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Biotransformation of plutonium complexed with citric acid

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Summary. The presence of organic ligands in radioactive wastes is a major concern because of their potential for increasing the transport of radionuclides from disposal sites. Biotransformation of radionuclides complexed with organic ligands should precipitate the radionuclides and retard their migration. We investigated the biotransformation of Pu(IV) (10^{-8} to 10^{-5} M), by *Pseudomonas fluorescens* in the presence of excess citric acid. Analysis of ^{242}Pu-citrate by electrospray ionization-mass spectrometry (ESI-MS) indicated the presence of biligand Pu-cit$_2$ as the predominant complex. XANES and EXAFS analyses showed that Pu was in the +4 oxidation state and associated with citric acid as a mononuclear complex. Citric acid was metabolized by *P. fluorescens* at a rate of 4.9 μM/h, but in the presence of 10^{-8} and 10^{-6} M Pu, this rate decreased to 4.0 and 3.8 μM/h, respectively. An increase in the ionic strength of the medium from 0.18 M to 0.9 M lowered citrate metabolism by ∼ 65%. Pu added to the growth medium in the absence of bacteria remained as Pu(IV) in solution as a complex with citric acid. However, solvent extraction by thenoyltrifluoroacetone (TTA) and microfiltration (0.03 μm) of the medium containing bacteria after citrate biodegradation revealed the presence of polymeric Pu. The extent of formation of the Pu polymer depended on the Pu : citrate ratio, the extent of citrate metabolism, and the ionic strength of the medium

Introduction

The presence of actinides (U, Np, Pu, and Am) together with natural organic compounds, including chelating agents, in transuranic (TRU)- and mixed-wastes are a major concern because of their potential for migrating from the waste repositories and contaminating the environment. Among the actinides, Pu is the major problematic radioactive component in TRU waste and has received considerable attention. It exists in several oxidation states (III, IV, V, VI) and as various chemical species (salts, organic complexes, colloids) with a very complex chemistry and environmental behavior. Soil pH, presence of organics, redox conditions, mineralogy, and microbial activity all affect the chemical speciation of Pu.

Chemical characterization of Pu at contaminated sites shows that its environmental form varies according to conditions at the site and in the waste stream. For example, at Rocky Flats (RF), CO, the predominant form appears to be as PuO_2(s); at the Nevada Test Site (NTS) Pu was associated with mineral colloids; while, at Oak Ridge, TN, it is associated with organic matter [1, 2]. Pu is generally considered to be relatively immobile; however, its transport, albeit at very low concentrations, was observed at several DOE sites including RF, Los Alamos National Laboratory (LANL), and NTS. Plutonium in surface waters at the RF site was shown to be associated with organic macromolecules [3].

Chelating agents, such as citric acid, are present in TRU and mixed wastes because they are widely used for decontaminating nuclear reactors and equipment, in cleanup operations, and in separating radionuclides. In addition to other organic ligands, an estimated 1950 moles of citrate is present in the Waste isolation Pilot Plant (WIPP) inventory of TRU waste [4]. Citric acid forms a strong complex with Pu(IV) and has been used in chelation therapy, and in extracting ^{239}Pu from contaminated soil [5–7]. Citric acid doubled the efficiency of removing plutonium and americium from contaminated soils at RF site [8]. Microorganisms bring about the dissolution of Pu by producing organic acids, such as citric acid, extracellular metabolites, and siderophores [9–11]. The microbial metabolite desferal (a polyhydroxamate chelate), and desferrioximine and enterobactin isolated from *Escherichia coli* were shown to dissolve plutonium hydroxide and plutonium oxyhydroxide, respectively [12]. However, citric acid was more effective at solubilizing amorphous plutonium hydroxide than the microbial siderophores [13, 14]. Microorganisms grown in the presence of plutonium produced complexing agents [15] that may transport plutonium into the cells [16–19]. Thus, the potential exists for microorganisms to solubilize plutonium in wastes, thereby increasing its bioavailability and mobility.

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 [20, 21]. ^{238,239,240}Pu (gross alpha activity of 1.7×10^5 pCi/L) was found in leachates collected from the low-level radioactive-waste disposal sites at West Valley, NY, and Maxey Flats, KY [22, 23]. Several aerobic- and anaerobic-bacteria were isolated from the leachate samples, among them, *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp. [21]. The radioactive- and organic-chemicals in the leachate were not toxic to the bacteria, which metabolized them, producing tritiated- and carbon-14 methane [20]. Metabolically active microbes

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were identified at the LANL's TRU waste burial site containing ^{239}Pu-contaminated soil and flammable waste [13]. Little is known of the biotransformation of Pu complexed with natural organic compounds and chelating agents. Biodegradation should precipitate the released ions as water-insoluble hydroxides, oxides, or salts, thereby retarding their migration. In this study, we investigated the biotransformation and fate of Pu complexed with citric acid under aerobic conditions. The factors affecting citrate metabolism and the nature of the Pu species following biodegradation also were investigated.

Experimental section

Culture

Pseudomonas fluorescens biovar II (ATCC 55241) was grown in a modified Simmon's citrate medium containing the following (g): citric acid, 2; $MgSO_4$, 0.2; NH_4Cl, 1; glycerol phosphate, 1.5; NaCl, 5; deionized water to one liter. The pH of the medium was adjusted to 6.5 with NaOH and 100 mL aliquots were transferred to 250 mL Erlenmeyer flasks. The medium was autoclaved, cooled, and inoculated with 4 mL of a 24-h old culture (OD 0.8). The culture was incubated at $26 \pm 1\,^{\circ}$C on a rotary shaker for 24 h. The cells then were separated by centrifugation, washed $2\times$ with sterile 0.1 M KCl/0.01 M PIPES solution adjusted to pH 6.5, and resuspended in KCl. Cell density was determined by measuring the turbidity at 600 nm; viable colony forming units (CFU) were assayed using the same medium with 1.5% agar.

Citric acid

Citric acid (anhydrous) was obtained from Sigma, MO. Radiolabeled 1-5-^{14}C-citric acid was obtained from Amersham Biosciences, NJ. A stock solution of citric acid was spiked with radiolabeled citric acid to give a final concentration of 2.23×10^{-6} M ^{14}C-citric acid, and 8.2×10^{-3} M ^{12}C-citric acid. The pH was adjusted to 6.5 with potassium hydroxide.

Pu-nitrate stock solution

Plutonium as $^{242}Pu(NO_3)_4$ (CRM No. 130) was obtained from New Brunswick Laboratories, Argonne, IL. One milligram of Pu was dissolved in 8 M nitric acid by heating in a Teflon bottle on a hot plate at 130 °C in a HEPA filtered hood; the sample was diluted to 10 ml with deionized water to give a final concentration of 4.13×10^{-4} M Pu. The solution was assayed by liquid scintillation counting (LSC; Wallac Winspectral Guardian 1414).

Pu-citrate complex

Plutonium-citrate complexes (1 : 10, 1 : 100, 1 : 1000, 1 : 10 000) were prepared by adding plutonium at varying concentrations (10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M) to a beaker containing a final concentration of 10^{-4} M citric acid in 10 mM PIPES buffer to maintain the pH at 6.5. The stability of Pu in the abiotic stock solutions containing 1 : 10 and 1 : 100 Pu : citric acid complexes was determined by analyzing its activity in 0.1 μm filtered and unfiltered aliquots. The activity of the Pu in solution was determined by LSC.

Pu speciation

The distribution of the various Pu(IV) species in the sample was determined by PHREEQCi, a computer program used for chemical speciation calculations [24]. The Pu(IV) speciation calculations included hydrolysis and carbonate complexation reactions [25, 26]. 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 [27]. In addition, the $Pu(OH)_2(CO_3)_2^{2-}$ species ($\log \beta_{1,2,2} = 44.76$ [28]) and the $Pu(CO_3)_3^{2-}$ species ($\log \beta_{1,3} = 31.5$ [29]) were included in the calculations. The stability constants for the 1 : 1 Pu-citrate complex ($Pu^{4+} + cit^{3-} \longleftrightarrow Pucit^{+}$ ($\log \beta_{1,1} = 19.11$) and for the 1 : 2 Pu-citrate complex ($Pu^{4+} + 2cit^{3-} \longleftrightarrow Pucit_2^{2-}$ ($\log \beta_{1,2} = 34.1$) were obtained from Kantar and Honeyman [30] and Nebel [31], respectively. The constants for the Pu(IV) species were corrected to zero ionic strength using the Davies equation [32].

Characterization of Pu : citric-acid complex

We used X-ray absorption near edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS) to determine the oxidation state of the Pu and the nature of Pu complexation with citric acid. Analysis was performed at the Pu L_{III} absorption edge (18.056 keV). An aqueous sample of 1 : 10 Pu : citric acid (as prepared above) was freeze-dried onto Kapton tape and mounted on an Al sample holder having a cut-out geometry of 2 mm(H) × 20 mm(L) × 1.5 mm thick. Fluorescence data was collected with a 13-element Ge detector on beamline X10C at the National Synchrotron Light Source (NSLS). The shift in the beam's energy position was monitored using a reference Zr foil and the absorption edge energy for the Pu-citrate was determined by comparison with the first inflection point of a Pu(IV)-nitrate standard. ATHENA software was used to analyze the data from the EXAFS spectra through a multistep procedure that included background subtraction using the AUTOBACK program and normalization to the edge-jump height followed by Fourier transformation of the k^3-weighted (2.5–12.5 Å) spectrum [33]. Theoretical phases and amplitudes were generated using FEFF6 and were used to fit the contributions from the single-scattering paths for the O and C neighboring atoms. The stoichiometry of the Pu : citric acid complex was assessed using electrospray ionization-mass spectroscopy (ESI-MS). One mg of plutonium nitrate was dissolved in 1.6×10^{-3} M citric acid by heating on a hot plate at 130 °C for several hours. Although the Pu-nitrate did not dissolve completely, the final concentration of the Pu in the solution was 1.6×10^{-4} M, as determined by LSC analysis. The Pu-citrate sample was introduced into a Thermo-Finnigan LCQ Advantage LC-MS system at a rate of 10 μL min^{-1} using a microsyringe pump and analyzed by ESI-MS. Operating parameters for the ESI, including gas flow rate, operating voltage, and current were

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optimized using an equimolar 10^{-3} M Cu(II)-citrate complex. The ESI source was operated in the negative-ion mode for citric acid, and in the positive-ion mode for the Pu-citrate complex.

Biodegradation of citric acid and Pu-citrate by *Pseudomonas fluorescens* cells

A 5 ml aliquot of the Pu-citrate complex solution was added to duplicate 25 ml incubation flasks (Kimble–Kontes, NJ), and the flasks were fitted with a polypropylene reservoir containing 0.35 ml 0.2 M potassium hydroxide solution suspended above the solution. Bacterial cells, obtained as described above, were resuspended in 0.1 M KCl/0.01 M PIPES buffer, and an aliquot was added to obtain a final OD of 0.1. The viable colony forming units (CFU) were $2.93 \pm 1.69 \times 10^7$ cells/ml. The samples were placed in an incubator in the dark at 26 °C.

Chemical and microbiological analyses

Citric acid metabolism. Periodically, the ^{14}C-carbon dioxide in the KOH solution was analyzed by LSC using Ultima Gold XR scintillation cocktail (Perkin Elmer, MA). The reservoir was replenished with fresh KOH solution after each sampling.

Speciation of plutonium. An aliquot of the sample was removed at periodic intervals, scintillation cocktail was added, and the total Pu in the unfiltered sample was determined by LSC. A separate aliquot was removed and filtered in parallel through 0.4 μm and 0.03 μm Poretics polycarbonate filters using an Amicon (Millipore, MA) stirred cell.

Pu oxidation state. The oxidation states for plutonium in solution after bacterial activity were determined by a solvent extraction technique using thenoyltrifluoroacetone (TTA) extraction at pH 0 and pH 4 [34].

pH. The pH of the culture solution was determined on an unfiltered aliquot using a Mettler–Toledo MP220 pH meter and a DG-111SC liquid-junction electrode filled with 3 M KCl (saturated with AgCl).

Results and discussion

Pu-citrate solubility and speciation

In Table 1 the solubility of 1 : 10 and 1 : 100 Pu-citrate species is shown. The extent of hydrolysis or polymerization of Pu is indicated by its retention on the 0.1 μm filter. Retention of Pu by 0.1 μm filter in the first 4 hours in the presence of 1 : 10 Pu : citric acid was 3% and at 100-h, it increased to 5%. In the presence of 1 : 100 Pu : citric acid, 3% was retained on the filter at 4-h and increased to 12% at 100-h. This indicates the extent of hydrolysis of Pu with both time and Pu concentration in the control sample.

Table 1. Distribution of ^{242}Pu in Pu-citrate stock solutions at pH 6.5 without the bacteria.

Complex	Treatment	^{242}Pu (dpm)		
		0 h	4 h	100 h
1 : 10 Pu : citric acid	unfiltered	1930	–	1750
	pass 0.1 μm	–	1750	1610
	retained by filter	–	59	88
1 : 100 Pu : citric acid	unfiltered	287	–	223
	pass 0.1 μm	–	257	199
	retained by filter	–	9	28

–: not determined.

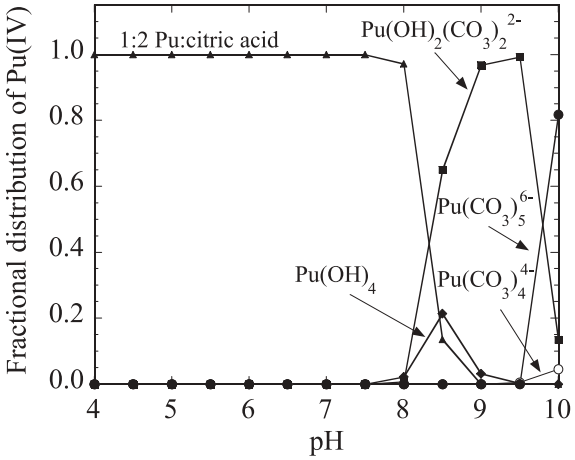


Fig. 1. Equilibrium thermodynamic modeling of the distribution of aqueous phase plutonium (10^{-5} M) species in equilibrium with air ($p_{CO_2} = 10^{-3.5}$ atm.) and ten-fold excess citric acid at pH 6.5.

Fig. 1 illustrates the result of a speciation calculation of the 10^{-5} M 1 : 10 Pu : citric acid complex as a function of pH. In the presence of excess citric acid, calculations show that the predominant form for Pu is as the biligand 1 : 2 Pu : citric acid complex. Carbonate and hydroxo species, including the amorphous $Pu(OH)_4$ phase, were considered in the calculations; they comprised less than 1% of the total plutonium species at pH 6.5.

Characterization of Pu : citric-acid complex

The XANES spectra for Pu(IV)-nitrate and Pu-citrate complexes are presented in Fig. 2. The first derivative of the absorption-edge energy for the Pu-citrate complex is at 18 060 eV. This is identical to that for Pu(IV)-nitrate, thereby confirming the tetravalent oxidation state of the Pu-citrate complex.

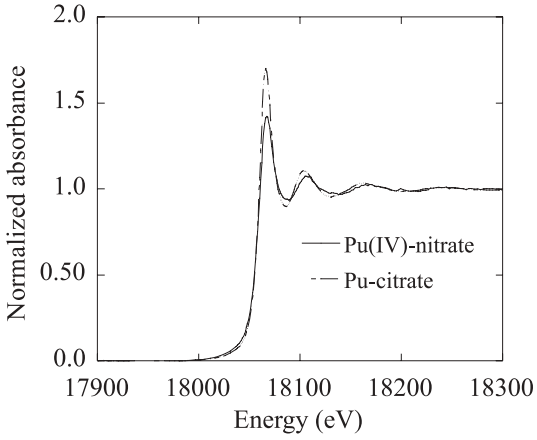


Fig. 2. Comparison of XANES spectra for Pu(IV)-nitrate standard with Pu : citric-acid complex.

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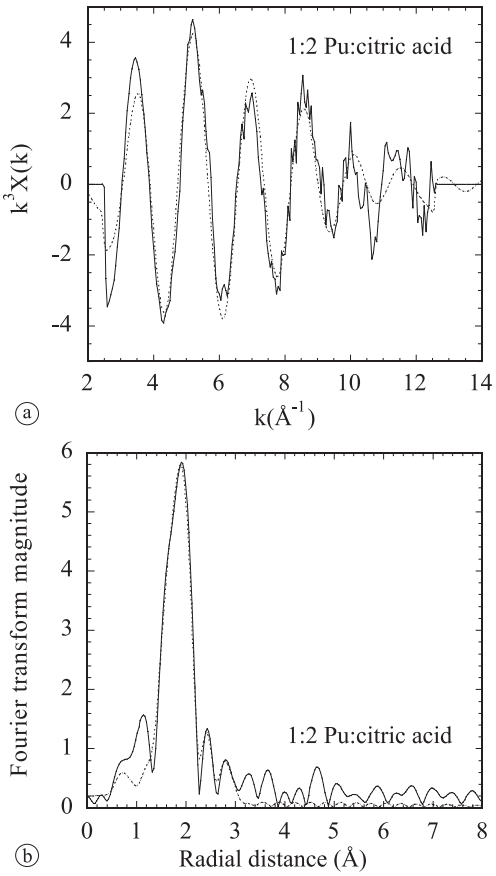


Fig. 3. EXAFS analysis for 1 : 2 Pu : citric acid complex at pH 6.5. Experimental data (- - - -); fitted data (——).

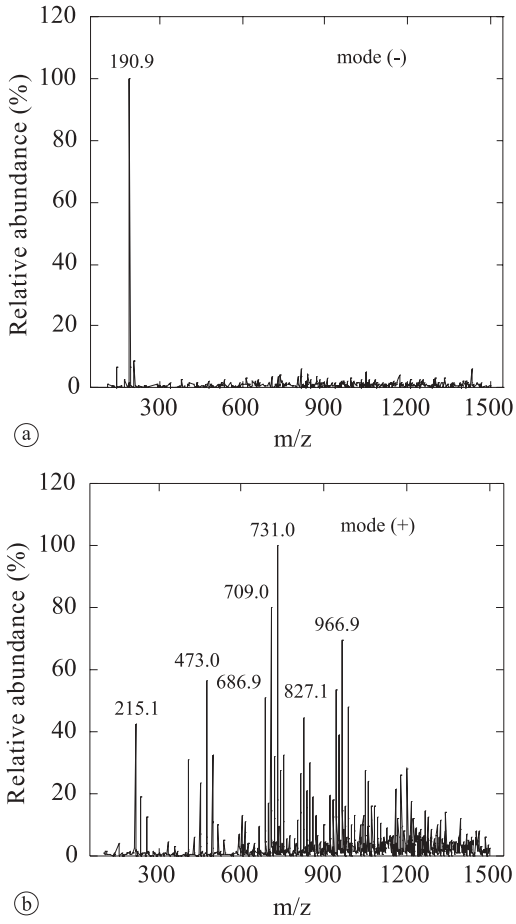


Fig. 4. Electrospray ionization (ESI-MS) spectra in the (-) mode (**a**), and (+) mode (**b**) showing the molecular ions (m/z) produced by citric acid and the Pu-citrate complex at pH 6.5.

Figs. 3a and b shows the k^3-weighted raw EXAFS spectrum and the Fourier transform for the Pu-citrate complex. Table 2 shows the fitting parameters for the complex. The inner-sphere coordination number for Pu is 10.0 and includes three distinct O shells. There are 5.0 ± 1.2 O's at 2.26 ± 0.02 Å, 3.5 ± 0.8 O's at 2.41 ± 0.02 Å, and 1.5 ± 0.8 O atoms at 2.69 ± 0.02 Å. The atoms closest to the Pu at 2.26 Å are due to the presence of water and other uncoordinated oxygens. The 3.5 O atoms at 2.41 Å are coordinated to the Pu through the carboxylate oxygens of two citric acid molecules. The O atoms at 2.69 Å are most probably due to coordination with the α-hydroxyl oxygens of citric acid. No Pu−Pu interaction typical for Pu(IV)-hydroxo complexes is observed. Based upon this analysis the best configuration for the molecule consists of a mononuclear biligand complex.

Figs. 4a and b give the ESI-MS spectra for citric acid and the 1.6×10^{-4} M Pu-citrate complex. Fig. 4a shows the spectrum for the singly charged negative ions generated by ionization of the citric acid (MW 192.0). The appearance of a peak at m/z 190.9 suggests that the [H_2cit]-species is present. Fig. 4b shows the spectrum generated for singly charged positive ions peaks for the Pu-citrate complex. The dominant peaks are due to the formation of a monomeric Pu-citrate complex at m/z 473.0 $[Pucit(H_2O)Na]^+$ and biligand complex formation is indicated by the presence of peaks at m/z 686.9 $[Pu(H_2cit)_2NO_3]^+$, m/z 709.0 $[Pu(Hcit)(H_2cit)NaNO_3]^+$, and m/z 731.0 $[Pu(Hcit)_2Na_2NO_3]^+$. The presence of a complex containing two Pu atoms is noted at m/z 966.9 $[Pu_2(Hcit)(cit)(H_2O)_4(OH)_2]^+$. The m/z values are considered accurate to within 1 or 2 amu's. Based upon the speciation calculations, X-ray absorption spectra, and LC-MS data, we propose a biligand [$Pu(cit_2)$] complex structure, similar to that suggested by Metivier and Guillaumont [5] (Fig. 5).

Table 2. EXAFS structural parameters for 1 : 2 Pu(IV) : citric acid complex at pH 6.5.

Sample	Atom	N	R (Å)	σ^2	E_0
1 : 2 Pu(IV) : citric acid	Pu−O	5.0 ± 1.2	2.26 ± 0.02	0.008 ± 0.002	6.5 ± 1.5
	Pu−O	3.5 ± 0.8	2.41 ± 0.02	0.005 ± 0.002	5.0 ± 2.3
	Pu−O	1.5 ± 0.8	2.69 ± 0.02	0.005 ± 0.002	7.0 ± 1.6
	Pu−C	4.2 ± 1.5	3.27 ± 0.02	0.010 ± 0.003	5.0 ± 1.3

N: coordination number;
R: interatomic distance;
σ^2: disorder parameter.

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Fig. 5. Proposed structure at pH 6 for the 1 : 2 Pu : citric-acid complex $[PuO(cit)_2]^{4-}$. The open circles represent oxygen and the filled circles represent carbon atoms.

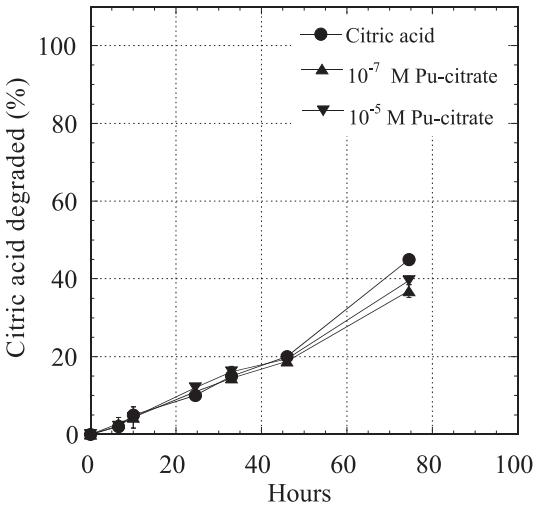


Fig. 7. Effect of adding ^{242}Pu on citrate metabolism (ionic strength 0.9 M). Citric-acid concentration is 10^{-4} M in all treatments.

Biodegradation of citric acid and Pu-citrate complexes

Fig. 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 acid (10^{-4} M) in the absence of Pu was metabolized completely at a rate of 4.9 μM/h. With 10^{-6} and 10^{-8} M Pu present as the Pu-citrate complex we observed a slight decrease in 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 μM/h and 3.8 μM/h, respectively. Increasing the ionic strength of the medium affected citrate metabolism. In high ionic-strength medium (0.9 M) containing 10^{-7} and 10^{-5} M Pu present as Pu-citrate complex, the rate and extent of citrate degradation was much lower at 0.8 μM/h in the 10^{-7} and 10^{-5} M Pu treatments with an overall degradation of 37% and 35%, respectively (Fig. 7). Without Pu, there was an increase in degradation to 45% of the total citric acid concentration.

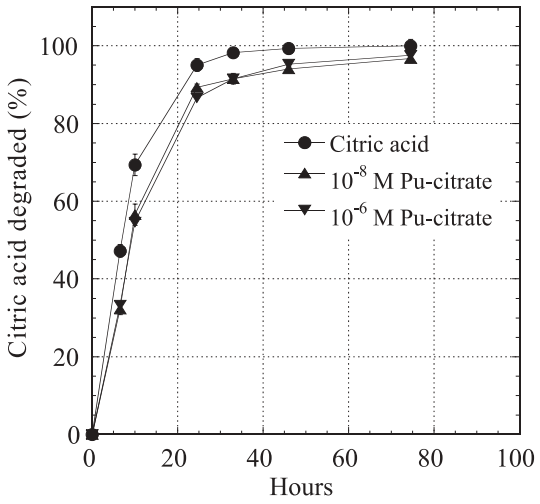


Fig. 6. Effect of adding ^{242}Pu on citrate metabolism (ionic strength 0.18). Citric-acid concentration is 10^{-4} M in all treatments.

Speciation of Pu before and after bacterial activity

Thenoyltrifluoroacetone (TTA) was used to determine the oxidation state of Pu in the unfiltered samples following 70 hours of bacterial activity. Extraction of the low ionic strength sample containing 10^{-6} M Pu at pH 4 showed 70% of the Pu was present as polymeric form with the remainder most probably as Pu^{4+} (Table 3). Although the TTA extraction also suggests the presence of Pu(V), it is more likely that the tetravalent form is present as indicated by the XANES data. Extraction of the medium at pH 0 reveals that the predominant form of Pu (76%) is the polymer species. Extracting the high ionic strength 10^{-5} M Pu at pH 4 indicated that Pu was predominantly polymeric, similar to the 10^{-6} M Pu sample. The speciation at pH 0 is similar to the results obtained at pH 4, with the polymeric species comprising 80% of the total Pu in the sample. The predominance of polymeric form of Pu in both treatments is most probably due to the biodegradation of citric acid which was almost completely metabolized at 10^{-6} M Pu and 40% biodegraded in the 10^{-5} M Pu treatments. In addition, the higher concentration of Pu in the 10^{-5} M sample may enhance polymerization reactions.

Table 3. Speciation of Pu after 70 hours incubation with *P. fluorescens*.

Analysis	Oxidation state	Pu	
		Total activity (dpm)	Total extracted (%)
10^{-6} M Pu			
TTA_{org} (pH 4)	+3, +4, +6	64.5 ± 3.5	30 ± 5
TTA_{aq} (pH 4)	+5, polymer	154 ± 17	70 ± 11
TTA_{org} (pH 0)	+4	66 ± 12	24 ± 5
TTA_{aq} (pH 0)	+3, +5, +6, polymer	206 ± 10	76 ± 4
10^{-5} M Pu			
TTA_{org} (pH 4)	+3, +4, +6	501 ± 44	19 ± 9
TTA_{aq} (pH 4)	+5, polymer	2110 ± 220	81 ± 10
TTA_{org} (pH 0)	+4	443 ± 18	20 ± 4
TTA_{aq} (pH 0)	+3, +5, +6, polymer	1740 ± 180	80 ± 10

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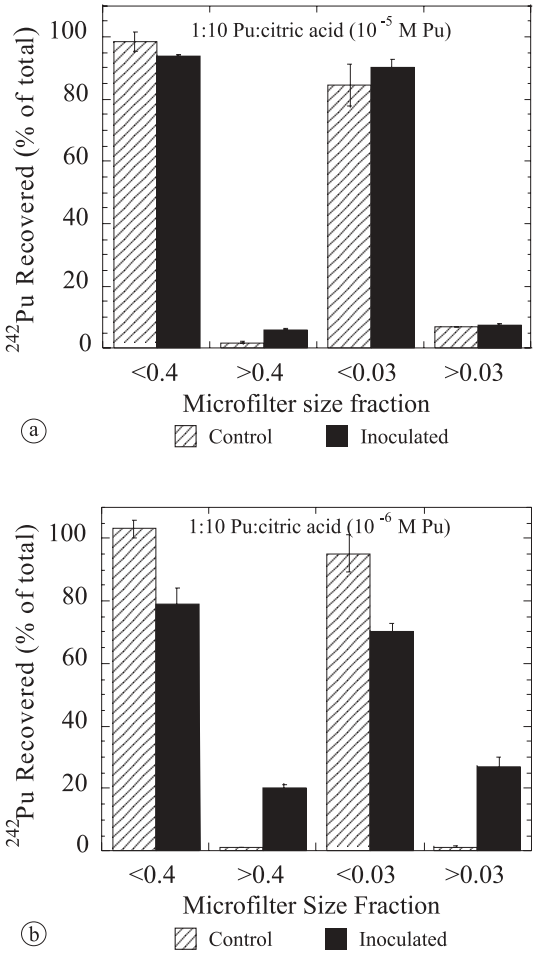


Fig. 8. Microfiltration results at 72-h incubation for (**a**) 1 : 10 (10^{-5} M), and (**b**) 1 : 100 (10^{-6} M) Pu : citric-acid complexes.

The microfiltration data at 72 hours for 10^{-5} and 10^{-6} M Pu samples are presented in Fig. 8. The results for the 10^{-5} M Pu-citrate complex shows 6% and 7% of the Pu retained on the $> 0.4\,\mu m$ and $> 0.03\,\mu m$ filters, respectively; similar findings were obtained with 10^{-7} M Pu (data not shown). Both of these Pu concentrations had a high ionic strength that inhibited citrate degradation, and therefore, the majority of the Pu passed through both filters either as colloidal or soluble form. With 10^{-6} M Pu, 20% was retained on the $0.4\,\mu m$ filter, and 27% on the $0.03\,\mu m$ filter; similar results were found with 10^{-8} M Pu, at 43% and 57%, respectively (data not shown). The treatments in which citrate degradation was significant (10^{-6} and 10^{-8} M Pu) exhibited a relatively greater amount of Pu associated with the filters compared to treatments containing 10^{-7} and 10^{-5} M Pu wherein citrate was slowly degraded due to the high ionic strength. Comparison of the 10^{-6} M Pu samples containing cells with the control samples (without cells) shows that there is greater retention on the $> 0.4\,\mu m$ 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 i) a particle-reactive species that adsorbed to the cells ($> 0.4\,\mu m$), and possibly as ii) a colloidal species, ($> 0.03\,\mu m$).

Tetravalent plutonium forms a 1 : 1 Pu : citric acid ($K = 10^{19}$) complex and a 1 : 2 Pu : citric acid complex ($K = 10^{34}$) [30, 31]. In the presence of excess citric acid, their solubility and Pu(IV) oxidation state are stable [9]. At pH 6.5, we identified a monomeric [Pucit] species, biligand [$Pucit_2$] species, and a dimeric [Pu_2cit_2] species of tetravalent plutonium with citric acid. Speciation calculations and ESI-MS show that the biligand complex is the predominant form. In addition, analysis of the complex over 100-h demonstrated that citric acid inhibits polymer formation. Although the Pu(IV)-citrate complex is stable in the absence of microbial activity, adding bacteria to the 1 : 100 and 1 : 10 000 Pu(IV)-citrate complexes resulted in the retention of Pu species at 20%–43% by the $0.4\,\mu m$ (biomass associated)-filter, and 27%–57% by the $0.03\,\mu m$ (colloid fraction)-filter. Plutonium behavior during microfiltration of the 1 : 10 and 1 : 1000 treatments, where citrate metabolism was moderate, suggests that biosorption of the Pu-citrate complex alone is not responsible for removing Pu from solution because it remained in the soluble form ($> 93\%$). Yong and Macaskie [35] showed that the Th(IV)-citrate complex, an analog for Pu(IV)-citrate, does not interact with immobilized cells of the Gram-negative bacterium, *Citrobacter* sp.

Summary

We have shown that the metabolism of citric acid by *P. fluorescens* was not affected by the presence of up to 10^{-5} M Pu. However, the ionic strength of the medium affected its metabolism. The Pu-citrate complex is relatively stable in the absence of bacterial activity. In media of low ionic strength, citrate was completely metabolized and the Pu released was predominantly present in the polymeric form. In high ionic strength media, citrate was only partially metabolized, and Pu remained in solution complexed with citric acid.

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