetravalent and trivalent plutonium. Boukhalfa, Hakim; Reilly, Sean D. ; Smith, Wayne H ; Neu, Mary P. *Inorganic Chemistry*; (2004), 43,(19), 5816-5823.

Plutonium Speciation Affected by Environmental Bacteria. Neu Mary P.; Icopini Gary A.; Boukhalfa Hakim. *Radiochimica Acta*, **2005**, 93, 705-714.

Environmental reduction of Tc, U, Np, and Pu by bacteria and the stability of reduction products. Gary A. Icopini, Hakim Boukhalfa, and Mary P. Neu., Proceedings of the 2005 Actinides Conference, Royal Society of Chemistry, London, (in press).

Summary of results

Toxicity of actinides and other metals to aerobic bacteria

• We have determined the toxicity of actindes and other relevant metal ions and chelators to three aerobic bacteria (*D. radiodurans, P. putida,* and *B. licheniformis*). We found that plutonium is less toxic than U, and that actinides are less toxic than most transition metals, and will therefore pose less of a limitation to bioremediation.

Effects of siderophores and EDTA on actinide geochemistry and biotransformations

- We have determined the stoichiometry and the thermodynamic parameters for several Pu(IV)EDTA complexes using *potentiometric* and *spectrophotometric titration* methods. Our results show that EDTA effectively stabilizes Pu(IV) and forms bis EDTA, and ternary mixed ligand complexes under environmentally relevant conditions. The stability of the Pu(IV) EDTA complexes relative to other oxidation states depends on both pH and Pu:EDTA ratio.

- We have purified the siderophore produced by the environmental bacterium *Pseudomonas putida*, characterized its structure by ^{1}H, ^{15}N, ^{13}C NMR and mass spectrometry. This highly fluorescent binds Fe(III) and Th(IV) and Pu(IV) efficiently. The binding constant of Pu(IV) with pyoverdin and other natural siderophores such as desferrioxamine B were determined. We found that complexes of different stoichiometry are formed depending on the siderophore:Pu(IV) ratios. In 1:1 Pu(IV):siderophore and low pH (pH < 2.5) the complex Pu(IV)(siderophore)$(H_2O)_n$ is the main complex in solution. At higher pH, the complex hydrolyzes to form hydroxo complexes Pu(IV)(siderophore)$(OH)_n(H_2O)_m$. However, in excess siderophores relative to Pu(IV) the ternary complexes Pu(IV)$(siderophore)_2(H)_n(H_2O)_m$ form.

- We have measured the redox potentials of several Pu(IV)-siderophores complexes using cyclic voltammetry. The redox potential of Pu(IV/III)-siderophore complexes are slightly more negative than their iron(III/II) analogues. The complexes formed between Pu(IV) and siderophores are highly soluble, and under environmental conditions the formation of such complexes has a potential of making Pu bioavailable to microorganism and plants.

Siderophore-mediated translocation of Pu

- We examined the capacity of pyoverdin to mediate the transport and accumulation of plutonium by *Pseudomonas putida*. Our results indicate that Pu can be transported into the cells through the siderophore-mediated iron transport system.

<u>Condensed Results:</u>

Toxicity of Actinides and Metals to *B. Lichenformis, P. putida, and other Microorganisms:*

Although *D.rad* is an interesting microorganism in regards to actinide toxicity, we believe it has limited potential for use in bioremediation for a number of reasons (metal tolerance is low, nutritional needs are high, needs to be genetically engineered for metal reduction, is not a common soil species so cannot be used in monitored natural attenuation). We have expanded our toxicity studies to microorganisms more likely to be useful in bioremediaiton strategies: *P. putida*, a common Gram-negative soil aerobe found at many DOE sites; and *B. licheniformis*, a Gram-positive spore-former, and most recently *S. putrifacens*. We have performed solution toxicity tests with *P. putida* with numerous transition metals (As, Cd, Co, Cr, Cu, Ni, Pb, Zn) and have initial toxicity data for Pu and U. We have also examined the metal tolerance of *B. licheniformis.* As would be expected, the toxicity of metals varies greatly among different microorganisms (Tables 1-3). This is most likely a result of the different

mechanisms each microorganism uses to protect against toxicity (Efflux pumps, Exporters, blocking uptake, internal/external precipitation, methylation for Hg - are the most common ways bacteria mediate toxicity). Fe, Pu, U, and Al were generally least toxic metals. For most bacteria, *Pu & U contamination is unlikely to be a problem for bioremediation compared to other metals present at contaminated sites.* Pu is less toxic than U in most bacteria tested. However, for *S. putrifacens*, we found a Pu(VI) toxicity of 6 mM (compared to 4.2-5 mM for *D.rad)*, while it can grow in 10mM U, using U as its terminal electron acceptor (see research plan for more details on these results).

We have also begun to compare our results on these solution toxicity tests to "zone of inhibition" toxicity tests, which will allow us to examine more actinides and actinide species. Our results show the same order of toxicity as we observed in solution studies, showing that these studies give us qualitatively the same results . We will soon expand these studies to actinide species and actinide/metal or organic co-contaminant systems, for example: Pu(VI), Pu(V), Pu(IV), Pu(III) toxicity comparisons, Pu-chelators (NTA, EDTA, siderophore, nitrate) comparisons.

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Table 1: B. licheniformis Solution Toxicity

Metal	ppm	μM
Cd(II)	4 - 6	36 - 53
Co(II)	20-30	340 - 510
Zn(II)	50	760
CrO_4^{2-}	40	770
AsO_4^{3-}	50-100	670-1300
Cu(II)	150-200	2400-3100
Ni(II)	200	3400

Table 2: P. putida Solution Toxicity

Metal	ppm	μM
(II)	1 - 3	17 - 50
Co(II)	2 - 4	34 - 67
Pb(Acetate)	7.5-10	36 - 48
Cu(II)	5	80
CrO_4^{2-}	4 - 5	77 - 96
Cd(II)	30-40	260-350
^{238}U(VI)(Cit-)	300-400	1300-1700
Zn(II)	120-300	1800-4600
^{239}Pu(IV)(Cit-)	>870	>3600
Al(III)(Cit-)	>1620	>60000
AsO_4^{3-}	6000	80000
Fe(III)(Cit-)	11000	200000

Table 3: D. radiodurans Solution Toxicity

Metal	ppm	μM
Cd(II)	0.1-0.2	0.9-1.8
Pb(Acetate)	5-10	24-48
Co(II)	2.5-5	42-85
CrO_4^{2-}	2.5-5	48-96
Zn(II)	5-10	76-150
Cu(II)	20-30	320-470
AsO_4^{3-}	30	400
Ni(II)	60-70	1000-1200
^{237}Np(V)-	260-380	1100-1600

DFB		
^{238}U(VI)-DFB	600	2500
Al(III)(Cit)	81	3000
Ba(II)	500	3600
^{239}Pu(IV)-DFB	1000-1200	4200-5000
Fe(III)-DFB	1600	29000

Plutonium-EDTA chemistry*:*

Amino carboxylate ligands are nonspecific chelating agents with strong binding affinity for a variety of metal ions. Upon binding to metal ions, the chelating agents change the composition of the inner coordination shell of the metal ions and thereby alter their chemical speciation and behavior. Because of these properties, ethylenediaminetetra-acetic acid (EDTA) and other amino carboxylate ligands have been extensively used in the processing of radionuclides. As a consequence EDTA is codisposed with radionuclides and co-located with radionuclide contamination. If EDTA is released to the environment, it has the potential to significantly affect the solubility and overall behavior of radionuclides.[1] This is particularly true for low-valent actinides, such as plutonium, that would generally hydrolyze and precipitate or sorb to mineral surfaces in water and soils in the absence of strong chelators. The first step in predicting how EDTA would affect the environmental behavior of plutonium and impact the safe disposal of nuclear waste is determining the nature of the complexes formed. Previous work on EDTA complexation of plutonium was focused on acidic conditions and interpretation of bulk solubility studies.[2] Our approach was to determine the speciation, and thermodynamic stability of species formed, more directly and under near-neutral pH, and excess EDTA conditions.

Plutonium can exist in aqueous solution as ions in a range of oxidation states III-VI that can be complexed by EDTA. The specific affinity of aminocarboxylate ligands for Pu(IV) will tend to drive the complexation reaction toward the formation of Pu(IV) complexes through redox processes. Specifically, Pu(IV) EDTA complexes can form through the reduction of the higher oxidation states, as for Pu(VI) and Pu(V), and through oxidation of Pu(III). Plutonium(IV) is known to form a 1:1 complex with EDTA in aqueous solution; but this hexadentate ligand does not fully encapsulate the metal ion. Two to four waters complete the coordination sphere, providing coordination sites for hydrolysis, polymerization or the formation of mixed ligand complexes.

We have determined the stoichiometry and the thermodynamic parameters for several Pu(IV)EDTA complexes using *potentiometric* and *spectrophotometric titration* methods (Figure 1).

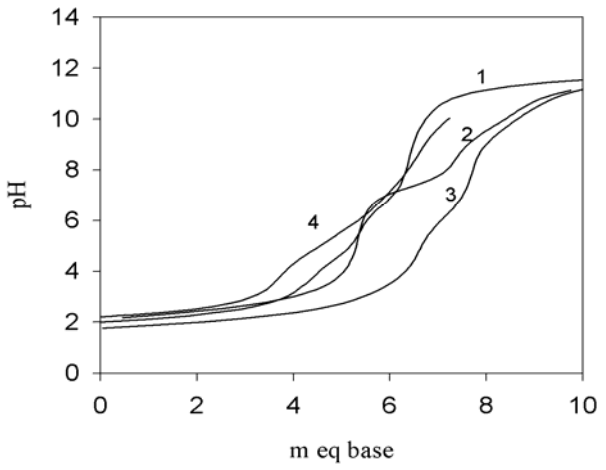

Figure 1. Potentiometric titrations of Pu(IV)-EDTA complexes. (1) [Pu(IV)] = 2.5 mM; [EDTA] = 2.53 mM, (2) [Pu(IV)] = 2.49 mM; [EDTA] = 5.0 mM, (3) [Pu(IV)] = 2.49 mM; [EDTA] = 2.5 mM, [citrate] = 2.52 mM and (4) [Pu(IV)] = 2.49 mM; [EDTA] = 2.5 mM,

alytical and Nuclear Chemistry (2001), 250(1), 47-53.
, G. R. *Radiochim. Acta* (2001**)**, *89*, 67-74. (b)Cauchetier, P.; Guichard, Guichard, C. *J. Inorg. Nucl. Chem.* (1975**)**, *37*, 1771-8.

[carbonate] = 2.52 mM. m represents the number moles of NaOH added per mol of plutonium(IV).

In acidic solution and at 1:1 Pu(IV):EDTA ratio, Pu(IV)-EDTA is formed ($\log\beta_{110}$ = 26.44), while at higher pH Pu(IV)-EDTA(OH) ($\log K_{11\text{-}1}$ = 25.6) and Pu(IV)-EDTA$(OH)_2$ ($\log K_{11\text{-}2}$ = 15.29) complexes are formed. Cyclic voltammetric examination of the Pu:EDTA system at pH below the first hydrolysis (pH < 4.3) shows that EDTA forms a very stable complex with Pu(IV), with a reduction potential of $E_{1/2}$ = 342 mV (*vs* NHE). The resulting Pu(III)-EDTA complex undergoes rapid protonation and dissociation within the pH range examined. The measured potentials also indicate that in acidic media and in the presence of O_2, EDTA will promote the oxidation of Pu(III) to Pu(IV). This oxidation is due to the difference in the EDTA affinity for Pu(IV) relative to Pu(III) as described by Nernst equation applied to this system.

In the presence of excess EDTA relative to Pu(IV), Pu(IV)-$(EDTA)_2$ is formed at neutral pH ($\log\beta_{120}$ = 35.39). In the presence of additional ligands like citrate or carbonate, mixed complexes Pu(IV)-EDTA-citrate ($\log\beta_{120}$ = 33.46) and Pu(IV)-EDTA-carbonate ($\log\beta_{120}$ = 35.51) are formed. The composition of the inner coordination sphere around Pu is probably very similar in all these complexes, where the remaining aquo ligands in the Pu(IV)-EDTA complex are displaced by the incoming ligand. Examination of these complexes by cyclic voltammetry reveals irreversible behavior (Figure 2).

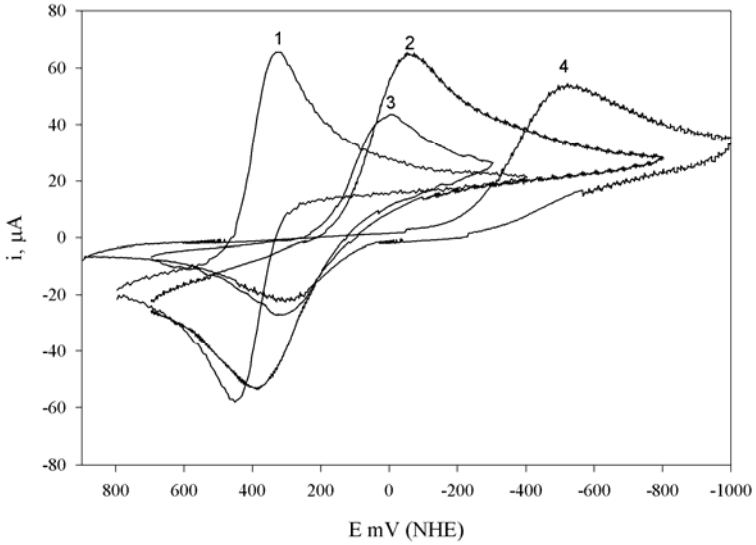

Figure 2. Cyclic voltammograms of 2.05 mM Pu(IV):EDTA solution with addition of 1 equivalent of either EDTA, citrate or carbonate. Curve (1) Pu(IV)-EDTA at pH 2.3, Curve (2) Pu(IV)-$(EDTA)_2$ at pH 6.75, Curve (3) Pu(IV)-EDTA-citrate at pH = 7.8, Curve (4) Pu(IV)-EDTA-carbonate at pH 8.2.

The reduction wave is shifted by about -400 mV as compared to the reduction wave of the complex Pu(IV)-EDTA, while the oxidation wave is observed at the same potential for all complexes. These results agree with the potentiometric and spectrophotometric results, suggesting that the Pu(IV) inner coordination sphere is more electron rich for the mixed-ligand complexes than the initial Pu(IV)-EDTA complex. The non reversible CV indicates that Pu(IV) can accommodate a higher coordination number from oxygen donor groups than can Pu(III).

Our results show that EDTA effectively stabilizes Pu(IV) discriminating Pu(III). The stability of the Pu(IV) EDTA complexes relative to other oxidation states depends on the pH and Pu:EDTA ratio. In excess EDTA over Pu(IV) the complexes formed have a 2:1 EDTA:Pu(IV) stoichiometry. In the presence of competing ligands like carbonate or citrate the addition of one ligand to Pu(IV)-EDTA

has been shown. Ternary Pu(IV)-EDTA-L complexes are formed when EDTA, OH-, citrate, carbonate are present at sufficiently high concentration. The formation constants for Pu EDTA species (Pu(IV)-EDTA, Pu(IV)-$EDTA_2$, Pu(IV)-EDTA-L (L = citrate or carbonate), Pu(IV)-EDTA(OH) , Pu(IV)-$EDTA(OH)_2$ have been determined and can now be included in thermodynamic databases and used to help predict the fate and transport of actinides in the environment.

Plutonium-Siderophores chemistry*:*

Compounds that solubilize plutonium or change its oxidation state can significantly increase its bioavailability and mobility, and therefore interfere with long term in situ stabilization. We have examined the fundamental inorganic chemistry of actinides with microbial siderophores in order to understand how they could affect actinide biogeochemistry. Siderophores are low molecular weight, strong metal chelating agents produced by most microbes to bind and deliver iron into microbial cells via active transport systems. We have focused on the tri-hydroxamate siderophores desferrioxamine E and B (DFB and DFE, or generically, DFO) because they are the most-well studied siderophores and are readily available. Hydroxamate siderophores have been estimated to be present in 0.1-0.01 μM concentrations in soils.[3] The stability constant for the Pu(IV)-DFB complex formed at neutral pH has been estimated to be $\log\beta_{110} = 30.8$.[4] In addition, we have isolated and characterized a pyoverdin type siderophore produced by *P. putida* strain ATCC 33015 a soil bacterium and studied its iron(III) and Pu(IV) complexes.

We examined the redox chemistry of Pu(IV) with the natural siderophores pyoverdine(I) which contains mixed functionalities(hydroxamate, catecholate and carboxylic acid), desferrioxamine E(III) and B(II) rhodotorulic acid(IV) and the synthetic bidentate aceto hydroxamic acid(V).

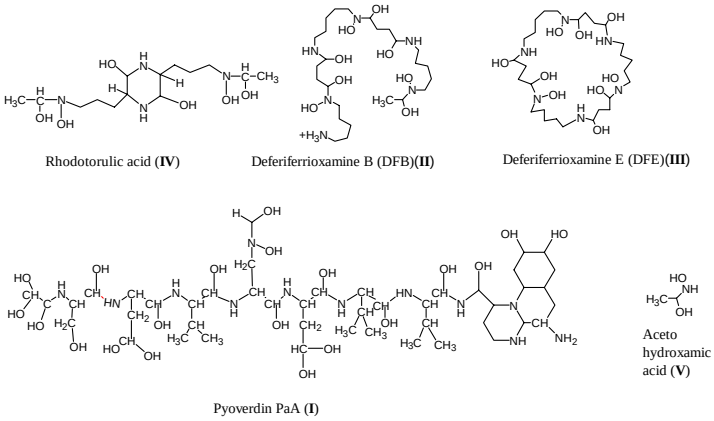

Figure ?. Structure of natural siderophores used.

We characterized the complexation of Pu(IV) ion by the natural siderophore desferrioxamine B (DFB) in aqueous solution was characterized by UV-visible spectroscopy and potentiometric measurements. The stability constants, complex protonation and hydrolysis constants and the formation of ternary complexes were determined for this system, thus providing accurate and full characterization of the Pu(IV) complexes formed in the presence of the natural siderophore desferrioxamine B. The initial complex Pu(IV)(H_2DFB) formed in acidic solution (pH < 1) undergoes a deprotonation reaction below pH 3 and hydrolyses at higher pH. The thermodynamic formation constants of the complexes Pu(IV)(H_2DFB) ($\log \beta_{211} = 34.78 \pm 0.20$) and Pu(IV)(HDFB) ($\log \beta_{111} = 33.79 \pm 0.93$) and the hydrolysis constant $\log K_{PuHDFB(OH)} = -5.94 \pm 0.2$ were determined from potentiometric and spectrophotometric titrations. In excess DFB over Pu(IV) a ternary complex Pu(IV)$(DFB)_2$ forms. The thermodynamic formation constant of the complex $\log \beta_{221} = 62.49 \pm 0.70$ was determined. The redox potential of the complex Pu(IV)$(DFB)_2$ was determined to be $E_{1/2} = -0.509$ V by cyclic volammetry. The redox potential of the complex Pu(IV)(DFB) was estimated to be $E_{1/2} = -0.269$ V by comparison with the reduction potentials of the ferric complex.

[3] P. E. Powell, G. R. Cline, C.P.P. Reid, P.J. Szanislo, *Nature* **1980**, *287*, 833-834.
[4] N. V. Jarvis, R. D. Hancock *Inorg. Chim. Acta* **1991**, *182(2)*, 229-232.

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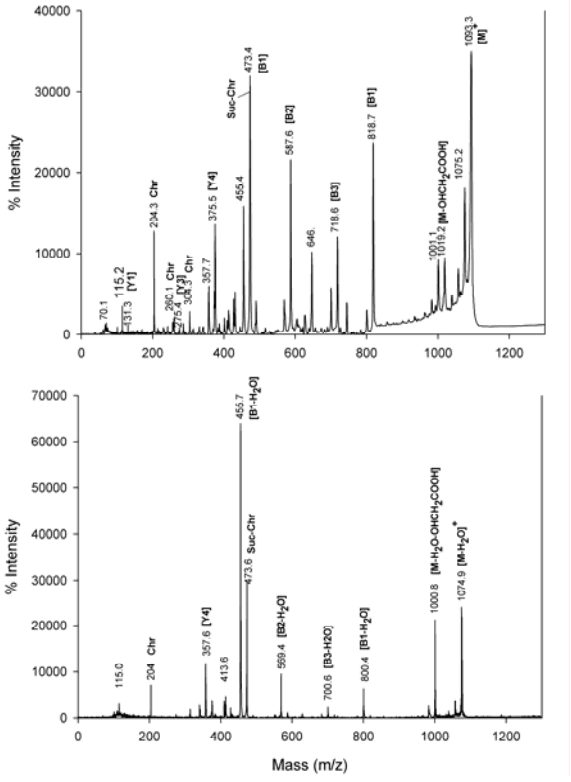
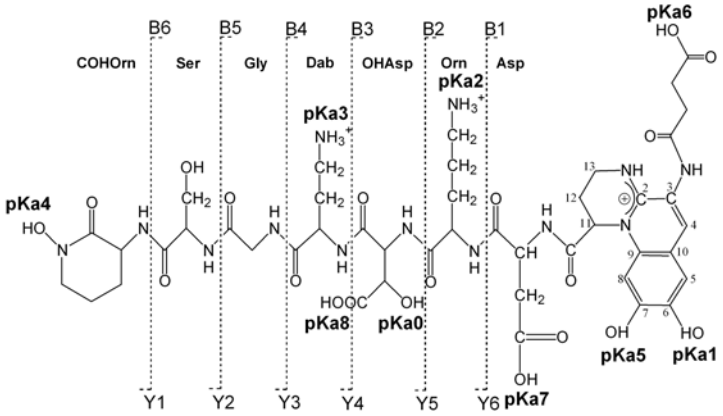
Pyoverdin purification and characterization: We have examined siderophore production by *Pseudomonas putida* strain ATCC 33015 grown in minimal succinate media. This micro-organism produces a green fluorescent siderophore under iron deficient conditions. Although *pseudomonas putida* reaches stationary phase growth after 24 h, siderophore production continues for over 2 weeks, and maximum siderophore production (60%) occurs in the first four days. Under our conditions of media composition and incubation duration, the amount of total siderophore siderophore produced in four days is about 300 mg/l. Analysis of the purified culture media by HPLC shows the production of three different siderophores, in which the main siderophore produced (MW = 1092) constitutes more than 70% of the total siderophore production. The separation of the main siderophore was achieved by preparative chromatography using a C18 reverse phase column. The pure siderophore fraction was eluted with 20%:80% v:v mixture of acetonitrile: 0.05 M acetate buffer solution at pH 5 containing 0.05 M pyridine.

The structure of pyoverdin was elucidated by mass spectrometry and NMR measurement performed on the pure product purified by HPLC. The siderophore fragmentation patterns obtained by MALDI PSD analysis(figure) was analyzed to determine the structure of the main siderophore(peak at m/z 1092). The mass spectrum of pyoverdin obtained by MALDI-PSD analysis shows a molecular ion peak at m/z = 1092.1, which is assigned to the parent molecule and a minor peak at M-18. The fragment peak observed at m/z 357, and assigned to the chromophore cleaved from the peptide chain, is followed by a peak at m/z = 473, which results from the addition of Asp, the first amino acid. The second amino acid is Orn, identified from the fragment peaks at m/z = 587. The peaks at m/z = 719 and 818 identify OHAsp and Dab as the following amino acids in the sequence. The remaining amino acids are identified from C-terminal fragments at m/z = 275 and 373. Their equivalent peaks in the ^{15}N labeled pyoverdin are shifted to a higher mass depending upon their nitrogen content.





The structure of pyoverdine (Figure), shows that this siderophore has a mixed functionalities, catecholate, hydroxamate and a carboxylic acid group. The binding groups bearing hard oxygen donor atoms are expected to enhance pyoverdin's blinding for hard metal ions like Fe(III) and Pu(IV).

Electochemical behavior of Pu(IV/III)-siderophore complexes. The cyclic voltammogram of Pu(IV/III) couple in 0.5 M HCl (shown in the previous section) shows a quasireversible behavior with $E_{1/2}$ = 935 mV(vs NHE), which is consistent with the formal potential for Pu(IV)-Pu(III) couple from literature data (953 mV, in 1 M HCl). We have shown that EDTA forms a strong complex with Pu(IV) and considerably less stable complexes with Pu(III). The cyclic voltammograms of Pu-EDTA show a quasi reversible couples with $E_{1/2}$ = 342 at pH 3.2. The shift in the redox potential of Pu(IV/III) upon EDTA binding (593 mV) is attributed to the differences in the affinity of EDTA toward Pu(IV) binding, which is attributed to the hard character of the oxygen donor groups on EDTA carboxylic acid binding groups. Similarly, siderophores are known for their hard oxygen donor groups character, favoring strong complexes formation with iron(III) and considerably less stable complexes with iron(II). The differences in siderophores binding for Fe(III)-Fe(II) result in redox potentials for Fe(III/II)-siderophore complexes in the range –350 to –700 mV depending on the structure of the siderophore. Pu(III)-siderophore complexes are considerably less stable than Pu(IV)-siderophore complexes. The cyclic voltammograms of Pu-siderophore complexes measured when one equivalent of siderophore is added to a solution of containing Pu(IV) shows an irreversible behavior. A reduction wave is observed around –200 mV(NHE) without its reoxidation counter part. The presence of excess siderophore is necessary to obtain a reversible voltammograms. The cyclic voltammograms of Pu(IV) complexes with the natural siderophores desferrioxamine E and B (DFB and DFE), pyoverdin, rhodotorulic acid and the synthetic aceto hydroxamic acid recorded in large excess of siderophore at neutral pH are shown in Figure (Figure**).** For comparison purposes, cyclic voltammograms of Pu-ferrioxamine B, Ferrioxamine E, Rhodotorulic acid, and Acido hydroxamic acid were also recorded under similar conditions. In all cases excess siderophore was used for cyclic votametry measurements. The redox potentials of the iron-siderophores and Pu-siderophore complexes shown in Table ? indicate that Pu-siderophore complexes undergo redox changes at potentials slightly lower than the iron complexes.

Most siderophores are hexavelent, and a single molecule ensures full iron coordination. The tetra valent siderophores like rhodotorulic acid were a single molecules can not satisfy full iron coordination, complexes with a 2:3 Fe:Siderophore stoichiometry are formed. Pu which can

accommodate a higher coordination number forms complexes with a 1:2 Pu:siderophore stoichiometry. In excess siderophore over Pu the formation of such complexes is favored over the mononuclear complexes1:1 Pu:siderophore. We have shown that in excess EDTA over Pu the formation of $Pu:EDTA_2$ complexes was favored around neutral pH and excess EDTA. The direct consequence of the formation of such complexes is the negative shift of the redox potential of the complexes formed. The cyclic voltammograms shown here, which were recorded in large excess siderophore concentration are assigned to Pu-siderophore complexes with a 1:2 Pu siderophore stoichiometry.

Table. Redox Potentials of Pu-Siderophore and Fe-Siderophore complexes.

Plutonium Complex	E1/2 mV NHE	Iron Complex	E1/2 mV NHE
Pu(IV/III)	0.953	Fe(III/II)	0.740
Pu(IV/III)-EDTA	0.342	Fe(III/II)-EDTA	- 0.134
Pu(IV/III)AHA	-	Fe(III/II)AHA	- 0.293
Pu(IV/III)Ferrioxamine B	-0.509 ± 25	Fe(III/II)Ferrioxamine B	- 0.482
Pu(IV/III)Ferrioxamine E	-0.550 ± 25	Fe(III/II)Ferrioxamine E	- 0.481
Pu(IV/III)Rhodotorulic acid	-0.455 ± 25	Fe(III/II)Rhodotorulic acid	- 0.422
Pu(IV/III)Pyoverdin	-0.440 ± 25	Fe(III/II)Pyoverdin	- 0.367 ± 15

These results show that siderophores effectively stabilizes Pu(IV) over Pu(III). In excess siderophores over Pu(IV) the complexes formed have a 1:2 Pu(IV):siderophore stoichiometry. These complexes are highly soluble and can promote Pu accumulation by micro-organisms.

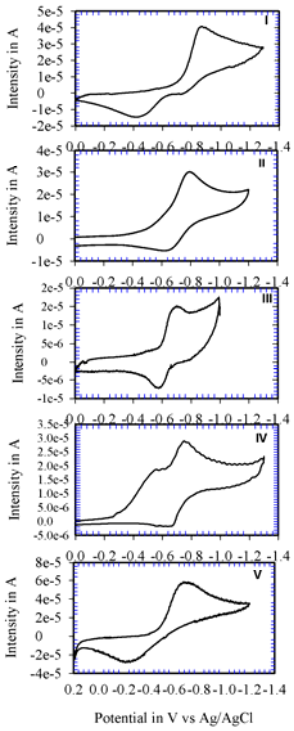


Figure ?. Cyclic voltammograms of 1.00 mM Pu(IV)in the presence of large excess siderophore. Curve (1) Pu(IV)-$(DFE)_2$ at pH 9.?? Curve (II) Pu(IV)-$(DFB)_2$ at pH ??, Curve (3) Pu(IV)-$(pyoverdine)_2$ at pH = ???, Curve (4) Pu(IV)-$(Rhorotorulic\ acid)_2$- at pH 8.2, Curve (V) Pu(IV)-$(AHA)_4$- at pH ???,

Siderophore-Actinide and Hydroxamate-Actinide Redox chemistry:

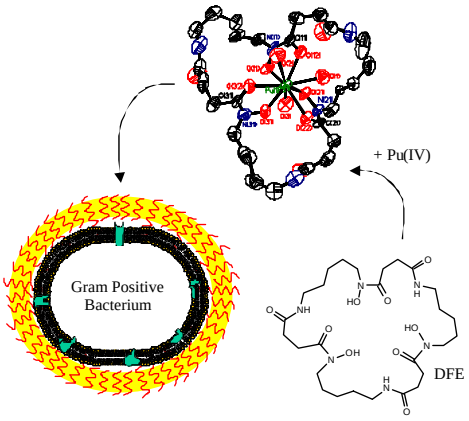
URANIUM: Using optical absorbance spectroscopy, cyclic voltammetry, and NMR we have determined the effect of the siderophores DFB and DFE on the oxidation state of U and Pu. When one equivalent of DFB is added to a solution of uranyl carbonate at neutral pH the optical spectrum changes; the local absorbance maximum changes from 425 nm (carbonate complex) to 378 nm (DFB complex) and the absorptivity increases. There is no evidence of reduction to U(IV). Cyclic voltammograms of the aqueous U(VI) DFB complex shows a quasi reversible wave at +0.060 V vs NHE corresponding to a U(VI/V) DFB couple and an irreversible wave at –1.18 V vs NHE corresponding to reduction to U(IV). These results confirm the thermodynamic stability of the U(VI) DFB complex and the electrochemistry results compare well with those determined for U(VI) acetate given the difference in corresponding solution complex formation constants.

PLUTONIUM: Siderophores that contain hydroxamic acids will have dramatic effects on Pu redox state, dependent on the concentration of the siderophore and the pH of the environment. Plutonium redox chemistry is generally very complicated. Because of the great thermodynamic stability of Pu(IV) DFO complexes, the overall behavior of Pu at near-neutral pH is simplified—regardless of the initial Pu oxidation state, the Pu(IV) DFO complex is formed. If Pu(III) is mixed with DFB or DFE in air, the Pu(IV) complex quickly forms. At pH >6, DFE, DFB, and even simpler hydroxamic acids (such as acetohydroxamic acid) rapidly and irreversibly reduce Pu(VI) to Pu(IV). The final form depends on the amount of chelator remaining in solution [Pu(IV)-chelator complex when excess chelator is used vs. Pu(IV) hydroxides when the chelator is used up]. At pH= 2 to pH = 6, all these hydroxamic acids (DFO, AcHa) rapidly reduce Pu(VI) to Pu(V), and eventually to Pu(IV). Up to 12 equivalents of Pu(VI) can be reduced to Pu(V) per DFE/DFB, so long as there is greater than 1 equivalent DFO per six equivalents plutonium. The higher the ratio of DFO to plutonium, the faster both reduction steps occur. Both reduction steps are pH and [DFO] dependent. Excess DFO in solution acts as a thermodynamic driving force for the formation of Pu(IV)-DFO from the Pu(V). There is no apparent binding of DFB to Pu(V), even when the ligand is in 1000x excess. At a ratio of 1 equivalent DFO to 12 equivalents plutonium, the initial reduction to Pu(V) takes over an hour. Up to 4 equivalents of Pu(VI) can be reduced to Pu(V) with acetohydroxamic acids. For both these reactions, the ratio is 4e-/hydroxamate moiety. At pH < 2, the reaction of Pu(VI) with DFO or AcHa proceeds to a mixture of Pu oxidiation states, including Pu(III); it appears that Pu(IV) reduction to Pu(III) is faster than other reductions steps. Based on optical absorbance, GC, and NMR spectroscopy, it appears that the hydroxamate moieties are oxidized and cleaved during the Pu(VI) reduction in a reaction with some similarities to reduction of actinides by hydroxylamines. When Pu(VI) is added to a solution of the Pu(IV)-DFB or DFE complex at pH > 2, the Pu(VI) is also rapidly reduced, despite the fact the DFO is already complexed to the Pu(IV). The reaction is slower than the reaction without Pu(IV) initially present. As expected, the Pu(VI) reduction by Pu(IV)DFO is faster at pH = 9 than at pH = 2; we would expect it to be faster due to the stronger binding of the DFO ligand to Pu(IV) at higher pH. Variable temperature NMR indicates that the Pu(IV)-DFO complexes are highly fluxional, and may involve equilibrium with free DFO, which would allow for DFO interaction with and reduction of the Pu(VI).

Actinide-Siderophore uptake into bacteria:

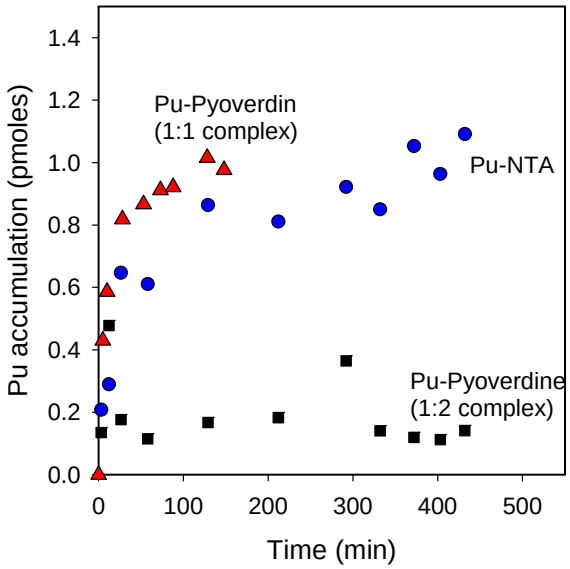
Once the formation, prevalence, and structure of the Pu(IV)DFO complexes were established, we studied their microbial uptake. Fe(III)-DFB and Pu(IV)-DFB complexes, but not U(VI)-DFB, are taken

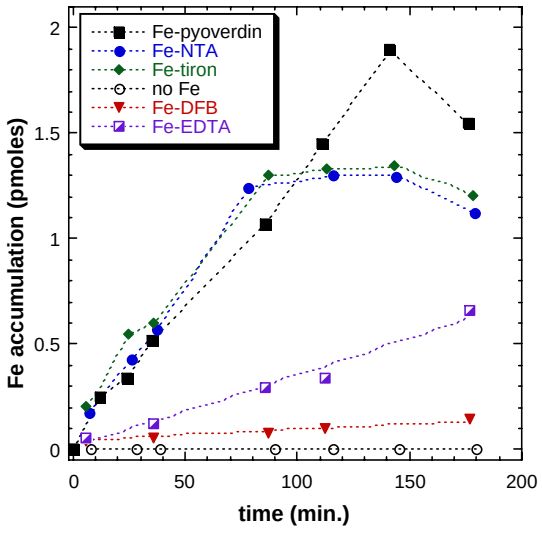
up by *Microbacterium flavescens* (JG-9).[5] We know the process is siderophore mediated, and not surface biosorption, because the siderophore complexes were taken up, but corresponding metal-nitrilotriacetic(NTA) acid complexes were not. (In addition, cells were washed with concentrated EDTA solutions to remove any loosely-bound or otherwise accessible metal or metal-complex). We discovered that only living, metabolically-active bacteria were capable of taking up the Pu siderophore complexes, just as with Fe-siderophore uptake (represented in the scheme below). Our studies also show that the two complexes (Pu(IV)-DFB and Fe(III)-DFB) mutually inhibit the uptake of the other, indicating that they compete for the same binding or transport sites in the microbe. Based on our results, we hypothesize that DFO-B mediated Fe uptake involves translocation of Fe into the interior of the cell. Pu accumulation appears to be, at least in large part, the result of surface binding at the uptake receptor. This accounts for its rapid dissociation from the cell when Fe-DFO-B is added. The fact that Pu-DFO-B competes with the same uptake receptors as Fe-DFO-B indicates that the Pu-DFO-B complex bears enough homology to the Fe-DFO-B complex to be recognized by the uptake channel receptors on the outside of the cell. However, the structural similarity does not appear to be enough to facilitate large amounts of Pu uptake into the cell interior.



Having demonstrated and quantified the microbial uptake of Pu-hydroxamate siderophore complexes, we have begun to determine if this phenomenon is general.

We examined the capacity of pyoverdin, ferrioxamine B and several synthetic iron chelator(NTA, tiron and EDTA) to mediate Fe(III) and Pu(IV) transport in *P. putida* by following the iron and plutonium uptake rate as function their chelate forms. We found that *P. putida* is able to acquire iron from all the chelate forms used (see figure ?). However, the amounts of iron accumulated by the cells depend on the chelator's affinity for iron. Pyoverdin, the siderophore produced by *P. putida* shows the highest accumulation levels in contrast with ferrioxamine B which is the least efficient in mediating iron transport. The dependence of iron accumulation by the cells with the ligand affinity for iron





[5] John, S.G., et al., *Siderophore Mediated Plutonium Accumulation by Microbacterium flavescens (JG-9).* Environmental Science and Technology, 2001. **35**(14): p. 2942-2948

suggests that the chelators release iron to pyoverdin which mediates its transport inside the cell. High iron accumulation level are observed for NTA and tiron which have the lowest affinity for iron, and are expected to readily release iron to pyoverdin in agreement with our interpretation. This is a critical point for metal uptake by microorganism since it shows that siderophores can mobilize metal irons for bacterial uptake through chemical exchange reactions.

Similarly, we found that pyoverdin mediates Pu(IV) uptake by *P. Putida* at rates similar to iron accumulation. We also observed Pu accumulation by *P. Putida* from Pu-NTA, which has a lower affinity for Pu(IV) binding and is expected to exchange Pu(IV) to pyoverdin. Our competition experiment performed by simultaneous addition of Pu-NTA and iron-NTA shows that iron and plutonium are taken up efficiently and simultaneously by the cells. This result suggest that Pu and iron are transported inside the cell with the same efficiency. Our studies of Pu uptake by *Microbacterium flavescens* (JG-9) and *P. putida* show that plutonium-siderophore complexes translocation across the cell membrane is as efficient as iron transport.