

## Microbial Transformations of Plutonium

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Microorganisms have been detected in transuranic- and low-level radioactive wastes, Pu-contaminated soils, and in nuclear waste-repository sites. Microbial activity could affect the chemical nature of the Pu by altering the speciation, solubility, and sorption properties and thus could affect the stability and mobility of Pu in the environment. We investigated the biotransformation of Pu(IV)-citrate by *Pseudomonas fluorescens* under aerobic conditions. Characterization of <sup>242</sup>Pu(IV)-citrate by electrospray ionization-mass spectrometry (ESI-MS) showed the presence of 1:1 mononuclear, 1:2 mononuclear biligand, and 2:2 dinuclear complex. X-ray absorption near edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS) analysis of Pu-citrate confirmed Pu in the IV oxidation state and predominantly present as a 1:2 mononuclear biligand complex. Citric acid was rapidly metabolized by *P. fluorescens* resulting in the formation of Pu polymer. Under anaerobic conditions, reductive dissolution of Pu(IV) to Pu(III) by *Clostridium* sp. was observed. The presence of Pu(III) was confirmed by XANES. Addition of Pu(VI) to a mixture consisting of bacterial cells and kaolinite clay showed that the Pu was preferentially associated with bacterial cells as Pu(IV) and that Pu(VI) was reduced to Pu(V) and Pu(IV) only in the presence of bacteria. Studies with Pu contaminated soils from Nevada Test Site showed remobilization of Pu and other radionuclides due to enhanced microbial activity.

### 1. Introduction

The presence of Pu in transuranic (TRU)- and mixed-wastes is a major concern because of the potential for migration from the waste repositories and contaminated environments. Plutonium exists in several oxidation states (III, IV, V, VI) and as various chemical species with a very complex chemistry and environmental behavior. Soil pH, presence of organic ligands, redox conditions, mineralogy, and microbial activity can affect the chemical speciation of Pu. Chemical characterization of Pu at contaminated sites shows that it varies according to the site and the waste stream. At Rocky Flats, CO, the predominant form appears to be PuO<sub>2</sub>(s).<sup>1</sup> Pu generally is considered to be relatively immobile; however, the transport of Pu, albeit at very low concentrations, as colloids was observed at Rocky Flats, Los Alamos National Laboratory (LANL), Nevada Test Site (NTS), and Mayak Production Association, Urals, Russia.<sup>1-4</sup> Plutonium was found associated with mineral colloids at the NTS; bound to iron oxide colloids at Mayak; at Oak Ridge National Laboratory (ORNL) with organics and at Rocky Flats as Pu(IV) mostly associated with organic colloids.<sup>3-6</sup> At Maxey Flats Kentucky, a former low-level radioactive waste site Pu was present as dissolved species, primarily as Pu(IV) complexed with organic ligands.<sup>7</sup> Chelating agents, such as citric acid, are present in TRU waste at the Waste Isolation Pilot Plant (WIPP).<sup>8</sup> Citric acid forms a strong complex with Pu(IV) and has been used in the extraction of <sup>239</sup>Pu from contaminated soil.<sup>9,10</sup> In this paper, we briefly review the microbial transformations of Pu and in particular describe our initial studies on the biotransformation of Pu-nitrate and Pu-citrate, the interactions of Pu with bacteria and clay, and the remobilization of Pu from contaminated soil due to enhanced microbial activity.

### 2. Microbiology of Plutonium Contaminated Sites

Microorganisms have been detected in low-level radioactive

wastes, TRU wastes, Pu-contaminated soils, and in waste-repository sites being considered for disposal of nuclear waste. Leachates collected from the low-level radioactive-waste sites contained <sup>238,239,240</sup>Pu (gross activity of  $1.7 \times 10^5$  pCi/L), aerobic- and anaerobic bacteria (*Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp.) and organic compounds.<sup>11,12</sup> The radioactive- and organic chemicals in the leachate were not toxic to the bacteria. Metabolically active microbes were identified at the LANL's TRU waste burial site containing <sup>239</sup>Pu-contaminated soil.<sup>13</sup> Microorganisms present in the radioactive wastes can affect the long-term stability of radionuclides.<sup>14-17</sup>

### 3. Microbial Mobilization and Immobilization of Plutonium

Under appropriate conditions, dissolution or immobilization of Pu is brought about by direct enzymatic or indirect non-enzymatic action of microorganisms. These include changes in the Eh, pH, oxidation state, production of extracellular metabolites (e.g., citric acid, siderophores), biosorption, bioaccumulation, biotransformation of Pu complexed with organic and inorganic ligands, and Pu colloid formation or destabilization. Plutonium can exist simultaneously as Pu(IV), Pu(V), and Pu(VI) under oxidizing conditions. Reduced Pu species has a high ionic charge, and tends to undergo hydrolysis, leading to the formation of polymers in systems with pH > 2.

Microorganisms directly or indirectly affect the oxidation and reduction of Pu (Figure 1). A slight increase in microbial activity (respiration) can alter the oxidation state of Pu(VI) to Pu(IV) because of the very small differences in the reduction potential between Pu(VI), Pu(V), and Pu(IV). The chemical

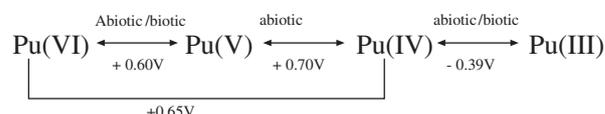


Figure 1. Plutonium oxidation states and redox potentials at pH 8.<sup>18</sup>

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form of Pu (e.g., oxide, ionic, organic- or inorganic complexes, co-precipitates), the availability of electron donors, electron acceptors ( $\text{Fe}^{3+}$ ,  $\text{Mn}^{4+}$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , organic compounds), nutrients (nitrogen, phosphorus), and environmental factors (pH, Eh, temperature, moisture) all can affect microbial activity and the biotransformation of Pu. The extent of Pu dissolution could rise considerably with an increase in microbial activity, particularly under anaerobic conditions due to the formation of stable complexes with metabolites and organic degradation products. Conversely, microorganisms can bring about a decrease in soluble Pu or immobilization due to reduction from higher to lower oxidation state, bioaccumulation and bioprecipitation reactions.

#### 4. Dissolution of Plutonium by Microbial Extracellular Metabolites

The dissolution of Pu by microorganisms is brought about by their production of organic acids, such as citric acid, extracellular metabolites, and siderophores. Microorganisms grown in the presence of plutonium produced unidentified compounds capable of dissolving and mobilizing Pu in soils. These compounds also may be involved in transporting plutonium into the cells.<sup>19–23</sup>

Siderophores are low-molecular-weight iron chelators produced by microbes in response to a limited availability of soluble iron. As Pu(IV) and Fe(III) have chemical and biochemical similarities, and because of the ubiquity of siderophore-producing microbes, iron-sequestering agents produced by such microorganisms could be important in the complexation of Pu and other actinides, thus increasing their solubility and bioavailability. Desferal, a polyhydroxamate chelate produced by microorganisms enhanced the dissolution of  $\text{PuO}_2$ .<sup>13</sup> The production of extracellular chelating agents resembling siderophores was observed with *Pseudomonas aeruginosa* grown in the presence of uranium and thorium.<sup>24</sup> Brainard et al.<sup>22</sup> determined rate-constants for the solubilization of hydrous  $\text{PuO}_2(\text{s})$  by the siderophores enterobactin and desferrioxamine B, and by selected carboxylate amino polycarboxylate, and catecholate ligands. They showed that siderophores are extremely effective in solubilizing actinide oxides; on a per-molecule basis, enterobactin was  $\sim 10^3$  times more effective than the other chelators tested in increasing the rate of solubilization of hydrous plutonium oxide.

Several bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that increased the concentration of Pu in soil-column eluates relative to controls. Elution through soil effectively removed positively charged Pu complexes. The increased mobility of Pu in soil resulted from the formation of neutral and negatively charged Pu complexes. In the presence of known microbial metabolites such as citric acid and synthetic ligands (DTPA, EDTA, EDDHA), Pu(VI) was reduced to Pu(IV) before complexation, suggesting that the latter valence state would be the dominant one associated with organic complexes in soils. Studies with selected organisms indicated that Pu was both actively transported into, and sorbed onto the cell, and these phenomena, as well as complexation of Pu by extracellular metabolites, were a function of the form of Pu supplied (hydrolyzed and complexed forms), the organisms' type and growth characteristics, and the ability of the organism to alter extracellular pH.<sup>20,21,23,25</sup>

#### 5. Immobilization of Plutonium by Biosorption and Bioaccumulation

Immobilization of Pu, on the other hand, may be caused indirectly, by changing the Eh, and facilitating precipitation of Pu, reduction of Pu(VI) and Pu(V) to Pu(IV) oxidation state, biosorption, and bioprecipitation reactions. Naturally occur-

ring soluble organics affected  $^{237}\text{Pu}$  uptake by the fresh-water bacterium *Aeromonas hydrophila* and by the alga *Scenedesmus obliquus*.<sup>26</sup> Low Pu (and Am) concentrations observed in the water column corresponded with maximum bacterial activity and it was attributed to bioaccumulation by the biomass.<sup>27</sup> The biosorption of  $^{239}\text{Pu}$  from aqueous nitrate medium by the fungus biomass *Rhizopus arrhizus* was maximal at pH 6–7 and that under optimal conditions a strain of *Rhizopus* species removed 99% of the  $^{239}\text{Pu}$  from the wastewater.<sup>28,29</sup> Alpha-energy spectral analyses in combination with SEM observations showed that the absorption occurred mainly on the cell walls of the fungus. *Halomonas* sp. isolated from the WIPP site biosorbed  $\sim 9\%$  ( $0.17 \times 10^{-9}$  M) of the total  $^{241}\text{Pu}$  in solution ( $1.8 \times 10^{-9}$  M); *Acetobacterium* sp. isolated from Grimsel groundwater biosorbed  $\sim 7\%$  ( $0.11 \times 10^{-9}$  M) of the total  $^{241}\text{Pu}$  ( $1.5 \times 10^{-9}$  M) in solution.<sup>30</sup> On a dry-weight basis, *Acetobacterium* sp. sorbed 145 ng  $^{241}\text{Pu}$  g<sup>-1</sup> dry cells, and *Halomonas* sp. sorbed 351 ng  $^{241}\text{Pu}$  g<sup>-1</sup> dry cells.

Sorption studies of Pu(VI) with bacterial resting cells have shown that the interaction with bacteria caused changes in the oxidation state to Pu(V) due to endogenous respiration. Due to its weak complexation ability, the cell bound Pu(V) was released into solution; disproportionation of Pu(V) to Pu(IV) and Pu(VI); and complexation of Pu(IV) with the biomass was observed.<sup>25,31</sup> EXAFS analysis of Pu associated with the cells showed that Pu(VI) is primarily bound to the phosphate groups on the cell surface.<sup>32</sup>

#### 6. Reductive Dissolution of Pu(IV) to Pu(III) by Bacteria under Anaerobic Conditions

The mobility and bioavailability of Pu is limited by its solubility. Plutonium is present in the environment mostly as the oxides and hydroxides of Pu(IV) that have low solubility. The solubility products of Pu(IV) oxyhydroxides are estimated to be  $10^{-56.8}$  and  $10^{-57.8}$ . The solubility of plutonium (III) hydroxide is much greater,  $K_{\text{sp}} = 10^{-22.6}$ . Consequently, the reduction of Pu(IV) oxyhydroxides to Pu(III) is expected to increase the solubility of plutonium in the environment. The electrochemical potential for this reduction reaction is similar to that for iron reduced by many microorganisms suggesting that those microbes able to reduce iron and other metals also might reduce Pu(IV) oxyhydroxides under similar conditions and, thus, reductively solubilize Pu(IV) to Pu(III). The persistence of the reduced soluble Pu(III) is affected by its stability, as well as by its interactions with organic and inorganic materials. Rusin et al.<sup>33</sup> showed that an iron-reducing bacterium *Bacillus* sp. solubilized up to 90% hydrous  $\text{PuO}_2(\text{s})$  in about 7 days under anaerobic conditions in the presence of the chelating agent nitrilotriacetic acid (NTA). In these studies, Pu(III) was present as a Pu-NTA complex. However, without NTA, only 40% of Pu was solubilized suggesting that a strong complexing agent is needed to keep the Pu in solution. Little dissolution of  $\text{PuO}_2$  was observed in sterile culture media or in the presence of non-iron-reducing bacteria, such as *Escherichia coli*.

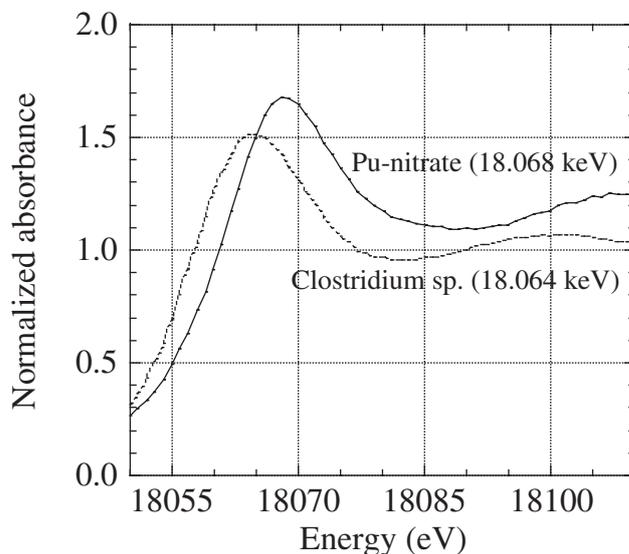
Francis et al. (*manuscript in preparation*) investigated the reductive dissolution of Pu(IV) by the anaerobic spore-forming bacterium *Clostridium* sp. known to reduce U(VI) to U(IV); Tc(VII) to Tc(IV); and Fe(III) to Fe(II). Addition of  $1 \times 10^{-7}$  M  $^{242}\text{Pu}$  (IV)-nitrate had no effect upon the growth and metabolism of glucose by *Clostridium* sp. Pu added to the bacterial growth medium (uninoculated control) resulted in its precipitation and was removed by 0.4  $\mu\text{m}$  filtration. Speciation calculations showed that Pu most likely existed as  $\text{Pu}(\text{OH})_4$  at pH 6.2 due to hydrolysis and polymerization. The growth of *Clostridium* sp. lowered the Eh ( $-180$  mV), and the pH from 6.2 to 2.8, concomitant with the production of acetic and butyric acids and of carbon dioxide (225  $\mu\text{mol}$ ) and hydrogen. After 14 h of growth, 70% of the Pu passed through a 0.4  $\mu\text{m}$

filter and 55% through a 0.03  $\mu\text{m}$  filter suggesting a significant portion of Pu was solubilized. Solvent extraction by thenoyltrifluoroacetone (TTA) confirmed a decrease in the polymeric form of Pu and an increase in the soluble fraction, suggesting the presence of  $\text{Pu}^{3+}$ . XANES analysis of the culture at the Pu  $L_{\text{III}}$  edge (18.057 keV) confirmed that the oxidation state was  $\text{Pu}^{3+}$  (Figure 2). The Eh of the medium was low and the  $\text{CO}_2$  concentration high, thus favoring the reduction of Pu from the tetravalent to the trivalent state. These results suggest that under appropriate conditions Pu can be reduced to Pu(III) by anaerobic bacterial activity.

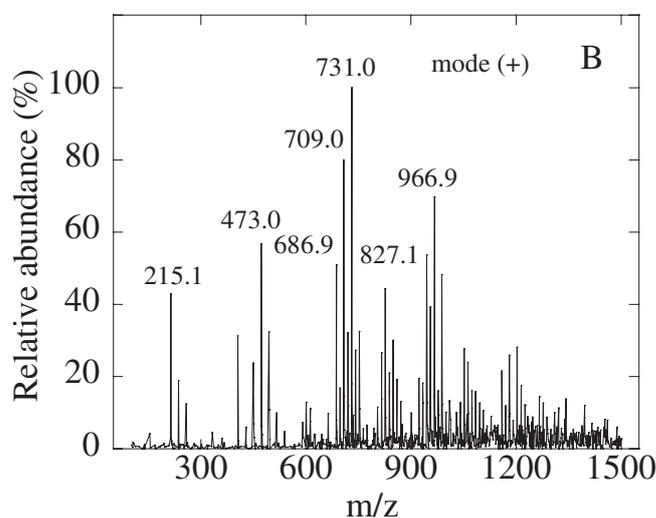
## 7. Biotransformation of Plutonium-organic Complexes

Chelating agents are present in TRU and mixed wastes due to decontamination of nuclear reactors and equipment, cleanup operations, and chemical separation of radionuclides. Plutonium forms very strong complexes with a variety of organic ligands. Naturally occurring organic complexing agents, such as humic and fulvic acids, and microbially produced complexing agents, such as citrate, and siderophores, as well as synthetic chelating agents can affect the mobility of Pu in the environment. The presence of organic ligands in radioactive wastes is a major concern because of the potential for increasing the transport of radionuclides from disposal sites. Biotransformation of the radionuclide-organic complex should precipitate the radionuclide from solution thereby retarding its mobility in the environment. Chelating agents, such as citric acid, are present in TRU and mixed wastes. In addition to other organic ligands, an estimated 1,950 moles of citric acid is present in the WIPP inventory of TRU waste.<sup>8</sup> Citric acid forms a strong complex with Pu(IV) and has been used in exploring chelation therapy, and in extracting  $^{239}\text{Pu}$  from contaminated soil.<sup>9,10,34,35</sup> We investigated the biotransformation of Pu-citrate (cit) under aerobic conditions by *Pseudomonas fluorescens*.<sup>36</sup>

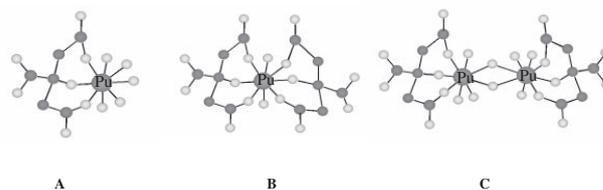
**7.1. Characterization of Pu-citrate.** The complexation of Pu(IV) with excess citric acid at pH 4.5 and 6.5 was determined by solvent extraction and potentiometric titration and the mononuclear biligand  $\text{PuO}(\text{cit})_2$  was proposed.<sup>34</sup> Analyses of Th(IV)-citrate by potentiometric titration and conductimetric measurements showed the formation of  $[\text{Th}_2\text{cit}_3]^{4+}$  (Reference 37). Tetravalent plutonium forms a 1:1 Pu: citric acid ( $K = 10^{15}$ ) complex and a 1:2 Pu: citric acid complex ( $K = 10^{30}$ ).<sup>38</sup> In the presence of excess citric acid, Pu(IV) oxidation state is stable.<sup>19</sup> At pH 6.5, we identified a monomeric  $[\text{Pucit}]$  species, two forms of biligand  $[\text{Pucit}_2]$  species, and a dimeric  $[\text{Pu}_2\text{cit}_2]$  species of tetravalent plutonium with citric acid. Speciation calculations showed that the biligand complex is the predominant form. In addition, analysis of the complex over 100 h demonstrated that citric acid inhibits polymer formation. We determined the complexation of Pu-citrate by ESI-MS and EXAFS. Figure 3 shows the ESI-MS spectra for  $1.6 \times 10^{-4}$  M Pu-citrate complex. The spectrum shows the presence of three forms of the Pu-citrate complex. The dominant peaks in the spectrum can be attributed to the formation of a monomeric Pu-citrate complex ( $m/z = 473.0$ )  $[\text{Pucit}(\text{H}_2\text{O})\text{Na}]^+$  (Figure 4A) and the biligand complex is indicated by the presence of the three peaks at  $m/z = 686.9$   $[\text{Pu}(\text{H}_2\text{cit})_2\text{NO}_3]^+$ ,  $m/z = 709.0$   $[\text{Pu}(\text{Hcit})(\text{H}_2\text{cit})\text{NaNO}_3]^+$ , and  $m/z = 731.0$   $[\text{Pu}(\text{Hcit})_2\text{Na}_2\text{NO}_3]^+$  (Figure 4B). The differences in molecular weight for each form are due to the presence of anion (nitrate) and cation (hydrogen, sodium) species which result in formation of singly-charged species. The presence of a complex containing two Pu atoms is noted at  $m/z = 966.9$   $[\text{Pu}_2(\text{Hcit})(\text{cit})(\text{NO}_3)_2]^+$  (Figure 4C) indicating the formation of a dinuclear species. XANES and EXAFS determine the oxidation state of the Pu and the nature of Pu complexation with citric acid. XANES analysis confirmed that the Pu was present as tetravalent form (data not shown). Figure 5 shows the  $k^3$ -weighted raw EXAFS spectrum



**Figure 2.** XANES analyses of Pu before and after bacterial action (Francis, Dodge, Gillow; manuscript in preparation).



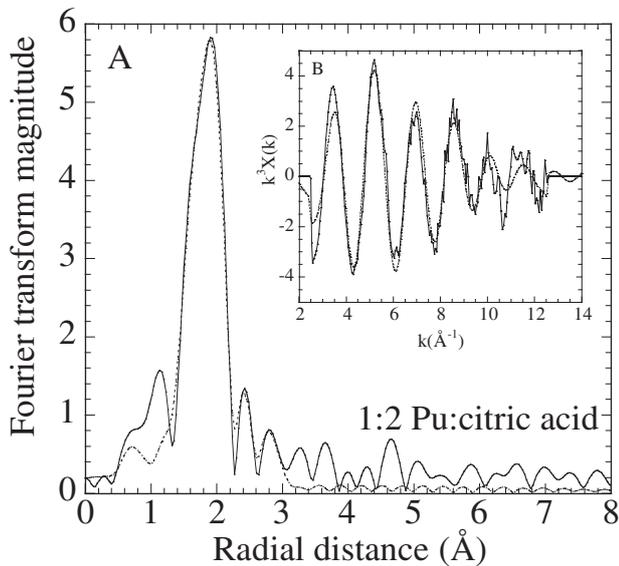
**Figure 3.** Electrospray ionization mass spectrum (ESI-MS) in the (+) mode showing the molecular ions ( $m/z$ ) produced by citric acid and the Pu-citrate complex at pH 6.5.<sup>36</sup>



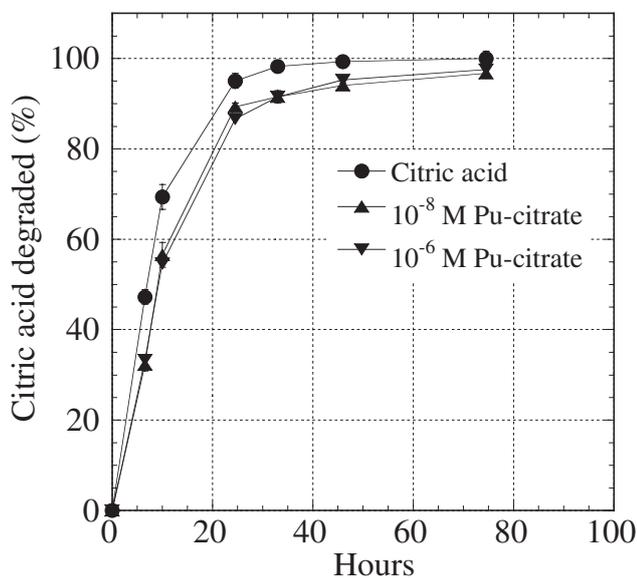
**Figure 4.** Proposed structures at pH 6 for the monoligand 1:1 Pu: citric-acid complex (A); biligand 1:2 Pu: citric-acid complex  $[\text{PuO}(\text{cit})_2]^{4+}$  (B); and dimeric 2:2 Pu: citric-acid complex (C). The open circles represent oxygen and the filled circles represent carbon atoms.

and the Fourier transform for the Pu-citrate complex. Based upon this analysis the best configuration for the molecule consists of a mononuclear biligand complex (Figure 4B). The presence of 1:1 Pu-citrate complex at pH 4 was reported by Kantar and Honeyman.<sup>39</sup> However, the dimeric complex has not been previously reported.<sup>36</sup>

**7.2. Biodegradation of citric acid and Pu-citrate complexes.** Figure 6 depicts the rate and extent of citrate degradation in samples containing  $10^{-6}$  M and  $10^{-8}$  M Pu present as Pu-citrate complexes at an ionic strength of 0.18 M. Citric

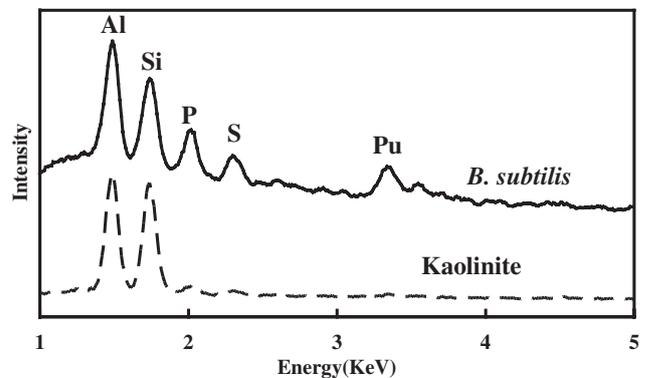
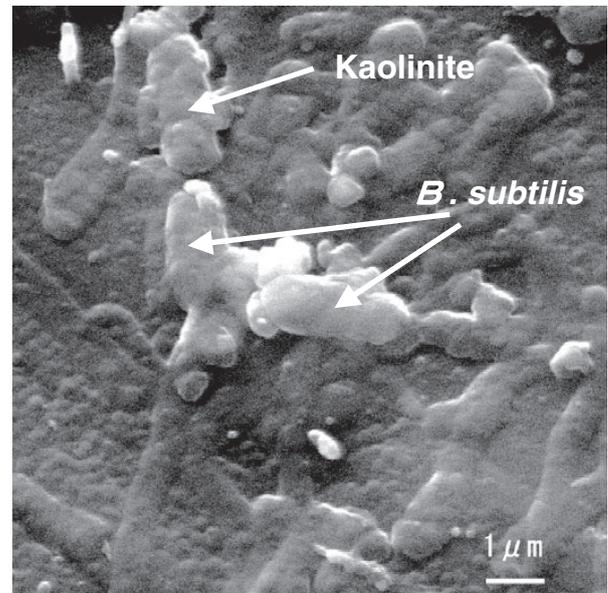


**Figure 5.** EXAFS analysis for 1:2 Pu: citric acid complex at pH 6.5. Experimental data (solid line); fitted data (dashed line).<sup>36</sup>



**Figure 6.** Effect of adding  $10^{-6}$  and  $10^{-8}$  M  $^{242}\text{Pu}$  on citrate metabolism. Citric-acid concentration is  $10^{-4}$  M in all treatments.<sup>36</sup>

acid ( $10^{-4}$  M) in the absence of Pu was metabolized completely at a rate of  $4.9 \mu\text{M}/\text{h}$ . With  $10^{-6}$  and  $10^{-8}$  M Pu present as the Pu-citrate complex we observed a slight decline on the rate and extent of citrate degradation in comparison to the sample lacking Pu. In both samples, citrate was degraded  $>96\%$ , and at the rate of  $4.0 \mu\text{M}/\text{h}$  and  $3.8 \mu\text{M}/\text{h}$ , respectively. In the presence of  $10^{-6}$  M Pu, 20% was retained on the  $0.4 \mu\text{m}$  filter, and 27% on the  $0.03 \mu\text{m}$  filter; similar results were found with  $10^{-8}$  M Pu, at 43% and 57%, respectively (data not shown). Comparison of the  $10^{-6}$  M Pu samples inoculated with bacteria with that of the control samples (uninoculated sample) showed that there is greater retention on the  $>0.4 \mu\text{m}$  filter in the former. These data suggest that the soluble  $10^{-6}$  and  $10^{-8}$  M Pu-citrate complexes underwent significant biodegradation with Pu released as a particle-reactive species that adsorbed to the cells ( $>0.4 \mu\text{m}$ ), and possibly as a colloidal species ( $>0.03 \mu\text{m}$ ). Although the Pu(IV)-citrate complex is stable in the absence of bacteria, adding bacteria to the 1:100 and 1:10000 Pu(IV)-citrate complexes resulted in the retention of Pu species at 20–43% by the  $0.4 \mu\text{m}$  filter (biomass associated), and 27–57% by



**Figure 7.** SEM-EDS analysis of the bacteria and clay mixture showing that Pu was preferentially associated with bacterial cells.<sup>42</sup>

the  $0.03 \mu\text{m}$  filter (colloid fraction). Yong and Macaskie<sup>40</sup> showed that the Th(IV)-citrate complex, an analog for Pu-citrate, does not interact with *Citrobacter* sp. immobilized cells.

## 8. Interactions of Plutonium with Bacteria and Clay

Kudo et al.<sup>41</sup> investigated the interactions between the anaerobic sulfate-reducing bacteria and Pu in the presence and absence of bentonite, a backfill material for nuclear waste disposal. The bentonite was obtained from Japan, and the anaerobic sulfate-reducing bacteria were isolated from a pulp- and paper-wastewater treatment plant. Distribution coefficients ( $K_d$ , in mL/g) were used as indices of radionuclide behavior. Pu  $K_d$  values for living bacteria ranged from  $1.8 \times 10^3$  to  $1.1 \times 10^5$  depending on the pH, while the  $K_d$  values for dead bacteria were  $1.2 \times 10^3$  to  $5.9 \times 10^3$ . Furthermore, higher  $K_d$  values of  $4.0 \times 10^4$ – $1.1 \times 10^5$  for living bacteria were obtained for pHs between 6.83 and 8.25, while pH had no effect on the dead bacteria. In contrast, Pu  $K_d$  values for mixtures of living bacteria with bentonite, and sterilized (dead) bacteria with bentonite were  $1.2 \times 10^3$  to  $8.4 \times 10^4$  and  $6.2 \times 10^2$  to  $1.7 \times 10^4$ , respectively. The presence of live anaerobic bacteria with bentonite increased the  $K_d$  values of  $^{239}\text{Pu}$  by 50-fold, in comparison to the mixture of dead bacteria with bentonite. The Pu  $K_d$  values for bentonite alone, both nonsterilized and sterilized, were approximately  $1.0 \times 10^4$ . The results suggest that anaerobic bacteria within the engineered barrier system (in this case, bentonite) will play a significant role in modulating the behav-

ior of Pu in geologic repositories.

Exposure of kaolinite clay to Pu(VI) showed rapid adsorption and was desorbed readily with change in the oxidation state. In contrast, *Bacillus subtilis* cells exposed to Pu(VI) showed absorption of Pu(VI) with subsequent reduction to Pu(V) and then to Pu(IV). Incubation of Pu(VI) with a mixture consisting of bacterial cells and kaolinite showed that Pu was preferentially associated with bacterial cells as Pu(IV) and little Pu was found with kaolinite as evidenced by SEM and EDS analyses (Figure 7).<sup>42</sup> The bacterial cell surfaces consist of the negatively charged anionic functional groups of phosphoryl, carboxyl, and hydroxyl moieties with affinity to bind Pu. The mechanisms of accumulation of Pu(IV) may entail its precipitation as insoluble Pu hydroxides and/or complexation with the functional groups of the cellular surfaces or phosphate released from the bacteria. Panak et al.<sup>32</sup> showed that Pu(IV) was predominantly associated with the bacterial phosphates.

### 9. Mobilization of Plutonium from Contaminated Soils Due to Microbial Activity

Characterization of Pu at contaminated sites shows that its environmental form varies according to the site and the waste stream. For example, at Rocky Flats, CO, the predominant form appears to be as PuO<sub>2</sub>(s)<sup>1</sup> at the Nevada Test Site (NTS) Pu was associated with mineral oxides;<sup>3</sup> while, at Oak Ridge, TN it is associated with organic matter.<sup>5</sup> Pu is generally considered to be relatively immobile; however, its transport to varying distances at low concentrations as colloids was observed at Rocky Flats (RF), Los Alamos National Laboratory (LANL), and NTS.<sup>1-4,6</sup> Plutonium in surface waters at the RF site was shown to be associated with organic macromolecules.<sup>6</sup> Studies with Pu contaminated soils show that Pu and other radionuclides are remobilized due to enhanced aerobic or anaerobic microbial activity (Francis and Dodge, unpublished results). Several bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that increased the concentration of Pu in soil-column eluates relative to controls. Elution through soil effectively removed positively charged Pu complexes. The increased mobility of Pu in soil resulted from the formation of neutral and negatively charged Pu complexes. In the presence of known microbial metabolites and synthetic ligands (DTPA, EDTA, EDDHA), Pu(VI) was reduced to Pu(IV) before complexation, suggesting that the latter valence state would be the dominant one associated with organic complexes in soils.<sup>20</sup> We examined the effect of microbial activity on the mobility of Pu from a contaminated soil.

**9.1. Characterization of Pu-contaminated soil.** Plutonium-contaminated soil (HP-11) was obtained from Area 11 soil of the Double Track test shot area at the NTS and had a gross activity of 50 nCi/g. The individual  $\alpha$  components of the total activity were determined and are given in Table 1. The  $\beta$ -emitter <sup>241</sup>Pu has also been detected but was not quantified. Minerals present in the soil were determined using  $\mu$ -X-ray diffraction on beamline X7A at the National Synchrotron Light Source (NSLS). The predominant minerals consisted of various forms of iron oxides and aluminosilicates. The uranium was present in the soil as hexavalent form associated as the minerals schoepite (UO<sub>3</sub>·2H<sub>2</sub>O) and liebigite (Ca<sub>2</sub>UO<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub>·11H<sub>2</sub>O).

**9.2. Mobilization of Pu, Am, and U in NTS soil due to microbial activity.** The effect of bacterial activity on the mobilization of radionuclides in NTS soil is presented in Figure 8. Under anaerobic conditions glucose was metabolized with decrease in pH to 4.8 and an increase in  $\alpha$  activity in solution. However, the appearance of the actinide in solution was concomitant with decrease in pH (Figure 8). In addition, the alkaline nature of the soil suggests that mobilization may be due to

TABLE 1: Total  $\alpha$  activity in NTS soil

Isotope	$\alpha$ activity in NTS soil	
	/ nCi g <sup>-1</sup>	/ $\mu$ g g <sup>-1</sup>
<sup>233,234</sup> U	<0.18	<6.1 × 10 <sup>-2</sup>
<sup>235,236</sup> U	<0.15	<3.6 × 10 <sup>1</sup>
<sup>238</sup> U	<0.14	<4.2 × 10 <sup>2</sup>
<sup>241</sup> Am	3.8 ± 0.7	1.1 × 10 <sup>-3</sup>
<sup>238</sup> Pu	0.44 ± 0.02	2.6 × 10 <sup>-5</sup>
<sup>239,240</sup> Pu	69.2 ± 6.5	1.1 × 10 <sup>-1</sup>

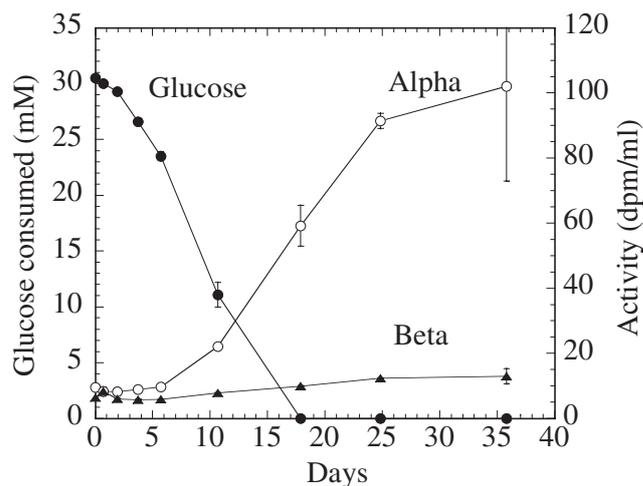


Figure 8. Mobilization of actinides due to microbial activity (Dodge and Francis, manuscript in preparation).

dissolution of carbonate species present in the soil.

Addition of citric acid to the soil showed metabolism of citric acid and an increase both  $\alpha$  and  $\beta$  activity in solution. However with increase in time there was sudden decrease in  $\alpha$  and  $\beta$  activity (data not shown). This loss of activity in solution coincided with the complete utilization of citric acid by the bacteria. There was only a slight decrease in pH to 7.7 in the bacterially active sample. This observation suggests that citric acid forms a soluble complex with the actinide and that its removal due to bacterial metabolism releases the actinide, resulting in its precipitation. These results also suggest that the type of carbon source will have a different effect on microbial mobilization of actinides.

### 10. Summary

A slight increase in microbial activity can alter the oxidation state of Pu(VI) to Pu(IV) because of the very small differences in the reduction potential between Pu(VI), Pu(V), and Pu(IV). We observed (i) reductive dissolution of Pu(IV) to Pu(III) by the anaerobic bacterium *Clostridium* sp.; (ii) biodegradation of Pu-citrate complexes by *Pseudomonas fluorescens* resulted in the formation of polymeric Pu; and (iii) incubation of Pu(VI) with *Bacillus subtilis*, kaolinite clay, or their mixture showed that Pu was reduced to Pu(V) only in the presence of bacteria and that the oxidation state of Pu associated with *Bacillus subtilis* and the mixture was Pu(IV); no changes in Pu oxidation state was observed with clay. Studies with Pu-contaminated soils from NTS show that Pu and other radionuclides are remobilized due to enhanced aerobic or anaerobic microbial activity and the type of carbon source affected the rate and extent of Pu in the solution phase.

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