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Reductive Dissolution of Pu(IV) by *Clostridium* sp. Under Anaerobic Conditions

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An anaerobic, gram positive, spore-forming bacterium *Clostridium* sp., common in soils and wastes, capable of reduction of Fe(III) to Fe(II), Mn(IV) to Mn(II), Tc(VII) to Tc(IV), and U(VI) to U(IV), reduced Pu(IV) to Pu(III). Addition of ^{242}Pu (IV)-nitrate to the bacterial growth medium at pH 6.4 resulted in the precipitation of Pu as amorphous $Pu(OH)_4$ due to hydrolysis and polymerization reactions. The Pu (1×10^{-5} M) had no effect upon growth of the bacterium as evidenced by glucose consumption; carbon dioxide and hydrogen production; a decrease in pH of the medium from 6.4 to 3.0 due to production of acetic and butyric acids from glucose fermentation; and a change in the Eh of the culture medium from +50 to −180 mV. Commensurate with bacterial growth, Pu was rapidly solubilized as evidenced by an increase in Pu concentration in solution which passed through a 0.03 μm filtration. Selective solvent extraction of the culture by thenoyltrifluoroacetone (TTA) indicated the presence of a reduced Pu species in the soluble fraction. X-ray absorption near edge spectroscopic (XANES) analysis of Pu in the culture sample at the Pu L_{III} absorption edge (18.054 keV) showed a shift of −3 eV compared to a Pu(IV) standard indicating reduction of Pu(IV) to Pu(III). These results suggest that, although Pu generally exists as insoluble Pu(IV) in the environment, under appropriate conditions, anaerobic microbial activity could affect the long-term stability and mobility of Pu by its reductive dissolution.

Introduction

The presence of plutonium in contaminated soils, and transuranic (TRU) and mixed wastes is a major concern because of its potential for migration from the waste-repository resulting in long-term contamination of the environment. The toxicity of Pu and the relatively long half-lives of its isotopes (87.7–8.0 × 10^8 y) are the primary causes for concern. Significant microbial activity is expected in the waste due to the presence of organic compounds and nitrate, which are used as carbon and nitrogen sources by a variety of microorganisms. Microorganisms have been detected in low-level radioactive wastes containing Pu, transuranic (TRU) wastes, Pu contaminated soils, and in waste-repository sites under consideration for the disposal of nuclear waste (*1*). Low-level radioactive and TRU wastes contain low levels of Pu along with other radionuclides and organic compounds (*2*). ^{238,239,240}Pu (gross alpha activity 1.7×10^5 pCi/L) isotopes were detected in leachate samples collected from the low-level radioactive-waste disposal sites at West Valley, NY and Maxey Flats, KY (*3–5*). Several aerobic and anaerobic bacteria were isolated from the leachate samples and included *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp. (*6*). The radioactivity and the organic chemicals present in the leachate were not toxic to the bacteria and were metabolized producing-tritiated and ^{14}C-methane (*2, 7*). Viable, metabolically active microbes were detected at the Los Alamos National Laboratory (LANL) TRU waste burial site containing ^{239}Pu contaminated soil and flammable waste (*8*).

Dissolution of Pu by microorganisms is brought about by their production of organic acids, extracellular metabolites, and siderophores (*9–14*). Immobilization of Pu by bacteria may be due to biosorption and bioprecipitation reactions (*15–21*). Solubilization of Pu(IV) hydrous oxide by Fe(III)-reducing strains of *Bacillus polymyxa* and *B. circulans* under anaerobic conditions has been reported (*22*). Although the physical, chemical, and geochemical processes affecting dissolution, precipitation, and mobilization of actinides have been investigated, we have only limited information on the interactions of microorganisms with actinides and the effect of microbial processes on the reductive precipitation or dissolution of Pu in TRU wastes (*1, 13, 14, 17*). A fundamental understanding of these processes would help to assess their impact on the behavior of radionuclides and on the long-term stewardship of contaminated sites.

In this study we report that an anaerobic, gram-positive, spore-forming bacterium *Clostridium* sp. (ATCC 53464), common in soils and wastes and capable of affecting the solubility of U(VI), Fe(III), Mn(IV), and Tc(VII) by reduction from higher to lower valence state (*23–25*), also reduces Pu(IV) to Pu(III).

Materials and Methods

Culture. *Clostridium* sp. (ATCC 53464), a gram positive, strict anaerobic bacterium isolated from a coal cleaning waste was grown in medium containing the following ingredients (per liter): glucose, 5 g; NH_4Cl, 0.5 g; glycerol phosphate, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 2.8 mg; peptone, 0.1 g; yeast extract, 0.1 g; pH 6.8. The medium was prereduced by boiling and purging with nitrogen gas, and was dispensed into 60 mL serum bottles inside a glovebag. The bottles were sealed with butyl rubber stoppers and autoclaved.

Pu-Nitrate. ^{242}Pu-nitrate (CRM 130) was obtained from New Brunswick Laboratory (Argonne, IL). One milligram of $^{242}Pu(NO_3)_4$ was dissolved in 10 mL of 8 M nitric acid by heating in a HEPA filtered hood for 30 min at 130 °C. The solution was diluted with deionized water to obtain a 1×10^{-5} M stock solution and prior to use the pH was adjusted to 6.3 with KOH in a glovebag using sterile, prereduced, deionized water. Total activity was determined on a 0.5 mL aliquot using a Wallac Guardian model 1414 liquid scintillation counter (LSC). The minimum detectable amount (MDA) for ^{242}Pu by alpha counting was 0.9 ρCi/mL (*26*).

Biotransformation of Pu by *Clostridium* sp. A 0.1 mL aliquot of pH-adjusted ^{242}Pu-nitrate stock solution was added to a 20 mL serum bottle containing 10 mL growth medium at pH 6.3 to give a final concentration of 1×10^{-7} M ^{242}Pu. Medium with and without ^{242}Pu was inoculated with a 0.25 mL aliquot of a 24 h old culture of *Clostridium* sp. grown in

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the medium described above. The samples were placed in an incubator in the dark at 26 °C.

Microbiological and Chemical Analyses. At periodic intervals the head space pressure was measured using a digital pressure gauge, and carbon dioxide was analyzed by gas chromatography, as previously described (*23*). The bottles were opened inside the glovebag and the pH was determined using a Beckman Φ350 pH meter with a Beckman 511275-AB combination electrode. The Eh was determined using a Mettler Toledo InLab 501/170 combination oxidation–reduction potential (ORP) electrode which was calibrated using a standard solution (ThermoOrion, MA). The solutions were filtered through a 0.45 μm filter, and glucose and organic acids were analyzed by high pressure liquid chromatography (HPLC) using a Shimadzu LC-10AS liquid chromatograph with a Bio-Rad HPX-87H column and 0.003 N H_2SO_4 mobile phase. Glucose and organic acids were determined using a refractive index and a UV-vis detector, respectively.

Pu Speciation. We used microfiltration, selective solvent extraction, X-ray absorption spectroscopy, and liquid scintillation counting to determine the speciation of Pu in solution phase.

Microfiltration. At selected intervals during the growth of the bacteria the samples were shaken and an aliquot was removed and counted by LSC to determine total ^{242}Pu in the unfiltered sample. In addition, 2.0 mL aliquots were parallel filtered through 0.4 μm and 0.03 μm Poretics polycarbonate filters, respectively, into preweighed LSC vials using an Amicon stirred cell under argon gas.

Selective Solvent Extraction Techniques. The speciation of the Pu in the solution was determined by extraction with thenoyltrifluoroacetone (TTA) at pH 0 and pH 4 (*27*). At selected times a 0.5 mL aliquot was removed from the sample bottle and transferred to a silanized glass vial inside an anaerobic glovebag. The organic and aqueous components of the extracts were separated and assayed for Pu by liquid scintillation.

X-ray Absorption Near-Edge Spectroscopy (XANES) Analysis of Pu. In a parallel experiment the oxidation state of Pu in the culture sample after anaerobic bacterial activity was determined using XANES analysis. Two 60 mL serum bottles containing 500 μg ^{242}Pu-nitrate each were placed inside an anaerobic glovebag and 40 mL prereduced growth medium was added to each one. This corresponds to a total concentration of 5.0×10^{-5} M Pu in each sample. The bottles were then sealed with butyl rubber stoppers. Two milliliters of a 24 h old culture of *Clostridium* sp. was added, and the samples were placed in an incubator in the dark at 26 °C. After incubation for 42 h the Eh was measured and the entire culture was freeze-dried using a Labconco Freezone 4.5 freeze-drier. The freeze-dried material was placed in an anaerobic glovebag, transferred to an Al sample holder, and analyzed for Pu at the Pu L_{III} absorption edge (18.054 keV) with a 13-element Ge detector at beamline X18B at the National Synchrotron Light Source (NSLS). The beamline energy position was monitored using a Zr metal foil, which has a nearby K absorption edge energy of 17.998 keV. Seven scans were collected and averaged to minimize the signal-to-noise ratio. The combined XANES spectrum was background-subtracted and normalized to the edge jump and the oxidation state was determined by comparing the energy position at the inflection point with tetravalent plutonium nitrate.

Plutonium Speciation Calculations. The aqueous speciation of Pu in bacterial growth medium was calculated using the geochemical modeling program PHREEQC (*28*). The thermodynamic constants for the Pu(IV) hydroxide species and the Pu(IV) carbonate species were obtained from the Nuclear Energy Agency Thermochemical Database Project (*29*). In addition, the $Pu(OH)_2(CO_3)_2^{2-}$ species (*30*) and the $Pu(CO_3)_3^{2-}$ species (*31*) were considered. The reduction potential for the Pu(IV)/Pu(III) couple was obtained from Lemire (*32*). All of the constants for the Pu(IV) species were corrected to an ionic strength of $I = 0$ (*33*).

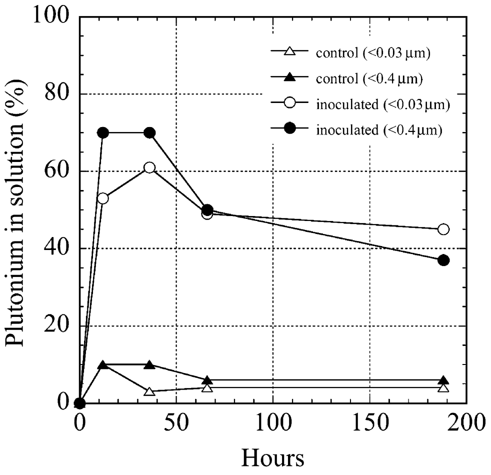

FIGURE 1. Dissolution of ^{242}Pu(IV) (1×10^{-7} M) by *Clostridium* sp.

Mechanisms of Pu Dissolution. Direct (enzymatic) and indirect (nonenzymatic) mechanisms of Pu dissolution in the bacterial growth medium was determined. Dissolution of Pu in the growth medium by *Clostridium* sp. was determined as described elsewhere (*24*). Briefly, 5×10^{-7} M ^{242}Pu-nitrate was added to (i) uninoculated prereduced, autoclaved growth medium (control), (ii) prereduced synthetic spent medium containing organic acids in the proportions found at logarithmic growth phase of the culture, (iii) unfiltered cell-free spent medium obtained from a 24 h bacterial culture (cells removed by centrifugation), (iv) 0.45 μm filtered cell-free spent medium, and, (v) to a 18 h-old culture. The samples were incubated for 24 h and were filtered through a 0.45 μm filter prior to analysis of Pu by LSC. All manipulations were performed inside an anaerobic glovebag.

Results

Dissolution of Pu by *Clostridium* sp. Figure 1 shows the solubilization of Pu concomitant with the growth of the bacteria. Adding ^{242}Pu (IV)-nitrate to the bacterial growth medium initially precipitated Pu as amorphous $Pu(OH)_4$ due to its hydrolysis and polymerization. After 12 h of incubation ~70% (7×10^{-8} M) of the total Pu passed through the 0.4 μm filter, while 53% (5.3×10^{-8} M) passed through the 0.03 μm filter; after 36 h of growth the values were 70% (7×10^{-8} M) and 61% (6.1×10^{-8} M), respectively. However, after 186 h of incubation the Pu passing through the 0.4 μm filter decreased to 45% (4.5×10^{-8} M) and 37% (3.7×10^{-8} M) through the 0.03 μm filter. Throughout the experiment, the total Pu remaining in the unfiltered control (uninoculated) sample was 1.8×10^{-8} M (18%) and 1.9×10^{-8} M (19%) in the inoculated sample which indicates the amount of Pu that was adsorbed on the surfaces of the serum bottles. At this time, ~6% (6×10^{-9} M) of the total Pu in the control sample was detected in solution after filtration through the 0.4 μm filter, and 4% (4×10^{-9} M) after passing through the 0.03 μm filter. The remaining Pu was retained on the filters.

The metabolism of glucose, change in pH, and production of carbon dioxide and organic acids by *Clostridium* sp. in the presence and absence of 1×10^{-7} M ^{242}Pu are shown in Figure 2A−D. Glucose was completely consumed at the rate of 0.4 mM h^{-1} in both samples in the presence and absence of Pu (Figure 2A). The pH of the Pu-containing uninoculated control sample remained constant at 6.4 during the experiment, while it decreased in the inoculated sample, both with

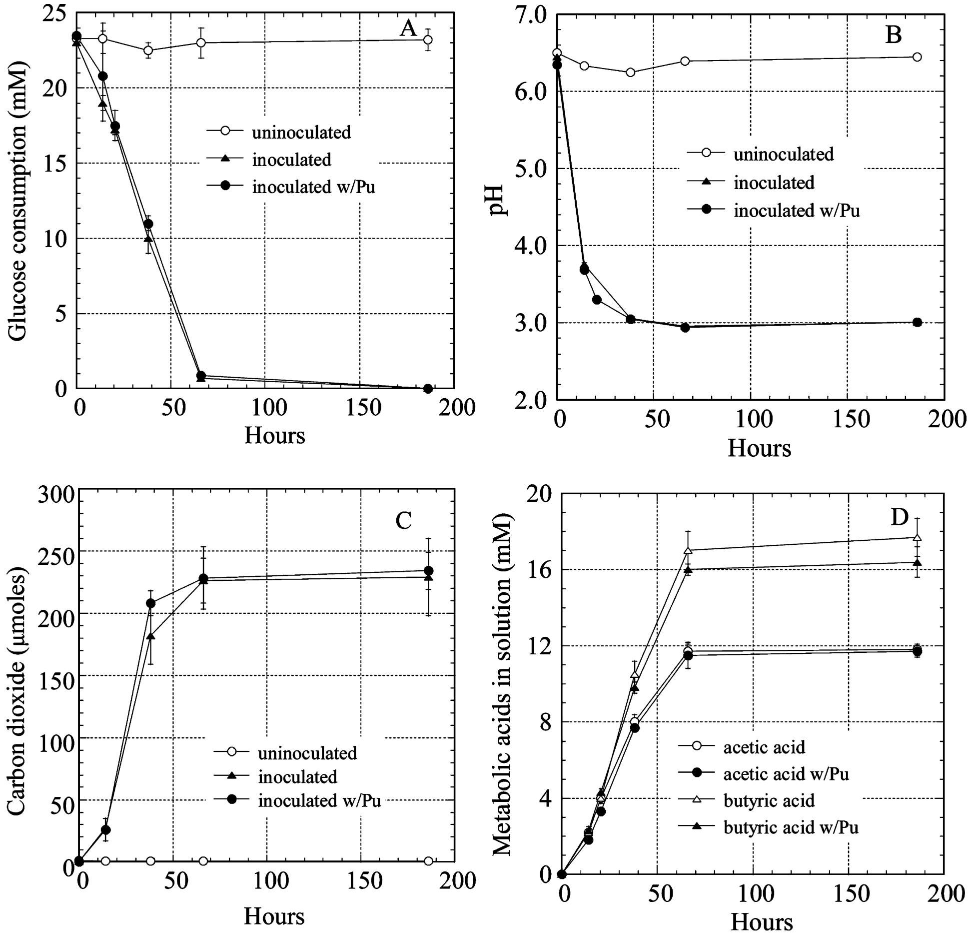

FIGURE 2. Glucose consumption (A), change in pH (B), production of carbon dioxide (C), and production of organic acids (D) during growth of *Clostridium* sp. in the presence of 1×10^{-7} M ^{242}Pu-nitrate.

and without Pu, from an initial value of 6.45 to a final value of 3.01 ± 0.02 at 186 h, indicating that Pu did not affect bacterial growth (Figure 2B); over the same time, the Eh of the medium decreased from +50 mV to −180 mV. Carbon dioxide production was similar in the presence (234 ± 15 µmoles) and absence (229 ± 31 µmoles) of Pu (Figure 2C). Hydrogen was also produced in stoichiometric amounts of 240 ± 28 and 227 ± 21 µmoles, respectively. The fermentation of glucose produced acetic and butyric acids (Figure 2D), the quantities of which were similar in the presence and absence of Pu. In a related experiment designed for oxidation state determination by XANES we also observed that addition of 5×10^{-5} M of ^{242}Pu(IV)-nitrate had no effect on the growth of *Clostridium* sp. as evidenced by similarities in quantities of glucose consumed, the decrease in pH and Eh, and in the amounts of carbon dioxide, hydrogen, and organic acids produced.

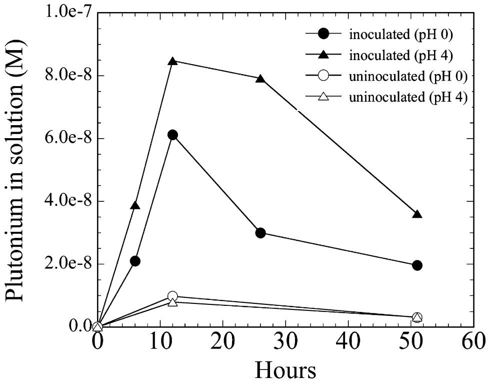

FIGURE 3. Selective extraction and speciation of Pu in the TTA organic fractions at pH 0 and 4 in 0.45 µm filtered solution from the uninoculated (control) and inoculated with *Clostridium* sp. samples. Total Pu concentration in the samples: 9.2 ± 1.2 x 10^{-8} M.

Plutonium Speciation by Selective Solvent Extraction. TTA extraction into the organic fractions at pH 0 and pH 4 after 0.45 µm filtration of the uninoculated (control) and inoculated medium with *Clostridium* sp. revealed the presence of a reduced Pu species in the soluble fraction (Figure 3). At pH 0 the Pu(IV) oxidation state is expected to be extracted into the organic fraction, while at pH 4 in addition to the Pu(IV), the Pu(III) and Pu(VI) oxidation states are also extracted. No detectable Pu was extracted from the control sample into the organic fraction at either pH. After 12 h incubation, a small amount of Pu(IV) was extracted at pH 0 (9.9 ± 0.9 × 10^{-9} M), and slightly less at pH 4 (8.0 ± 0.6 × 10^{-9} M). After 51 h incubation, the Pu concentration fell to 3.1 ± 0.1 × 10^{-9} M in the pH 0 and to 3.2 ± 0.8 × 10^{-9} M in the pH 4 fractions. In contrast, in the inoculated samples, we observed a significant increase in Pu concentration in both fractions. After 12 h of incubation at pH 0, 6.1 ± 1.0 × 10^{-8} M was extracted; after 51 h, activity had decreased to 2.0 ± 0.2 × 10^{-8} M. In the pH 4 fraction, the amount of Pu in solution reached a maximum value of 8.5 ± 1.1 × 10^{-8} M at 12 h, and decreased to 3.6 ± 0.3 × 10^{-8} M at 51 h. The greater activity observed at pH 4 reflected the presence of either Pu(III) or Pu(VI). However, these samples are in a highly reducing environment that favors the formation of the Pu(III) oxidation state. Total activity in the unfiltered sample was 9.2 ± 1.2 × 10^{-8} M. Less than 15% of the total Pu ($<1.3 \times 10^{-8}$ M) was

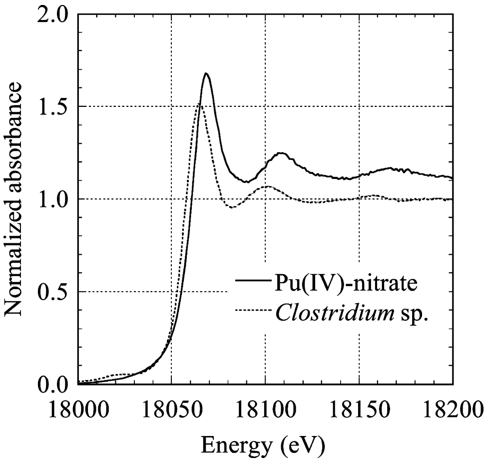

FIGURE 4. XANES analysis of Pu before (—) and after (····) anaerobic bacterial activity. Comparison of the sample's absorption edge position at (18.059 keV) with that for Pu(IV)-nitrate (18.062 keV) showed a shift of –3 eV, confirming the presence of Pu(III).

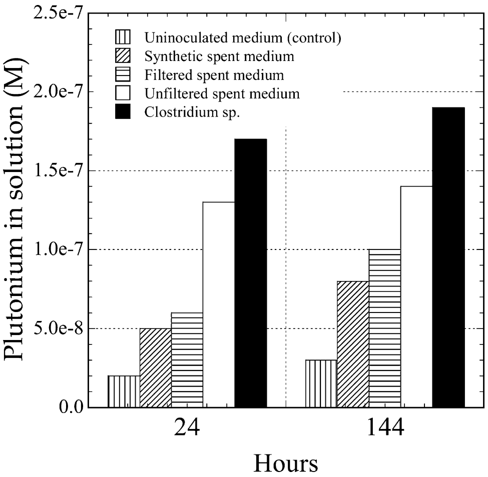

FIGURE 5. Dissolution of Pu(IV) by uninoculated medium (control), (ii) prereduced synthetic spent medium, (iii) unfiltered cell-free spent medium, (iv) 0.45 μm filtered cell-free spent medium, and (v) in the presence of actively growing cells.

extracted into the aqueous phases at both pHs, signifying that the polymeric forms were removed by filtration.

Plutonium Speciation by XANES Analysis. We determined the oxidation state of Pu in the freeze-dried culture sample by XANES analysis. The addition of 500 μg ^{242}Pu had little effect on growth of *Clostridium* sp., as described above. At 42 h the pH in the samples was 3.02, total gas production of 17 ± 2 mL, and the Eh was −185 mV. The inoculated control (no Pu) sample had similar values with a pH of 2.98, total gas production of 19 ± 2 mL, and an Eh of −182 mV. Prior to freeze-drying an aliquot of the solution was filtered through a 0.45 μm filter and the filtrate contained 63% of the added Pu while 27% of the Pu was retained on the filter, similar to the results obtained in the time-course experiment. The speciation of Pu by XANES analysis in samples incubated with *Clostridium* sp. is shown in Figure 4. Comparison of the XANES spectra shows the amplitude of the solid line for the Pu(IV) standard is greater than the bacterially treated sample (dotted line). The sample absorption edge position is displaced by 3 eV to lower energy at (18.059 keV) compared to the Pu(IV) nitrate (18.062 keV). This shift is similar to that observed for the trivalent Pu oxidation state and confirms the presence of Pu(III) (*34, 35*). No shoulder on the high-energy side of the absorption peak was observed ruling out the presence of a Pu(V) or Pu(VI) actinyl oxidation state.

Speciation calculations showed that $Pu(OH)_4$ dominates in the growth medium at pH 6.5. This poorly soluble species exists as an amorphous colloid that can be removed by filtration (log $K_{sp} = -58$) (*31, 36*). At pH 2.8, various Pu(IV) hydroxide species of the general formula $Pu(OH)_x^{4-x}$ (where $x = 1, 2$, or 3) may be formed. The acidic conditions (pH 3), low Eh (−180 mV), and elevated CO_2 levels (234 mM) in the culture's headspace space under pressure demonstrate that conditions for formation of Pu(III) is favored.

Mechanisms of Pu Dissolution. Figure 5 depicts the extent of Pu dissolution by the various biotic and abiotic treatments. After 144 h, 15% of the Pu from the uninoculated growth medium (control) at pH 5.7 was in solution, whereas in the synthetic medium at pH 3.3, 41% was present in solution. In the filtered spent medium 50% of the Pu was solubilized, whereas in the unfiltered spent medium (pH 3.1) 70% was solubilized. In the presence of cells at pH 2.9 95% of Pu was in solution. These results suggest that increase in bacterial activity as well as the components of bacterial activity enhanced the dissolution of Pu by reduction of Pu(IV) to Pu(III).

Discussion

The solution chemistry of plutonium is very complex, as it can exist in several oxidation states (III−VI). Under oxidizing condition Pu undergoes a disproportionation reaction and can exist simultaneously as Pu(IV), Pu(V), and Pu(VI). Although Pu is relatively immobile in the environment, its transport, albeit at very low concentrations has been observed at several Department of Energy sites and Mayak Production Association, Urals, Russia. For example, at Los Alamos National Laboratory's (LANL) waste site it was detected as Pu colloids (*37*), at the Nevada Test Site (NTS) it was associated with mineral colloids (*38*), at Rocky Flats, CO, the predominant form in the soil is $PuO_2(s)$ (*39*), and as colloids associated with organic macromolecules in surface waters (*40*); at Mayak Pu was bound to iron oxide colloids (*41*) , and at Maxey Flats, a former low-level radioactive waste site, it was observed in colloidal form and as a soluble tetravalent species complexed with organic ligands (*5*).

Microorganisms have been detected in low-level radioactive and TRU-wastes in Pu contaminated soils and in waste-repository sites being considered for nuclear-waste disposal (*1*). Microbes can affect the solubility and mobility of Pu (*1, 13*). Microbial activity can alter the oxidation states of Pu because of the very small differences in the redox potential between Pu(III), Pu(IV), Pu(V), and Pu(VI) (*32, 42*). In this study we have observed that the growth of *Clostridium* sp. lowered the Eh of the growth medium to −180 mV, and the pH from 6.2 to 3.0, concomitant with the generation of acetic- and butyric-acids, carbon dioxide, and hydrogen. After 14 h of growth, 70% of the Pu passed through a 0.4 μm filter and 55% passed through a 0.03 μm filter, suggesting that Pu (IV) is solubilized by *Clostridium* sp. Solvent extraction by thenoyltrifluoroacetone (TTA) confirmed a decrease in the polymeric form of Pu and an increase in the soluble fraction, indicating the presence of Pu(III). XANES analysis of the Pu-containing culture at the Pu L_{III} edge (18.054 keV) verified that the oxidation state was Pu(III). Brookins (*44*) has shown that trivalent Pu exists under low Eh and pH conditions; and that reduction took place only in the presence of growing cells, indicating direct bacterial action. In addition, thermodynamic calculations predict that Pu is transformed from amorphous $Pu(OH)_4$ to Pu(III). Pu (III) is relatively more soluble ($K_{sp} = 10^{-22.6}$) than Pu(IV) ($K_{sp} = 10^{-57.8}$) (*44, 45*). However, Pu(III) is unstable and is readily reoxidized to Pu(VI) which can exist as colloidal form (*5, 22*). Other work has shown that Pu(V) can exist along with Pu(III) at the low Eh and pH conditions (*46, 47*). However, we did not observe the presence of Pu(V). Although reductive dissolution of Pu(IV) by *Bacillus* sp. has been implied (*22*), to our knowledge, this is the first study to afford evidence of the bacterial reduction of Pu(IV) to Pu(III). Trivalent Pu has been detected in low-level radioactive waste leachates containing a variety of organic compounds and anaerobic bacteria including *Clostridium* sp. in a highly reducing environment (*5, 6, 48*). However, the dissolution of seemingly insoluble Pu(IV) by anaerobic bacteria under certain conditions, such as alternating oxic- and anoxic-conditions or under wet and dry cycles, can indeed remobilize Pu and facilitate its movement, even for short distances. Accordingly, the management of TRU wastes and long-term stewardship of remediated sites still containing residual Pu must take these processes into consideration.

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Literature Cited

(1) Francis, A. J. Microbial transformations of Pu and implications for its mobility. In *Plutonium in the Environment*; Kudo, A., Ed.; Elsevier Science Ltd.:New York, 2001.

(2) Francis, A. J. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. *Experientia* **1990**, *46*, 840–851.

(3) Husain, L.; Matuszek, J. M.; Hutchinson, J.; Wahlen, M. *Chemical and Radiochemical Character of a Low-Level Radioactive Waste Burial Site*; Carter, M. W., Moghissi, A. A., Kahn, B., Eds.; Pergamon Press:New York, 1979.

(4) Weiss, A. J.; Francis, A. J.; Colombo, P. *Management of Low-Level Radioactive Waste*; Carter, M. W., Moghissi, A. A., Kahn, B., Eds.; Pergamon Press: New York, 1979.

(5) Cleveland, J. M.; Rees, T. F. Characterization of plutonium in Maxey Flats radioactive trench leachates. *Science* **1981**, *212*, 1506–1509.

(6) Francis, A. J.; Dobbs, S.; Nine, B. J. Microbial activity of trench leachates from shallow-land, low-level radioactive waste disposal sites. *Appl. Environ. Microbiol.* **1980**, *40*, 108–113.

(7) Francis, A. J.; Dobbs, S.; Doering, R. F. Biogenesis of tritiated and carbon-14 methane from low-level radioactive waste. *Nucl. Chem. Waste Manage.* **1980**, *1*, 153–159.

(8) Barnhart, B. J.; Campbell, E. W.; Martinez, E.; Caldwell, D. E.; Hallett, R. *Potential Microbial Impact on Transuranic Wastes under Conditions Expected in the Waste Isolation Pilot Plant (WIPP)*, LA-8297-PR; Los Alamos National Laboratory: Los Alamos, NM, 1980.

(9) Au, F. H. F. The role of microorganisms in the movement of plutonium. In *The Dynamics of Plutonium in the Desert Environments*, Nevada Applied Ecology Group Progress Report NVO-42; 1974.

(10) Beckert, W. F.; Au, F. H. F. Plutonium uptake by a soil fungus and transport to its spores. In *Transuranium Nuclides in the Environment*, IAEA-SM-199/72; 1976.

(11) Brainard, J. R.; Strietelmeier, B. A.; Smith, P. H.; Unkefer, P. J.; Barr, M. E.; Ryan, R. R. Actinide binding solubilization by microbial siderophores. *Radiochim. Acta* **1992**, *58/59*, 357–363.

(12) Neu, M. P.; Ruggiero, C. E.; Francis, A. J. Bioinorganic Chemistry of Plutonium and Interactions of Plutonium with microorganisms and lants. In *Advances in Plutonium Chemistry 1967–2000*; Hoffman, D. Ed.; ANS and University Research Alliance,: La Grange Park IL and Amarillo, TX, 2002.

(13) Neu, M. P.; Icopini, G. A.; Boukhalfa, H. Plutonium speciation affected by environmental bacteria. *Radiochim. Acta* **2005**, *93*, 705–714.

(14) Wildung, R. E.; Garland, T. R. The relationship of microbial processes to the fate and behavior of transuranic elements in soils, plants, and animals. In *Transuranic Elements in the Environment*, DOE/TIC-22800; Hanson, W. C.,Ed.; Technical Information Center/US Department of Energy: Washington, DC,1980.

(15) Ruggiero, C. E.; Matonic, J. H.; Reilly, S. D.; Neu, M. P. Dissolution of Pu(IV) hydroxide by desferrioxamine siderophores and simple organic chelators. *Inorg. Chem.* **2002**, *41*, 3593–3595.

(16) Francis, A. J.; Gillow, J. B.; Dodge, C. J.; Dunn, M.; Mantione, K.; Strietelmeier, B. A.; Pansoy-Hjelvik, M. E.; Papenguth, H. W. Role of microbes as biocolloids in the transport of actinides from a deep underground radioactive waste repository. *Radochim. Acta* **1998**, *82*, 347–354.

(17) Banaszak, J. E.; Rittmann, B. E.; Reed, D. T. Subsurface interactions of actinide species and microorganisms: Implications for bioremediation of actinide-organic mixtures. *J. Radioanal. Nucl. Chem.* **1999**, *241*, 385–435.

(18) John, S. G.; Ruggiero, C. E.; Hersman, L. E.; Tung, C. S.; Neu, M. P. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). *Environ. Sci. Technol.* **2001**, *35*, 2942–2948.

(19) Panak, P. J.; Booth, C. H.; Caulder, D. L.; Bucher, J. J.; Shuh, D. K.; Nitsche, H. X-ray absorption fine structure spectroscopy of plutonium complexes with *Bacillus sphaericus*. *Radiochim. Acta* **2002**, *90*, 315–322.

(20) Keith-Roach, M. J.; Day, J. P.; Fifield, L. K.; Bryan, N. D.; Livens, F. R. Seasonal variations in interstitial water transuranium element concentrations. *Environ. Sci. Technol.* **2000**, *34*, 4273–4277.

(21) Ohnuki, T.; Yoshida, T.; Ozaki, T.; Kozai, N.; Sakamoto, F.; Nankawa, T.; Suzuki, Y.; Francis, A. J. Chemical speciation and association of plutonium with bacteria, kaolinite clay, and their mixture. *Environ. Sci. Technol.* **2007**, *41*, 3134–3139.

(22) Rusin, P. A.; Quintana, L.; Brainard, J. R.; Strietelmeier, B. A.; Tait, C. D.; Ekgerg, S. A.; Palmer, P. D.; Newton, T. W.; Clark, D. L. Solubilization of plutonium hydrous oxide by iron-reducing bacteria. *Environ. Sci. Technol.* **1994**, *28*, 1686–1690.

(23) Francis, A. J.; Dodge, C. J. Anaerobic microbial dissolution of transition and heavy metal oxides. *Appl. Environ. Microbiol.* **1988**, *54*, 1009–1014.

(24) Francis, A. J.; Dodge, C. J.; Lu, F.; Halada, G.; Clayton, C. R. XPS and XANES studies of uranium reduction by *Clostridium* sp. *Environ. Sci. Technol.* **1994**, *28*, 636–639.

(25) Francis, A. J.; Dodge, C. J.; Meinken, G. E. Biotransformation of pertechnetate by *Clostridia*. *Radiochim. Acta* **2002**, *90*, 791–797.

(26) Currie, L. A. Limits for qualitative and quantitative determination: Application to radiochemistry. *Anal. Chem.* **1968**, *40*, 116–131.

(27) Schramke, J. A.; Rai, D.; Fulton, R. W.; Choppin, G. R. Determination of aqueous plutonium oxidation states by solvent extraction. *J. Radioanal. Nucl. Chem.* **1989**, *130*, 333–346.

(28) Parkhurst, D. L.; Appelo, C. A. J. *User's Guide to PHREEQC (Version 2)-A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations*, Report 99–4259; United States Department of the Interior: Denver, Colorado, 1999.

(29) Nuclear Energy Agency (NEA). Thermochemical Database Project (TDB); 2005; http://www.nea.fr/html/dbtdb/.

(30) Rai, D.; Hess, N. J.; Felmy, A. R.; Morre, D. A.; Yui, M.; Vitorge, P. A thermodynamic model for the solubility of PuO_2(am) in the aqueous K^+-HCO_3^--CO_3^{2-}-OH^--H_2O system. *Radiochim. Acta* **1999**, *86*, 89–99.

(31) Neck, V.; Kim, J. I. Solubility and hydrolysis of tetravalent actinides. *Radiochim. Acta* **2001**, *89*, 1–16.

(32) *Chemical Thermodynamics of Neptunium and Plutonium*; Lemire, R. J.,Ed.; Elsevier:New York, 2001.

(33) Essington, M. E. *Soil and Water Chemistry: An Integrative Approach*; CRC Press: New York, 2004.

(34) Allen, P. G.; Bucher, J. J.; Shuh, D. K.; Edelstein, N. M.; Reich, T. Investigation of aquo and chloro complexes of UO_2^{2+}, NpO_2^+, Np^{4+}, and Pu^{3+} by X-ray absorption fine structure spectroscopy. *Inorg. Chem.* **1997**, *36*, 4676–4683.

(35) Conradson, S. D.; Mahamid, I. A.; Clark, D. L.; Hess, N. J.; Hudson, E. A.; Neu, M. P.; Palmer, P. D.; Runde, W. H.; Tait, C. D. Oxidation state determination of plutonium aquo ions using x-ray absorption spectroscopy. *Polyhedron* **1998**, *17*, 599–602.

(36) Fanghanel, Th.; Neck, V. Aquatic chemistry and solubility phenomena of actinide oxides/hydroxides. *Pure Appl. Chem.* **2002**, *74*, 1895–1907.

(37) Penrose, W. R.; Polzer, W. L.; Essington, E. H.; Nelson, D. M.; Orlandini, K. A. Mobility of plutonium and americium through shallow aquifer in a semiarid region. *Environ. Sci. Technol.* **1990**, *24*, 228–234.

(38) Kersting, A. B.; Efurd, D. W.; Finnegan, D. L.; Rokop, D. K.; Smith, D. K.; Thomson, J. L. Migration of plutonium in groundwater at the Nevada Test Site. *Nature* **1999**, *397*, 56–59.

(39) Clark, D. L.; Janecky, D. R.; Lane, L. J. Science-based cleanup of Rocky Flats. *Phys. Today* **2006**, *59*, 34–40.

(40) Santschi, P. H.; Roberts, K. A.; Guo, L. Organic nature of colloidal actinides transported in surface water environments. *Environ. Sci. Technol.* **2002**, *36*, 3711–3719.

(41) Noviko, A. P.; Kalmykov, S.N.; Utsunomyia, S.; Ewing, R. C.; Horreard, F.; Merkulov, A.; Clark, S. B.; Tkachev, V. V.; Myasoedov, B. F. Colloid transport of plutonium in the far-field of the Mayak Production Association, Russia. *Science* **2006**, *314*, 638–641.

(42) Choppin, G. R. Utility of oxidation state analogs in the study of plutonium behavior. *Radiochim. Acta* **1999**, *85*, 89–95.

(43) Brookins, D. G. Eh-pH Diagrams for Geochemistry; Springer-Verlag: New York,1988.

(44) Kim, J. I.; Kanellakopulus, B. Solubility products of plutonium(IV) oxide and hydroxide. *Radiochim. Acta* **1989**, *48*, 145–150.

(45) Morse L. R. Thermodynamic properties. In *The Chemistry of the Actinides Elements* , 2nd ed.; Katz, J. J., Seaborg, G. T. Morse, L. R., Eds; Chapman and Hall: New York, 1986.

(46) Neck, V.; Altmaier, M.; Seibert, A.; Yun, J. I.; Marquardt, C. M.; Fanghanel, Th. Solubility and redox reactions of Pu(IV) hydrous oxide: Evidence for the formation of PuO_{2+x}(s, hyd.). *Radiochim. Acta* **2007**, *95*, 193–207.

(47) Yun, J-.I.; Cho, H.-R.; Neck, V.; Altmaier, M.; Seibert, A.; Marquardt, C. M.; Walther, C.; Fanghanel, Th. Investigation of the hydrolysis of plutonium(IV by a combination of spectroscopy and redox potential measurements. *Radiochim. Acta* **2007**, *95*, 89–95.

(48) Francis, A. J.; Iden, C. R.; Nine, B. J.; Chang, C. K. Characterization of organics in leachates from low-level radioactive waste disposal sites. *Nucl. Technol.* **1980**, *50*, 153–163.

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