

Subsurface bio-mediated reduction of higher-valent uranium and plutonium

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Received 12 March 2007; received in revised form 15 May 2007; accepted 4 June 2007

Available online 12 June 2007

Abstract

Bio-mediated reduction of multivalent actinide contaminants plays an important role in their fate and transport in the subsurface. To initiate the process of extending recent progress in uranium biogeochemistry to plutonium, a side-by-side comparison of the bioreduction of uranyl and plutonyl species was conducted with *Shewanella alga* BrY, a facultative metal-reducing bacterium that is known to enzymatically reduce uranyl. Uranyl was reduced in our system, consistent with literature reports, but we have noted a strong coupling between abiotic and biotic processes and observe that non-reductive pathways to precipitation typically exist. Additionally, a key role of biogenic Fe²⁺, which is known to reduce uranyl at low pH, is suggested. In contrast, residual organics, present in biologically active systems, reduce Pu(VI) species to Pu(V) species at near-neutral pH. The predominance of relatively weak complexes of PuO₂⁺ is an important difference in how the uranyl and plutonyl species interacted with *S. alga*. Pu(V) also led to increased toxicity towards *S. alga* and is also more easily reduced by microbial activity. Biogenic Fe²⁺, produced by *S. alga* when Fe(III) is present as an electron acceptor, also played a key role in understanding redox controls and pathways in this system. Overall, the bioreduction of plutonyl is observed under anaerobic conditions, which favors its immobilization in the subsurface. Understanding the mechanism by which redox control is established in biologically active systems is a key aspect of remediation and immobilization strategies for actinides when they are present as subsurface contaminants.

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Keyword: Actinide alloys and compounds

1. Introduction

The role and importance of redox reactions in determining actinide subsurface mobility are beyond question. In the subsurface, redox control is often established by the iron mineralogy and associated aqueous chemistry [1,2]. There is also a growing recognition of the important role microbiological processes have in defining the redox chemistry of multivalent actinide species [3,4] by both direct and indirect means. The mechanisms by which redox control is established are a key aspect of remediation and immobilization strategies for actinides when they are present as subsurface contaminants.

The important effects of redox-active minerals (e.g. iron and iron oxides) and microbial processes on subsurface redox processes are not mutually exclusive [5]. Metal-reducing bacteria often modulate the oxidation state of aqueous iron species and can reduce iron(III) phases to increase the overall solubility of iron in environmentally relevant systems. From the perspective of actinide chemistry, solubilized reduced iron and reduced iron phases will compete, in some cases very effectively, with direct enzymatic reduction pathways.

It is this coupling of biological and geochemical processes, and correspondingly its effect on actinide speciation, that is the focus of our current studies. For uranyl systems, where much more data exist in the literature, we provide the general status of microbiological effects and some insight into abiotic pathways that can also result in the precipitation of uranium phases. Much less is known, however, about microbiological effects on pluto-

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nium speciation. Since higher-valent plutonium is more easily reduced than uranium, the expectation is that microbial systems that reduce higher-valent uranium will also reduce plutonium. To provide a comparison of the uranyl and plutonyl microbial system, we have investigated the bioreduction of these two actinides under both aerobic and anaerobic conditions with *S. alga BrY*, which is a facultative metal-reducing bacterium that is representative of the genus *Shewanella*; a family of soil bacteria that are common to many subsurface environments.

1.1. Bioreduction of higher-valent actinide species

That microbial activity, especially under anaerobic conditions, can reduce higher-valent actinides is well established, although the mechanism is not yet well understood. This is a key and important subsurface process that adds to, but is not sufficient for, the argument that natural attenuation is a defensible containment strategy for existing DOE contamination sites. In particular, questions about the coupling of abiotic and biotic processes as well as the longevity and permanence of the microbiological processes, still remain. These key questions continue to be the focus of DOE research program areas [6], and some general reviews on this subject have been published [7–9].

By far the most studied higher-valent actinide, with respect to bioreduction, is uranyl which exists as UO_2^{2+} inorganic/organic complexes when present as a contaminant in natural systems [10–15]. Direct enzymatic reduction of uranyl under anaerobic conditions has been demonstrated for wide variety of bacteria including the genera *Shewanella*, *Desulfovibrio*, and *Geobacter*. This generally occurs through a dissimilatory process that leads to cell growth but is, in general, not a well-understood mechanism. Enzymes (reductases) have been identified that can reduce uranyl and this reaction is a key step in the overall reduction mechanism.

In our research [8,4,16–18], we also have shown that neptunyl, as NpO_2^+ , is reduced in a wide variety of anaerobic systems. Methanogens and sulfate reducers actively precipitate neptunium from solution, and this was confirmed to be due to bioreduction to form Np(IV) precipitates by XANES analysis. For consortia, neptunium precipitation was most rapid when cultures were supplemented with hydrogen, rather than organic substrates, as the electron donor. For pure cultures, the rate of precipitation was linked to metabolic activity but, unlike the uranyl system, we could not demonstrate direct enzymatic pathways. The exact mechanism for this reduction process is, for this reason, the subject of further study.

In contrast, there has been very little published on the effects of microbiological activity on higher-valent plutonium species. Pu(VI) and Pu(V) are higher-valent plutonium species which exist as a wide variety of PuO_2^{2+} and PuO_2^+ inorganic/organic complexes when present as a contaminant in natural systems, e.g. subsurface groundwaters. By analogy with the neptunyl and uranyl systems, it is also the expectation that bioreduction of plutonyl will prevail under biologically active anaerobic systems.

1.2. Facultative metal-reducing bacteria

Metal-reducing bacteria play a key role in the subsurface since, as facultative bacteria, they are key players in the aerobic to anaerobic transition zones that are critical in defining the likely mobilization or immobilization of multivalent metals such as actinides. From a redox perspective, microbial processes under anaerobic conditions will likely reduce most higher-valent actinides. These microbes also play a key role in the solubilization and production of reduced iron, as Fe(II) phases or aqueous Fe^{2+} , which are also known to have a critical role in defining the subsurface speciation of multivalent actinides.

The genus *Shewanella* has recently received much attention as a fairly ubiquitous facultative metal-reducing genus [19]. This genus displays a wide-ranging respiratory versatility and can respire on oxygen, nitrate, nitrite, oxidized Mn and Fe, sulfite, thiosulfate, and higher-valent actinides. This generally occurs by coupling with the oxidation of hydrogen or organic carbon since many key organic species are typically present as complexants that co-exist with actinide contaminants. Under anaerobic conditions, enzymes (reductases and hydrogenases) are generated and have been shown to enable *Shewanella* to enzymatically reduce metals. *Shewanella* is especially adept at solubilizing iron from iron oxide minerals to derive energy, which is a very important process at near-neutral pH where Fe^{3+} is sparingly soluble. Most recently, long-range electron transfer capability has been demonstrated in extra-cellular polymeric substrates (EPS), including with nanowires, that are associated with many *Shewanella* species adding to the ability of these bacteria to facilitate electron transfer reactions [21]. Understanding the nature of the redox reactions that take place in the EPS may be key to the bioreduction processes observed for higher-valent actinides.

2. Experimental approach

Uranium, as a UO_2^{2+} stock in 0.1 M hydrochloric acid, was prepared by converting uranyl nitrate hexa-hydrate (Spectrum, >98% purity by mass) to the oxide by heating in a furnace to 600 °C and dissolving the oxide in reagent grade hydrochloric acid. Plutonium (99% Pu-242 by mass), was oxidized to PuO_2^{2+} by fuming in perchloric acid [1,20] and dissolved in 0.1 M hydrochloric acid. PuO_2^+ was synthesized by adding Pu(VI) to 0.1 M PIPES at pH ~ 7 and letting it sit overnight leading to essentially complete reduction to Pu(V) . Oxidation state purity was confirmed by absorption spectrometry and was >99% for Pu(VI) and >95% for Pu(V) . These uranyl and plutonyl stock solutions were the initial species added to the various buffered growth media or groundwater simulants (pH ~ 7) and did not cause a change in the pH. Aqueous iron(III), prepared by dissolving ferric oxide (99.999% Sigma–Aldrich) in 0.1 M hydrochloric acid, when present, was added as a stabilized Fe^{3+} -NTA complex. Lactate, as sodium L-lactate (98%, Sigma) was used as the electron donor. Total uranium and iron concentrations were determined by ICP-MS (Agilent Model 7500ce) specially configured for iron analysis. Plutonium concentration was determined by liquid scintillation counting (Beckman-Coulter LS 6500). Lactate was analyzed using a lactate reagent and standards kit (Trinity Biotech) based on the colorimetric technique as recommended by the manufacturer. The media used are given in Table 1 and are based on the media used by Caccavo et al. [11]. NTA and other organics were analyzed by ion chromatography (Dionex DX 500). Absorption spectrometry (CARY 500) was used to establish and confirm the actinide and iron oxidation states. An HDEHP extraction technique [22] was modified to work in *n*-heptane with 0.5 M HCl acid in the forward extraction step (for Fe^{2+}) and 3.95 M HCl acid for the back extraction (for Fe^{3+}) and combined with ICP-

Table 1
Composition of Fe-lactate growth media for *S. alga*

Aqueous species	Minimal media (mM/L) ^a	Fe-lactate growth (mM/L)
Na ⁺	30.10	60.10
K ⁺	12.36	12.36
NH ₄ ⁺	11.22	11.22
Fe ³⁺	~0	10.0
HCO ₃ ⁻ /CO ₃ ²⁻	29.76	29.76
Cl ⁻	13.14	13.14
PO ₄ species	11.02	11.02
NTA species	0.0545	10.05
Lactate species	~0	20.0
Mg, Mn, sulfate, Ca, Co, Zn, Cu, Al, borate, Mo, Ni, W	Trace level	Trace level

^a The macro media consisted of the sodium, potassium, ammonium, phosphate and chloride as specified in the minimal media above.

MS analysis to track oxidation state changes in the iron. This approach was used due to interference in the analysis of Fe³⁺ by NTA complexation in the more commonly used Ferrozine analytical approach [23] and our desire to analyze both Fe²⁺ and Fe³⁺ directly in our experiments.

The metal-reducing bacteria used in the bioreduction experiments was *S. alga* strain BrY [11]. This is a dissimilatory, Gram-negative, iron-reducing bacterium that was isolated from the Great Bay Estuary, New Hampshire. It is a facultative anaerobe that was grown aerobically in tryptic soy broth (TSB) prior to use in aerobic experiments [11]. Cells were harvested in log phase, rinsed in 0.1 M PIPES at pH 7, concentrated by centrifugation, and then re-suspended to prepare an inoculum stock prior to use. For anaerobic experiments, the cells were grown anaerobically in TSB for the Fe³⁺ reduction studies to minimize lag time or, in the Pu and U containing experiment, with air-added growth media in a negative pressure anoxic glovebox (MBraun Labmaster 130 with a re-circulating copper shaving oxygen purification system) prior to inoculation into de-aerated growth media. Air had to be added to the sealed vessels to help transition to anaerobic growth because *S. alga* would not grow when washed cells were placed in the strictly oxygen-free system present in our glovebox. All anaerobic experiments were maintained in the anoxic glovebox in the dark, in sealed screw-top glass vials, and sampled in the nitrogen atmosphere. Under the conditions of our experiments, Fe²⁺ was stable for over 4 months (<1% oxidation to Fe³⁺) and U⁴⁺ species were generated and stable for at least a period of weeks as confirmed by XANES analysis. Cell free and heat-killed cell blanks were run as abiotic and biologically inactive controls. Replicate or triplicate experiments were run in most cases. Cell viability was established by plating the cells onto TSB agar plates under aerobic conditions and counting sequential dilutions. Speciation modeling, when performed, was done using CCBATCH [24].

3. Results and discussion

The key interactions between the uranyl and plutonyl system with *S. alga* for aerobic and anaerobic growth conditions are compared.

3.1. Abiotic reduction of U(VI), Pu(V) and Pu(IV)

Higher-valent uranium and plutonium species can be stable towards reduction for relatively long periods of time in the absence of reducing agents and at near-neutral pH [1,20]. They also can persist for a fairly long time (weeks) as meta-stable species in the aqueous phase at apparent solubilities that are much higher than their true solubility. Dissolved uranium, in environmental systems, exists primarily in two oxidation states: U(IV) as sparingly soluble U⁴⁺ complexes (typically nanomolar range), and U(VI) as moderately soluble UO₂²⁺ complexes (typically micromolar range). The U(V) oxidation state exists as a

transient but it cannot be readily observed or stabilized in aqueous systems. In contrast to this, plutonium can exist as aqueous species in four oxidation states in environmental systems: Pu(III) as Pu³⁺ complexes, Pu(IV) as sparingly soluble Pu⁴⁺ complexes and colloids, Pu(V) as relatively soluble PuO₂⁺ complexes, and Pu(VI) as moderately soluble PuO₂²⁺ complexes. All of these oxidation states can be readily made and stabilized in the laboratory although in environmental systems, it is expected that the Pu(IV) and Pu(V) species will predominate.

There are primarily two abiotic pathways that could lead to the loss of uranium from solution in the growth media: (1) precipitation reactions to form U(VI) phases, and (2) abiotic reduction by reducing agents, e.g. reduced iron or ammonium. The solubility of UO₂²⁺ in the pH range of our growth experiments (pH 6–8) ranges between 3 and 10 μM in the absence of inorganic and organic complexants. In this context, there are a number of precipitation pathways that are possible if the initial concentration of uranyl is above this concentration (e.g. stabilized by a complexant). Changes in speciation during the growth experiments due to the biodegradation of organic complexants (e.g. lactate or NTA) can promote the precipitation of uranium from solution as a U(VI) phase even though the uranyl is stable towards precipitation in the unreacted growth media.

The stability of uranyl in the individual components of the growth media, at pH 7, was determined to establish potential abiotic pathways. These results are shown in Fig. 1. In the full experimental medium, uranium was stable at 10 μM. The removal of Fe³⁺, however, led to a significant destabilization of uranyl to an effective solubility of 0.2 μM. This instability was attributed to phosphate precipitation in the single-component studies we performed. The presence of phosphate in this case lowered the effective solubility of uranyl to levels below that observed in water.

The reduction of uranyl by divalent or zero-valent iron is also a key abiotic process that can lead to the precipitation of U(IV) phases. The very high sensitivity of U⁴⁺ species to re-oxidation by trace oxygen levels makes the complete removal of oxygen essential. We observed the reduction of U(VI) by zero-valent iron in simulated groundwater at pH 6.8. This was, however, a very slow process that occurred over a timeframe of months. The reaction of Fe²⁺ with uranyl at near-neutral pH is the subject of ongoing studies but is expected to be relatively rapid. In

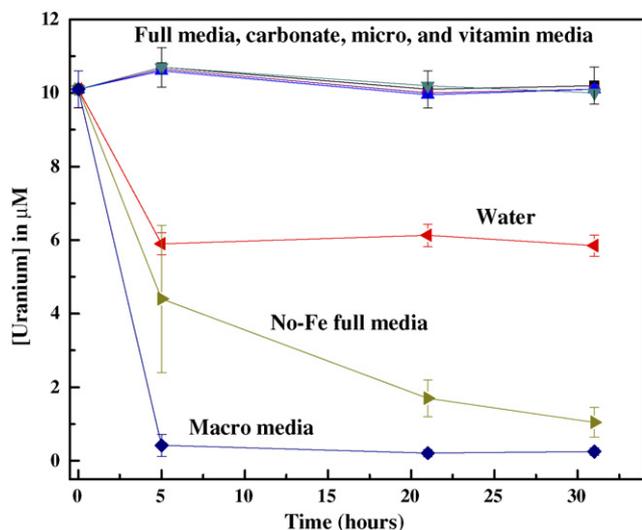


Fig. 1. Abiotic stability of uranium in the various components of the *S. alga* growth medium. Uranium was stable in the full medium, when carbonate was present, and when only trace metals and trace vitamins were present. U(VI) precipitation was noted in water (no complexants), the full media without Fe^{3+} , and in the macro media (ammonium, phosphate and potassium/sodium chloride).

the *S. alga* system Fe^{2+} is produced biogenically under anaerobic growth conditions from the Fe^{3+} initially present in our system. Uranyl added to the experiment after anaerobic growth of *S. alga* in the presence of Fe^{3+} as the electron acceptor was instantaneously reduced and precipitated, presumably as a U(IV) phase.

There are also a number of abiotic pathways towards reduction for higher-valent plutonium in environmental systems. Plutonium(V) and plutonium(VI) species are both more easily reduced than uranyl. Plutonium(VI), which exhibits similarly strong complexation, hydrolysis, and interaction tendencies analogous to uranium(VI) species, is reduced by organic species and acids that typify biologically active systems. The reduc-

tion of Pu(VI) by oxalate is shown in Fig. 2. In the absence of oxalate, Pu(VI) is stable as a hydrolytic species for weeks. This relatively rapid redox reaction is also observed for citrate, NTA, lactic acid, succinic acid, and acetic acid for not only Pu(VI) but Np(VI) as well. U(VI), under the same conditions, is not reduced by these organic complexants. This reduction by organics is also seen in brine systems over a broad pH range [25]. In biologically active systems, for these reasons, it is difficult to argue that Pu(VI) species will have a key and important role. In this context, the predominant higher-valent plutonium species is expected to be the Pu(V) species, which will have a chemistry that is much more analogous to Np(V) than U(VI). Plutonium(V), as the PuO_2^+ species, is relatively non-complexing (hydrolysis does not start until $\text{pH} > 8$) and will, in this context, be much less strongly complexed in near-neutral systems and is likely to be much more interactive with the metabolism of *S. alga*.

Higher-valent plutonium is also reduced by both aqueous Fe^{2+} , Fe(0), and Fe(II) oxides. Stabilized Pu(VI) and Pu(V) are reduced to Pu(IV) by zero-valent iron and Fe(II)-containing iron oxide phases [1] over a wide range of pH. This reduction process can be influenced, and usually slowed, by complexation effects. In the presence of reduced iron, essentially complete reduction was typically observed on a timeframe of less than 100 h. The most rapid reduction rate was observed with magnetite and attributed to the presence of Fe(II) in the oxide. At $\text{pH} \sim 1$ or lower, Pu(VI) and Pu(V) are rapidly reduced by aqueous Fe^{2+} to form aqueous Pu(III) species. This process is slowed by increased pH due to complexation. The reaction of Fe^{2+} with Pu(VI) is shown in Fig. 3 at $\text{pH} \sim 3$. The reduction to Pu(V) is instantaneous with a slower reduction to a stable Pu(III) species. At pH 7, the reduction of Pu(V) occurs over a timeframe of minutes rather than hours and leads to the formation of Pu(IV) precipitates rather than aqueous Pu(III) species as the final product. A more detailed study of this reduction process at pH 5–10 is in progress.

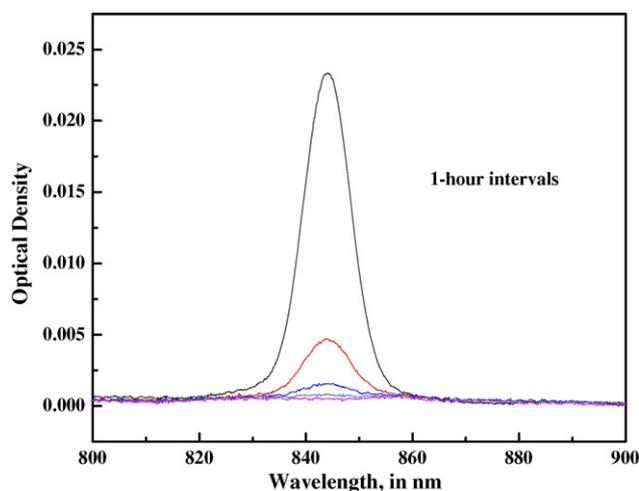


Fig. 2. Absorption spectrum of PuO_2^{2+} with oxalate at $\text{pH} \sim 6$ as a function of time. Oxalate reduced PuO_2^{2+} in a 4 h timeframe to form the PuO_2^+ oxalate complex.

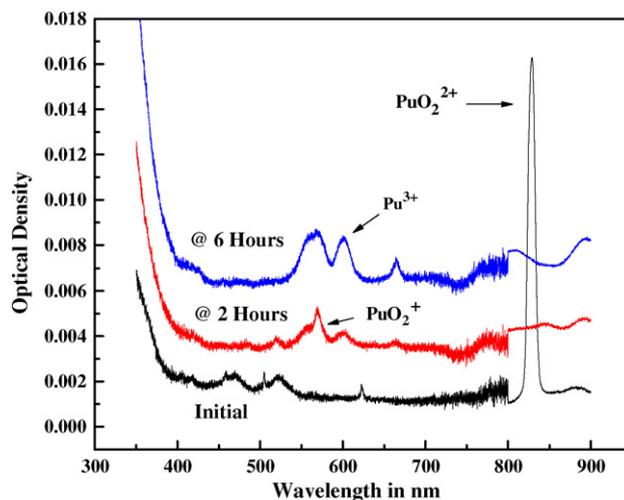


Fig. 3. Plutonium absorption spectra as a function of time showing the reduction of PuO_2^{2+} by aqueous Fe^{2+} . In the presence of excess Fe^{2+} , plutonium(VI) is reduced to Pu(III).

3.2. Toxicity of the uranyl and plutonium system towards *S. alga*

The toxicity of low-activity actinide isotopes, such as the U-238 and Pu-242 isotopes used in our experiments, is predominantly chemical, not radiolytic (i.e. due to radiolysis of the emitted alpha particle) [25]. Uranium, in both the oxidized UO_2^{2+} and reduced U^{4+} forms, is highly hydrolyzed at neutral pH and there is no significant amount of aquo species present. In our system, there are also organic complexants (e.g. NTA and lactate) and inorganic complexants (phosphate and carbonate/bicarbonate) present. Speciation calculations show the uranyl to primarily exist as an NTA complex at pH 7 in our growth medium. This will shift to a predominantly hydroxide and phosphate complex once uranium is reduced to U(IV). For all conditions investigated in our uranium–*S. alga* system, even in the absence of inorganic and organic complexants, no chemical toxicity was noted based on plate counts.

Strong chemical toxicity was, however, noted in the plutonium–*S. alga* system. As discussed in Section 3.1, the predominant form of higher-valent plutonium in our system was aquo PuO_2^+ , rather than PuO_2^{2+} , because of rapid reduction by the organics present. Unlike uranyl, and by analogy a strongly hydrolyzed PuO_2^{2+} , the PuO_2^+ species is much more weakly complexed, and significant amounts of the aquo species could be present in near-neutral systems. The fractional survival of *S. alga* is shown in Fig. 4 as a function of the initial concentration of PuO_2^+ and time for cells that were suspended in 0.1 M PIPES buffer at pH 7 under aerobic conditions. Cell viability, was determined by plate counts (TSB agar plates) as a function of time. For the higher plutonium concentrations, no cells remained viable longer than 20 h. When PuO_2^+ was present as a complex, rather than an aquo species, toxicity was suppressed. Complexation with NTA, oxalate, and citrate resulted in some

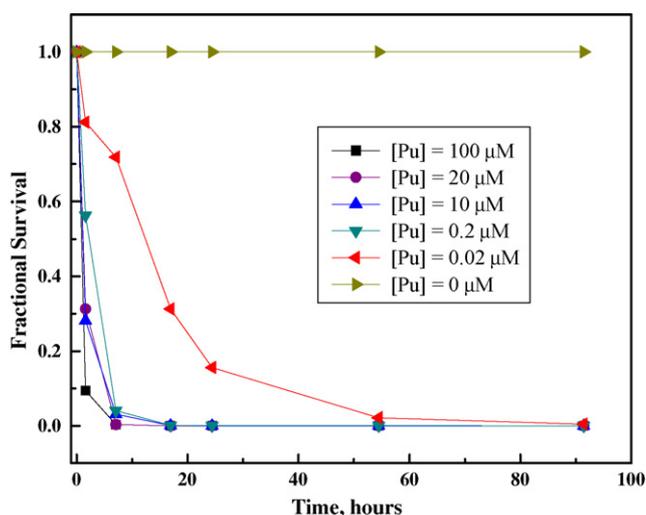


Fig. 4. Fractional survival of *S. alga* as a function of time and the initial concentration of PuO_2^+ in 0.1 M PIPES solution under aerobic conditions. The fractional survival was calculated as the number of viable cells divided by the initial number of cells based on plate counts. Overall uncertainty in each data point is $\pm 20\%$.

cell growth rather than toxicity even in nutrient depleted media (0.1 M PIPES). These data agree with the results reported by Ruggiero et al. [25] where Pu(VI), which is strongly hydrolyzed, was shown to be non-toxic at $\sim\text{mM}$ concentrations of plutonium. In our growth media (see Table 1), therefore, no significant toxicity effect was initially present due to the complexation of Pu(V) with complexants in the media (e.g. lactate, NTA and phosphate) although there are scenarios where microbiologically induced changes in complexant concentration and speciation could lead to toxicity.

3.3. Bioreduction of higher-valent uranyl and plutonyl

Aerobic experiments were performed by growing *S. alga* in TSB aerobically, harvesting the cells in the log-growth phase, rinsing the cells in 0.1 M PIPES and inoculating these cells into Fe-lactate growth media that had been pre-equilibrated with either uranyl or plutonyl (as PuO_2^+). Under these aerobic conditions, *S. alga* grew well but, as expected, did not reduce the Fe^{3+} -NTA complex to form an Fe^{2+} species. Concurrently, uranyl and plutonyl were also not reduced and remained stable in their higher-valent form throughout the ~ 1 -week duration of the experiments. In the longer term, based on the abiotic chemistry [20], a slow reduction of plutonyl is likely given the many organic species present and generated in this system but we did not observe this in the timeframe of our experiments.

Higher-valent plutonium and uranium were anaerobically reduced in the Fe-lactate system under our experimental conditions. These experiments were performed in an anoxic glovebox (<0.1 ppm O_2) with cells that has been initially grown on TSB aerobically, washed with 0.1 M PIPES, and transferred with some residual oxygen to Fe-lactate growth media and grown anaerobically, and then added without rinsing to growth media that had been pre-equilibrated with uranyl or plutonyl. In all cases, plate counts were taken to establish that growth had occurred and there was visual evidence of reduction due to the change in color from the highly colored Fe^{3+} complex to the colorless Fe^{2+} complex.

Under anaerobic conditions, lactate was utilized to reduce Fe^{3+} to Fe^{2+} . The normalized concentrations of Fe^{2+} , Fe^{3+} , lactate and viable biomass, as a function of time, are shown in Fig. 5. Lactate consumption was well correlated with the production of reduced iron. What is most important, from the perspective of uranyl and plutonyl, are the very high concentrations of reduced iron that are generated in this system. Plutonyl added to bio-reacted systems that had been filtered to remove biomass was reduced, and consequently precipitated instantaneously, presumably as a Pu(IV) precipitate. There is no question that soluble divalent iron will exhibit a strong influence over the redox chemistry of plutonyl in the environment.

The bioreduction of uranium(VI) was also observed under the conditions of our experiments. These data are shown in Fig. 6 in *S. alga* growth experiments where lactate utilization was coupled with Fe^{3+} as the electron acceptor. The addition of chloramphenicol, which inhibits the synthesis of proteins, suppressed the bioreduction process linking the reduction pathway to enzymatic activity.

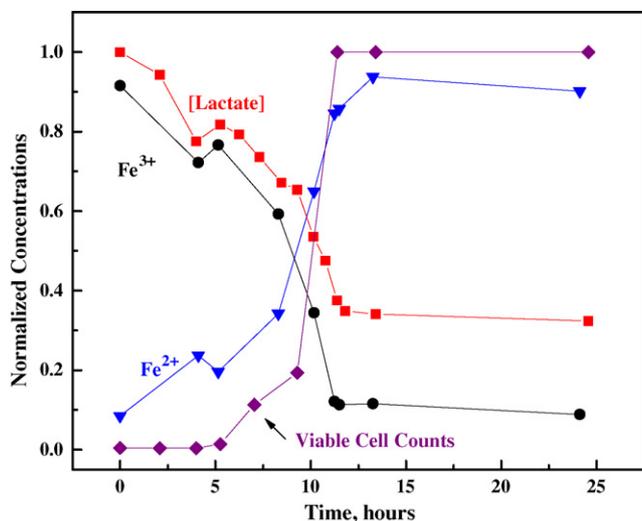


Fig. 5. Bioreduction of Fe^{3+} , initially present as an NTA complex, by *S. alga* utilizing lactate as the electron donor to form soluble Fe^{2+} . Uncertainties are $\pm 10\%$ for the $\text{Fe}^{2/3}$ data, $\pm 5\%$ for the lactate data, and $\pm 20\%$ for the viable cell count data.

It is important to note that in our system, the loss of uranium in solution was not always the outcome of bioreduction. In the presence of high carbonate concentrations, we did not see uranium precipitation indicating that the reduced uranium was persisting as a soluble complex (confirmed by extraction and XANES analysis). Also, there was a slow abiotic pathway for uranium precipitation when carbonate was absent from solution due to U(VI) phase precipitation. This is consistent with our previous discussion of abiotic pathways (note Fig. 1). Our results in this system are consistent with those published by others except that we note a greater degree of coupling between non-reductive abiotic precipitation pathways and bioreduction pathways at the lower uranium concentrations we used. A key point we are making is that precipitation, in and of itself, is not a sufficient indicator of bioreduction—it is important to use

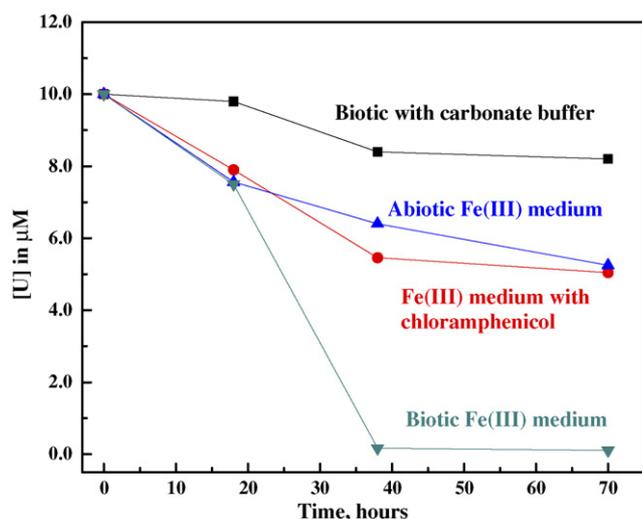


Fig. 6. *S. alga* growth experiments showing the uranium concentration as a function of time and experiment conditions. Coupled abiotic and biotic effects were noted. Uncertainty in the uranium concentration measurement is $\pm 15\%$.

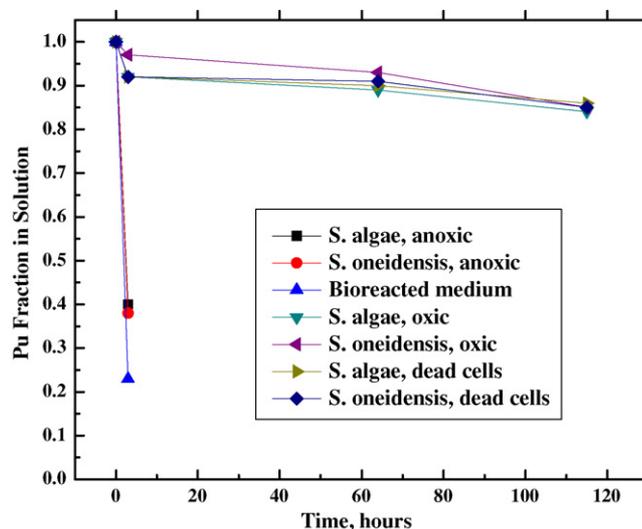


Fig. 7. Stability of PuO_2^+ in the presence of *S. alga* and *S. oneidensis*. Under oxic and dead cell conditions, only slight sorption of plutonium was noted. Anaerobically grown cells and filtered media, resulted in the reduction of plutonium. Uncertainty in the plutonium concentration data was $\pm 10\%$. Data point for the anoxic live cell experiments were taken at 60 and 120 h, but were below our detection limits ($< 10^{-8}$ M).

extraction or spectroscopic techniques (e.g. XANES) to confirm/establish reduction. It remains unclear in our experiments, where we know there is significant buildup of Fe^{2+} in solution, if uranyl reduction is by reaction with the biogenically formed Fe^{2+} , by direct enzymatic processes, or concurrently by both reduction pathways.

Plutonium, as PuO_2^+ , was also reduced anaerobically in our *S. alga* Fe-lactate system. These results are shown in Figs. 7 and 8. The reduction of plutonium led to its subsequent precipitation, yielding concentrations in solution that were several orders of magnitude lower.

In Fig. 7, data that compare oxic and anoxic experiments are shown. In actively growing aerobic *S. alga* and *S. onei-*

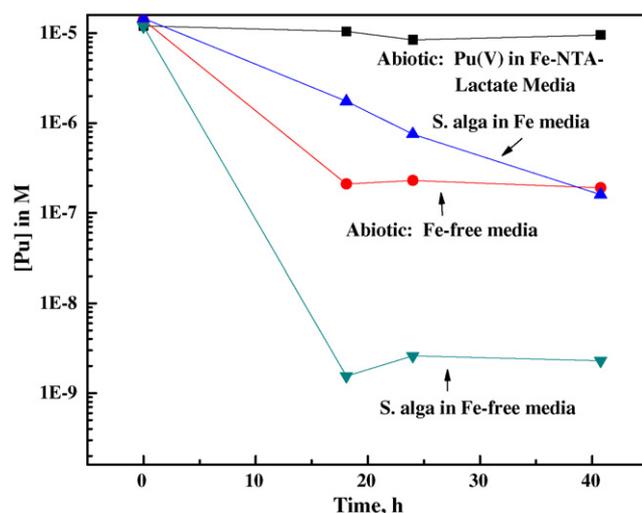


Fig. 8. Bioreduction of higher-valent plutonium by *Shewanella alga* BrY under anaerobic conditions in the presence and absence of iron. Uncertainty in the plutonium concentration data is $\pm 10\%$.

densis systems, as well as in the presence of dead cells under anoxic condition, PuO_2^+ solution concentrations were stable, other than the $\sim 10\text{--}15\%$ sorption observed. This was consistent with our NpO_2^+ results and re-extraction of the plutonium under these conditions gave us a Pu(V) complex. That reduction did not occur was evidenced by the stable concentrations seen and the absorption spectrum taken that confirmed that only Pu(V) species were in solution. Under anaerobic conditions, however, rapid precipitation of plutonium was noted. This occurred in the presence of microbial activity and in filtered abiotic reacted media. The reaction with biogenic Fe^{2+} and microbiologically produced enzymes potentially explain the observed reduction of plutonium.

The relative contribution of these two co-existing reduction pathways was also, in part, addressed. In Fig. 8, the reduction of plutonium in the *S. alga* system was determined in the absence and presence of iron. In both cases, biotic reduction of plutonium was noted. This was significantly more rapid when iron was not present. In this case it is likely that Fe^{3+} is the preferred electron acceptor, and the Fe^{2+} generated is causing the concurrent reduction of plutonium, since this process begins to occur before Fe^{3+} depletion. These results on PuO_2^+ system are analogous to those we observed with NpO_2^+ , which has a somewhat similar complexation behavior but a lesser tendency towards reduction.

4. Summary of observations

A comparison of uranium and plutonium chemistry, as it relates to the Fe-lactate *S. alga* system was provided. Both uranium and plutonium show strong reduction. Biogenic Fe^{2+} and enzymatic processes play a role in this reduction process. Further research is needed to quantify the relative contributions of each process as a function of growth conditions and actinide speciation.

Important differences, from the perspective of their biogeochemistry, exist between uranium and plutonium. First, they differ in the relative irreversibility of the reduction pathway in abiotic systems, something that is well established in the actinide literature. The importance of this, however, is uncertain in microbiologically active systems and needs to be demonstrated experimentally. Second, the predominance of plutonium(V), rather than plutonium(VI), in microbiologically active systems is also a key difference between the biogeochemistry of uranium and plutonium since only U(V) species do not persist in groundwater systems. Actinyl(V) species exhibit a very different complexation behavior, apparent extent of interaction, and toxicity towards *S. alga*, which, by analogy may be the case for many other soil bacteria. Lastly, the end-result of the bioreduction of plutonium may be the formation of Pu(III), rather than Pu(IV) species. There is no analogy between uranium and plutonium systems in this event since U(III) species are not expected in environmentally relevant systems. The role and likely formation of Pu(III) precipitates and species from higher-valent plutonyl species has also not been fully established.

Overall, these results show that higher-valent plutonium is more easily reduced than uranium in biologically active systems,

a result consistent with the redox chemistry of the uranium and plutonium in these systems. This is an important result from the perspective of subsurface containment and, to a lesser extent, the role of natural attenuation on plutonium containment in the subsurface. Future work is focused on a more quantitative measurement of the role of enzymatic and iron-reduction pathways, the modeling of these systems, and the nature and durability of the precipitates formed.

Acknowledgements

This work is performed in part under the Actinide Chemistry and Repository Science Program supported by the Waste Isolation Pilot Plant (DOE-CBFO) and the Environmental Remediation Science Program (DOE-OBER/OS).

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