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Bioreduction of U(VI)−Phthalate to a Polymeric U(IV)−Phthalate Colloid

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Phthalic acid, a ubiquitous organic ligand, formed soluble mono- and biligand complexes with a uranyl ion that was then reduced to a U(IV)-phthalate by a *Clostridium* species under anaerobic conditions. We confirmed the reduction of the hexavalent uranium to the tetravalent oxidation state by UV−vis absorption and X-ray absorption near edge structure spectroscopy. Sequential micro- and ultrafiltration of the solution revealed that the bioreduced uranium was present as a colloid with particles between 0.03 and 0.45 μm. Analysis with extended X-ray absorption fine structure revealed the association of the reduced uranium with the phthalic acid as a repeating biligand 1:2 U(IV):phthalic acid polymer. This is the first report of the formation of a U(IV) complexed to two phthalic acid molecules in the form of a polymeric colloid. Although it was proposed that the bioreduction and the precipitation of uranium might be an invaluable strategy to immobilize uranium in contaminated environments, our results suggest that the organic ligands present there might hinder the precipitation of the bioreduced uranium under anaerobic conditions and, thereby, enhance its environmental mobility as uranium organic complexes or colloids.

Introduction

The demonstrated presence of phthalic acid and its various esters in the marine and freshwater environments and the low-level radioactive and transuranic (TRU) wastes[1−3] have been attributed to the natural degradation of humic and fulvic acids and the leaching of components from synthetic plastic resins.[2] Phthalic acid is an organic compound which forms strong complexes with metal ions; in particular, with the uranyl ion it forms bidentate-, mononuclear-, and biligand complexes involving both carboxylate groups.[4−7]

A wide variety of bacteria present in radioactive wastes and aquatic and subsurface environments are involved in the reductive precipitation of uranium. They include phylogenetically diverse organisms like hyperthermophilic archaeons, thermophilic bacteria, mesophilic iron- and sulfate-reducing bacteria, and fermentative bacteria.[8] Among the several remediation strategies being explored to mitigate the movement of uranium in surface water, groundwater, and subsurface environments, the reductive precipitation of U(VI) to U(IV) by indigenous microbial communities is considered a promising treatment option.

While the bioreduction and precipitation of uranium offers an attractive strategy to immobilize uranium, the presence of complexing organic ligands in the environment might affect its mobility. For example, when citric or oxalic acid or Tiron are present, the bioreduction of U(VI) by *Shewanella putrefaciens*, *Desulfovibrio desulfuricans*, or *Shewanella alga*[9] generated soluble uranium species. The reduced uranium was presumed to be complexed with an organic ligand, although its oxidation state and the nature of the complex was not determined. In a similar study, Gu et al.[10] reported that, in the presence of humic materials, the bioreduction of U(VI) did not entail its precipitation; rather, the uranium remained in the solution phase as the U(IV)−humic acid complex. Recently, we demonstrated that under anaerobic conditions the *Clostridium* sp. reduced the biligand binuclear U(VI)-citrate to the biligand mononuclear U(IV)−citrate complex.[11] Furthermore, U(VI)-citrate was readily accessible as

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(1) Giam, C.; Chah, H.; Neff, G. *Science* **1978**, *199*, 419−421.
(2) Staple A.; Peterson, D.; Parkerton, H.; Adams, W. *Chemosphere* **1997**, *35*, 667−749.
(3) Francis, A. J.; Iden, C. R.; Nine, B. J.; Chang, C. K. *Nucl. Technol.* **1980**, *50*, 158−163.
(4) Rajan, K.; Martell, A. *J. Inorg. Nucl. Chem.* **1967**, *29*, 523−528.
(5) Kim, B.; Chie, M.; Imoto, S. *J. Inorg. Nucl. Chem.* **1974**, *36*, 2015−2021.
(6) Krauskopf, L. Monomerics for polyvinyl chloride (phthalate, adipate and trimellitakes. In *Plastics Additives and Modifiers Handbook*; Edenbaum, J., Ed. van Nostrand Reinhold: New York, 1992; pp 359−378.
(7) Vazquez, G. J.; Dodge, C. J.; Francis, A. J. *Inorg. Chem.* **2008**, *47*, 10739−10743.
(8) Wall, J. D.; Krumholz, L. R. *Annu. Rev. Microbiol.* **2006**, *60*, 149−166.
(9) Ganesh, R.; Robinson, K.; Reed, G.; Sayler, G. *Appl. Environ. Microbiol.* **1997**, *63*, 4385−4391.
(10) Gu, B.; Yan, H.; Zhou, P.; Watson, D.; Park, M.; Istok, J. D. *Environ. Sci. Technol.* **2005**, *39*, 5268−5275.
(11) Francis, A. J.; Dodge, C. J. *Environ. Sci. Technol.* **2008**, *42*, 8277−8282.

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an electron acceptor for the microorganisms, despite their inability to metabolize the organic ligand complexed to the actinide.

In this study, we investigated the ability of the *Clostridium* sp. to reduce uranium complexed with phthalic acid under anaerobic conditions. We show that the uranium was reduced to a tetravalent state and that it persisted as a colloidal polymeric U(IV)−phthalate biligand complex.

Materials and Methods

Bacterial Growth Medium. A *Clostridium* sp. (ATCC 53464) was grown in a medium containing the following ingredients (per liter): glucose, 5.0 g; glycerolphosphate, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; peptone, 0.1 g; yeast extract, 0.1 g; and, $FeSO_4 \cdot 7H_2O$, 5.6 mg. The pH was adjusted to 5.5, and the medium was prereduced by boiling and purging with a filtered ultra-high purity nitrogen gas for 15 min. Eighty ml of the medium was dispensed into a 125 ml acid-washed, glass serum bottle in an anaerobic nitrogen-filled glovebox. The bottles were fitted with sterile butyl rubber stoppers, sealed with aluminum crimp seals, and autoclaved. All manipulations were carried out in a nitrogen atmosphere inside a glovebox. In separate experiments, the medium was supplemented with 0.1 M phthalic acid.

U−Phthalic Acid Complex. Uranyl nitrate and phthalic acid stock solutions were prepared by dissolving $UO_2(NO_3)_2 \cdot 6H_2O$ (Analar, BDH Chemicals Ltd., Poole England) and anhydrous o-phthalic acid (ACS reagent grade, Aldrich, WI) in deionized water. To prepare the U−phthalic acid complex, we slowly combined equimolar concentrations of the phthalic acid and the uranyl nitrate solutions and adjusted the pH to 5.5 with sodium hydroxide. The solution was left to equilibrate in the dark for 24 h before using it.

Bioreduction of U(VI)−Phthalic Acid Complex. The growth medium supplemented with 0.1 M phthalic acid or without phthalic acid was inoculated with 2 mL of a 24 h old culture of the *Clostridium* sp. grown in a medium without the organic ligand. Triplicate samples of each treatment were incubated at 28 ± 1 °C in the dark. When the optical density (OD_{600}) of the culture reached 0.45, we added 1.5 mM of the U(VI)−phthalic acid complex. Periodically, a 3 mL sample was withdrawn from each culture and filtered through a 0.45 μm Millex-HV membrane filter (Millipore, MA). Immediately thereafter, we obtained a UV−vis spectrum (400−700 nm) of the filtered solution aliquots using a Hewlett-Packard 8452A UV−vis scanning spectrophotometer. The samples were analyzed for U(IV), U(VI), total uranium, and phthalic acid, as described below. At the end of the experiment, the remaining cultures were centrifuged at 8000xg for 15 min, the supernatants filtered through a 0.45 μm Millex membrane filter, and then separated into aliquots. The cell pellets and the supernates were stored at −20 °C for X-ray absorption spectroscopic analyses. All manipulations were performed under low light and with minimum exposure to air to prevent photodegradation or oxidation of the complex.

Ultrafiltration of Bioreduced Uranium. An equimolar U−phthalic acid complex (1.0 mM) was added to the late exponential-growth phase of the *Clostridium* sp. grown in a medium containing 0.1 M phthalic acid. After 48 h, the culture was centrifuged at 8000xg for 15 min. The supernate was filtered through a 0.45 μm filter, and 5 ml of the filtrate then was sequentially ultrafiltered through both a 0.03 and a 0.003 μm polycarbonate membrane filter (Poretics, CA) using an Amicon Model 8010 stirred cell. Total uranium and U(IV) was analyzed in each filtered aliquot. All manipulations were carried out in the glovebag.

Chemical Analysis. Reduced uranium was quantified immediately after filtration by a colorimetric method[12] based on the capacity of U(IV) to reduce Fe^{3+} at pH 3.5 in a mixed solution containing excess Fe^{3+}. The Fe^{2+} formed during this redox reaction was determined at 510 nm by the extent of the color developed due to its complexation with 1−10 phenanthroline (Sigma, MO). For calibration, a standard solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was used; we assumed that, in the samples, every 2 mols of Fe^{2+} formed were equivalent to the formation of 1 mol U(IV). The U(VI) was quantified in an anaerobic nitrogen-filled glovebag via the bromo-PADAP colorimetric method.[13] Total uranium was determined with the kinetic phosphorescence analyzer (Chemcheck, WA) after completely reoxidizing the bioreduced sample. The absorption spectrum of the reduced uranium sample was measured (400 to 700 nm) by transferring one milliliter of the sample into a 1 cm quartz cell. Phthalic acid was quantified by high-performance liquid chromatography (HPLC) using an Aminex HPX-87H (Bio-Rad, CA) ion-exclusion organic acid analysis column heated to 40 °C; a 0.1% H_3PO_4:10% acetonitrile solution was the mobile phase with a flow rate of 0.5 mL/min. We performed all the treatments and the measurements in triplicate.

X-ray Absorption Spectroscopy. The aqueous and solid uranium samples from the bacterially treated samples were freeze-dried under nitrogen gas using a Freezone 4.5 freeze drier (Labconco, MO). The resulting solids were ground to a fine powder and mixed with boron nitride. All manipulations were carried out in an anaerobic glovebag, and the samples stored in a nitrogen-filled anaerobic jar (Becton-Dickinson, MD). Uranyl nitrate and uranium dioxide were used as the standards for U(VI) and U(IV), respectively. The solids were mounted on Al sample holders with a cutout geometry of 2 mm height × 20 mm length × 1.5 mm thickness, sealed with Kapton tape, and placed in an argon-flushed chamber on the X-10C beamline at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL). The monochromator was equipped with a Si(2,2,0) double flat crystal, and the energy was calibrated by using the inflection point in the first derivative for uranium dioxide (17166 eV). Fluorescence spectra were collected using a seven-element Ge detector. The oxidation state of uranium in the samples was determined by using a X-ray absorption near edge spectroscopy (XANES). The background was subtracted with AUTOBACK, and the data were normalized to the edge jump with ATHENA software to determine the shifts in

(12) Streeton R.; Jenkins, E.The preparation, stabilization and analysis of uranium(IV) nitrate solutions. In *United Kingdom Atomic Energy Authority Research Group*; Atomic Energy Research Establishment, Harwell, Berks, England, 1962.

(13) Johnson, D.; Florence, T. *Anal. Chim. Acta* **1971**, *53*, 73−79.

Table 1. Effect of Phthalic Acid on Solubility of Uranium in Bacterial Growth Medium

	mM U(VI) in solution	
time (days)	without phthalic acid	with 0.1 M phthalic acid
0	1.09 ± 0.05[a]	1.12 ± 0.05
1	0.60 ± 0.02	0.97 ± 0.01
3	0.53 ± 0.02	0.92 ± 0.01
30	0.20 ± 0.01	0.79 ± 0.07

[a] Standard error of the mean (SEM) is ±1.

the peak energy.[14,15] We gained information on the type and the number of atoms surrounding the absorbing atom by an extended X-ray absorption fine structure (EXAFS).[16,17] With the theoretical EXAFS modeling code FEFF6, we calculated the backscattering phase and the amplitude information for the individual neighboring atoms. Four scans per sample were taken, and the data was averaged. The amplitude reduction factor (S_0^2) was fixed at 1.0 for all of the fits. The coordination numbers for the axial O atoms and the first coordination sphere for U(IV)-oxide were fixed at 2.0 and 8.0, respectively. The background was subtracted from the spectra, and the data were fitted in R space by the Fourier transformation of the X(k) data. We took into consideration the four-legged U→O→U→O→U multiple-scattering path for the uranyl ion during the fitting procedure.

Results

Solubility of Uranium in Bacterial Growth Medium. Table 1 shows the decrease in the concentration of soluble uranium with time in the uninoculated (control) bacterial growth medium supplemented with and without 0.1 M phthalic acid. In the latter, we recorded a 45% decrease in the uranium concentration after 1 day, 51% after 3 days, and ~82% after 30 days. In contrast, in the medium containing 0.1 M phthalic acid, the uranium concentration in the solution dropped by 13% after 1 day, 18% after 3 days, and 29% after 30 days, indicating that the U−phthalic acid complex is relatively stable in solution.

Bioreduction of U(VI)-Phthalate. Figure 1 depicts the rate and the extent of reduction of the uranyl phthalate by *Clostridium* sp. Adding equimolar U−phthalic acid complex to the growing culture in the medium containing no phthalic acid triggered a rapid decrease of U(VI) in solution from 1.5 to 0.62 mM in about 30 min; thereafter, it decreased slowly to 0.57 mM. At the same time, we observed U(IV) in the solution phase, which reached a maximum of 0.13 mM before declining to about 0.04 mM (Figure 1A). The bioreduction of U(VI) to U(IV) was incomplete most probably due to the precipitation of the uranium with the cell biomass, thus preventing further enzymatic reduction.

Figure 1B illustrates the bioreduction of the U(VI)−phthalate complex in a media supplemented with 0.1 M phthalic acid. The concentration of U(VI) decreased rapidly from 1.5 to ~0.9 mM in about 30 min, followed by a slower rate until its concentration was below the detection limit. Concomitant with the decrease in U(VI), the concentration of U(IV) in solution increased to a maximum of 1.46 mM. By 30 h, 98% of the total U remaining in solution was of the U(IV) species, indicating that the bioreduction was complete.

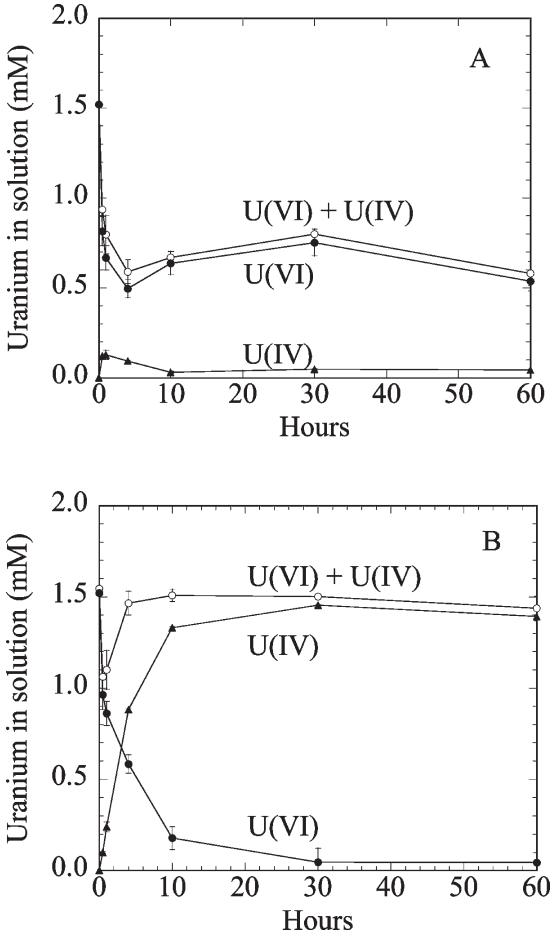

Figure 1. Reduction of U−phthalic acid complex by *Clostridium* sp. in a media without phthalic acid (**A**) and with 0.1 M phthalic acid (**B**).

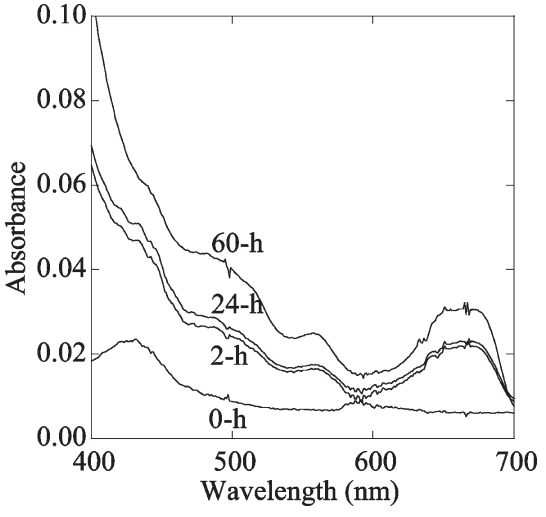

Figure 2. UV−vis spectra of the bioreduction of the U−phthalic acid complex by *Clostridium* sp. in a medium containing 0.1 M phthalic acid.

(14) Newville, M.; Livins, P.; Yacoby, Y.; Rehr, J.; Stern, E. *Phys. Rev. B: Solid State* **1993**, *47*, 14226−14131.
(15) Newville, M. *J. Sync. Rad.* **2001**, *8*, 322−324.
(16) Rehr, J.; de Leon, M.; Zabinsky, S.; Albers, R. *J. Am. Chem. Soc.* **1991**, *113*, 5135−5140.
(17) Zabinsky, S.; Rehr, J.; Ankudinov, A.; Albers, R.; Eller, M. *Phys. Rev. B: Solid State* **1995**, *52*, 2995−3001.

Table 2. Sequential Ultrafiltration of Bioreduced Uranium in Bacterial Culture Medium Containing Phthalic Acid

pore size, μm	total uranium, mM	U(IV), mM
0.45	1.17 ± 0.17^a	0.98 ± 0.09
0.03	0.14 ± 0.01	0.13 ± 0.02
0.003	0.05 ± 0.01	0.04 ± 0.01

[a] Standard error of the mean (SEM) is ±1.

The UV−vis spectra of uranium reduction by *Clostridium* sp. in a medium supplemented with 0.1 M phthalic acid is shown in Figure 2. The spectrum at 0 h represents the U(VI)−phthalate complex with its predominant peak at ~440 nm, typical of the presence of the U(VI) species. The disappearance of this peak and the appearance of two peaks at 550 and 660 nm in the absorbance spectrum of the filtrates obtained at 2, 24, and 60 h is diagnostic for the U(IV) species, reflecting the bacterial reduction of uranium.

Analysis of Colloidal Uranium by Ultrafiltration. Table 2 shows the distribution of U(VI) and U(IV) after sequential filtration of the bioreduced uranium solution through a 0.45, 0.03, and 0.003 μm filter membrane. The 73% of total uranium retained on the 0.03 μm filter demonstrates that the uranium was present in a colloidal form with a particle size between 0.45 and 0.03 μm. The 3% of the total bioreduced uranium species retained after filtration through the 0.003 μm filter membrane indicates that it had a particle size between 0.03 and 0.003 μm; the remaining 24% passed through the 0.003 μm filter. These findings point to the polymeric structure of the reduced form of the complex, consisting of a number of size fractions, thereby suggesting that polymerization may be time dependent.

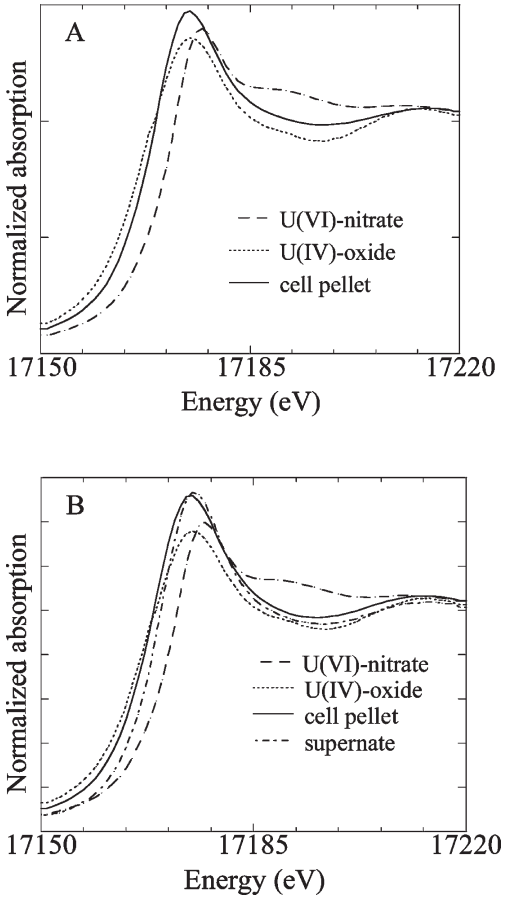

Figure 3. XANES spectra at the U L_{III} edge of the U−phthalic acid complex bioreduction in media without phthalic acid (**A**) and with phthalic acid (**B**).

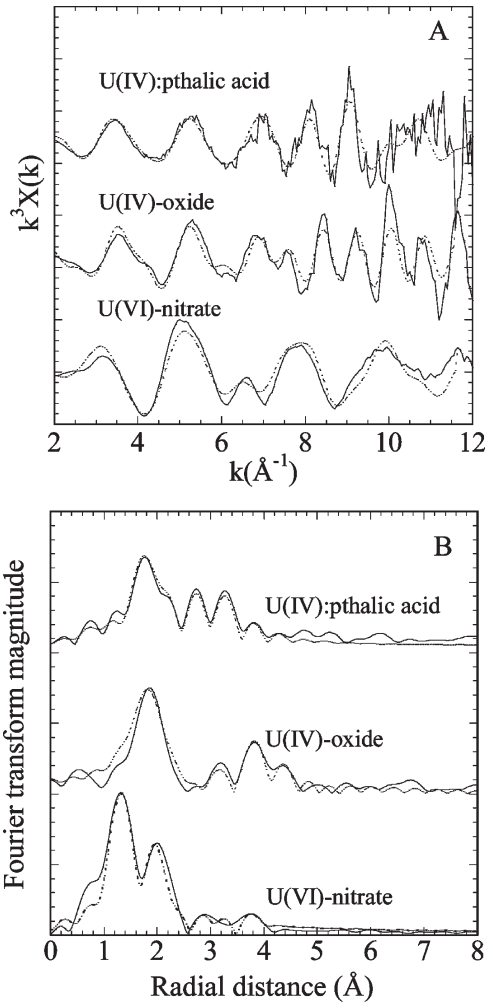

Figure 4. EXAFS analysis of the U(IV)−phthalate complex and the uranium standards. k^3-weighted EXAFS (**A**), and Fourier transforms EXAFS spectra (**B**) at the U L_{III} edge. Experimental data (—); theoretical fit (---).

X-ray Absorption Near Edge Spectroscopy (XANES). To confirm the oxidation state of the uranium after bacterial action, we analyzed the precipitated uranium consisting of the bacterial cells and the supernatant by XANES. Figure 3A demonstrates that in the absence of phthalic acid there was a shift in the absorption edge energy of the uranium precipitate to 17168 eV compared to that of U(VI)-nitrate (17172 eV); that value is close to that of the U(IV)-oxide standard (17166 eV), indicating uranium was present predominantly as U(IV). The amount of uranium in the supernate was too low to be measured by XANES. Figure 3B illustrates our analysis of the oxidation state of uranium in the supernatant and the uranium-containing cell pellet in the presence of phthalic acid. The absorption edge energy for uranium has shifted to 17167 eV in both the liquid and the solid phases; again, this is close in energy to that of the reduced uranium standard, denoting the presence of reduced uranium.

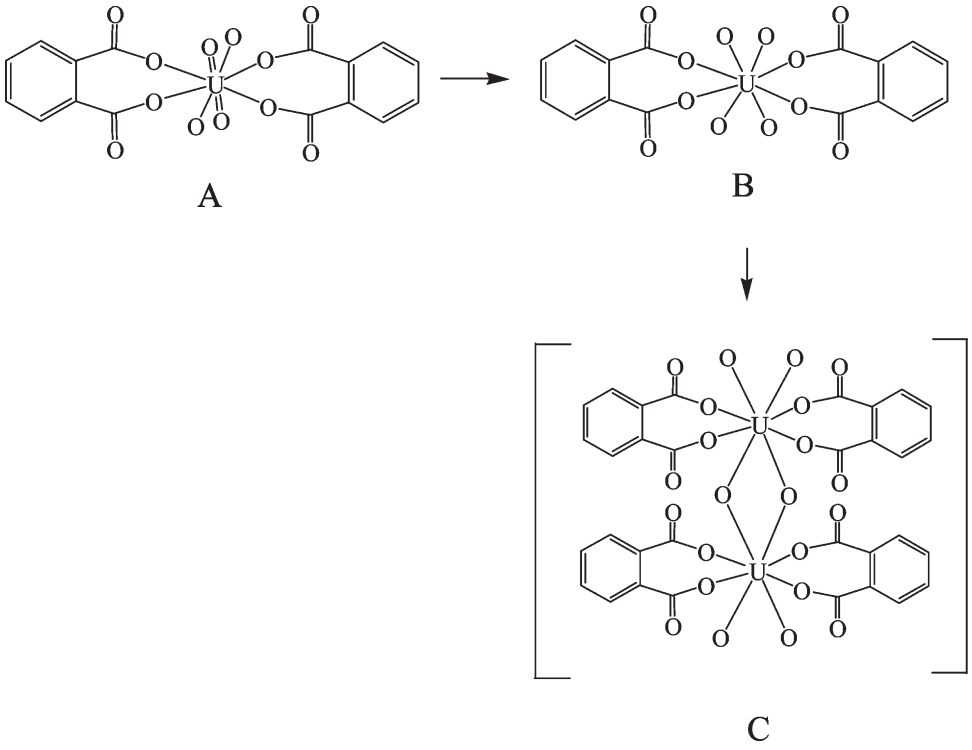

Figure 5. Reduction of U(VI) phthalate (**A**) to U(IV)-phthalate (**B**), and proposed structure for polymeric U(IV)-phthalate colloid (**C**).

Table 3. EXAFS Fitting Parameters for Uranyl Nitrate, Uranium Dioxide, and Uranium Phthalate Complex

sample	atom pair	coordination number	R (Å)	σ^2 ($\times 10^{-3}$ Å^2)	$\triangle E^0$ (eV)
U(VI)-nitrate	U−O_{ax}	2	1.77 ± 0.02	2.0 ± 0.1	8.3 ± 1.0
	U−$O_{eq\ 1}$	5.1 ± 0.6	2.47 ± 0.03	1.1 ± 0.3	7.3 ± 2.9
	U−U	2.0 ± 0.9	3.89 ± 0.06	7.4 ± 0.3	−5.9 ± 3.1
U(IV)-oxide	U−O	8	2.38 ± 0.01	1.7 ± 0.2	7.9 ± 1.7
	U−U	9.7 ± 2.2	3.90 ± 0.03	9.1 ± 1.2	1.6 ± 1.7
	U−O	20.4 ± 3.7	4.53 ± 0.01	6.5 ± 2.3	−0.3 ± 1.5
U(IV)-phthalate acid	U−O_1	4.3 ± 0.3	2.29 ± 0.03	5.1 ± 0.7	7.8 ± 2.7
	U−O_2	3.9 ± 0.4	2.45 ± 0.03	5.7 ± 0.1	−4.4 ± 3.7
	U−C_1	4.7 ± 1.2	2.89 ± 0.01	7.5 ± 0.7	−2.9 ± 1.6
	U−O_1	4.6 ± 1.6	3.51 ± 0.05	7.5 ± 1.6	−10.8 ± 7.6
	U−C_2	4.1 ± 2.3	4.29 ± 0.01	0.9 ± 0.1	4.0 ± 0.5
	U−U	2.1 ± 0.9	4.23 ± 0.02	7.4 ± 0.7	−4.4 ± 7.7

Extended X-ray Absorption Fine Structure (EXAFS) Spectroscopic Analysis. We carried out EXAFS analyses to determine the molecular association of the bioreduced uranium remaining in the solution containing phthalic acid. Figures 4A and B, respectively, show the k^3-weighted EXAFS spectra (2.0−12.0 Å^{-1}) and the Fourier-transformed spectra for the U standards (uranyl nitrate and uranium dioxide) and the U−phthalate complex after bacterial activity. Table 3 lists the fitting parameters. The uranyl nitrate structure consisted of two axial oxygens at 1.77 ± 0.02 Å and an equatorial O shell with 5.1 ± 0.6 oxygens at 2.47 ± 0.03 Å. The uranyl dioxide standard had eight oxygens at 2.38 ± 0.01 Å, a U−U interaction with 9.7 ± 2.2 uranium atoms at 3.90 ± 0.03 Å, and a second O shell with 20.4 ± 3.7 oxygens at 4.53 ± 0.01 Å. In contrast to this standard, bioreduced uranium exhibited a split O shell with 4.3 ± 0.3 oxygens at 2.29 ± 0.03 Å and 3.9 ± 0.4 oxygens at 2.45 ± 0.03 Å. Splitting of the U−O shell is indicative of the uranium bonding to the carboxylate from the phthalic acid as well as to the water and, possibly, the hydroxide groups. There are two shells showing U−C interactions, one at 2.89 ± 0.01 Å with 4.7 ± 1.2 carbon atoms that correspond to the bonding of carboxylate carbons to uranium and the other at 4.29 ± 0.01 Å from the aromatic ring structure with 4.1 ± 2.3 distal carbons. Finally, there are 2.1 ± 0.9 U−U interactions at 4.23 ± 0.04 Å. From these fitting results, we deduce that uranium occurs as a polymeric form with U−U bonding, and each uranium is associated with two phthalic acid molecules. The structures for the oxidized and the reduced biligand complexes and the proposed structure for the polymeric U(IV)-phthalate colloid are shown in Figure 5.

Discussion

The conditions in the subsurface environments at nuclear waste disposal sites are expected to be reducing. The occurrence of reduced uranium in solution under anoxic conditions

seems not uncommon because anaerobic systems with high concentrations of dissolved organic matter were shown to contain significant amounts of uranium as U(IV). Although tetravalent uranium in reducing environments should be relatively stable and immobile, the presence of organic ligands can mobilize U(IV) by forming soluble complexes or colloids. Thus, the precipitation of bioreduced U(IV) was attenuated by the presence of organic ligands.[9−11,18,19] Bacterial reduction of uranyl citrate resulted in the formation of a mononuclear biligand complex that remained as a soluble species in solution, in contrast to the reduction and the precipitation of uranium.[11]

Phthalic acid, a synthetic as well as naturally occurring compound present in radioactive wastes, forms mono- and biligand complexes with uranyl ions, with the latter being the predominant species.[7] Kim et al.[5] used infrared, UV−vis, and nuclear magnetic resonance spectroscopy to show that such bidentate complexes were formed through the charge transfer between the ligand and the metal. The stability constant (log *K*) of the U−phthalate complex ranged from 4.3 to 4.8.[4] Goncalves et al.[20] and Rajan and Martell[4] reported the formation of a uranium bidentate complex involving the two carboxylates of phthalic acid, while Vazquez et al.[7] showed that both 1:1 and 1:2 U−phthalic acid complexes form, with the mononuclear biligand 1:2 U−phthalic acid complex being the predominant species.

Bioreduction of U(VI) by *Clostridium* sp. in the absence of phthalic acid resulted in its precipitation as predominantly U(IV); in contrast, the bacterium reduced the U(VI)−phthalate complex to U(IV)-phthalate, and it remained in the solution phase as a colloid. The formation of a colloid from the U(IV)-organic ligand complex has not been previously reported. No consumption or degradation of the organic ligand was detected in the present or the previous studies.

Uranium in groundwater has been shown to associate with low molecular weight humic acid colloids.[21] Reduction of U(VI) to U(IV) by naturally occurring organic matter (NOM) and complexation with NOM has been reported.[10,19,22,23] In anoxic environments, the sparingly soluble U(IV) may form intrinsic colloids or be deposited on carrier colloids (pseudocolloids associated with iron, alumina, or organic compounds, such as humics) and become mobile.[24,25] Haas and Northrup[18] reported that the type of organic complexing agents affected the bacterially mediated reductive precipitation of U(IV), and when strong complexing agents were present, such as citric and humic acids, the bioreduced U(IV) remained in solution phase. Gu et al.[10] and Luo and Gu[19] reported that in the presence of humic materials, the bioreduction of U(VI) did not cause its precipitation; rather, the uranium remained in the solution phase as a U(IV)−humic complex; they proposed this occurred primarily through coordination with the chelating functional groups of the humates, such as carboxyls, hydroxyls, and ketones. However, we lack specific structural information about the U(IV) complexation with organic ligands and, in particular, with NOM. Further, there is little data on the stability of U(IV)−organic complexes. Carey and Martell[26] reported that the log *K* for a mixed ligand 1:1:1 U(IV)−EDTA−phthalate complex was 4.2.

We noted the presence of a colloidal U(IV) species following microfiltration; the majority of the reduced uranium was associated as a colloid with a particle size between 0.03 and 0.45 μm. Also, approximately 5% of the total bioreduced uranium passed through a 0.003 μm membrane, highlighting that this small fraction could be a highly mobile species. These results reflect the nature of the bioreduced uranium as a complex, wherein some U(IV) coordination sites are free for polymerization reactions that lead to the formation of these colloidal particles. The variation in particle size of the colloid suggests its time-dependent growth.

EXAFS analysis showed the molecular structure had a split shell indicating different bonding modes with oxygen. The $U-O_1$ is most likely due to the presence of solvent water or hydroxide, while the $U-O_2$ points to the bidentate association of carboxylic acid with two molecules of phthalic acid; this latter association is confirmed by the presence of four carboxylate carbons at 2.89 Å. Such a split in the O shell was demonstrated earlier for the U(IV) complexed with citrate.[11] Although we cannot determine the exact structure of the colloid based upon the EXAFS information alone, it most likely has the general formula $[U(IV)O\text{-}OCC_6H_4CO_2H]_n$ (Figure 5C). This is the first report of a reduced uranium−organic complex species which is present in a colloidal form.

In conclusion, our research suggests that enhancing the bioreduction process in an environment with a high concentration of complexing organic ligands may not entail the precipitation of uranium. It also indicates that in the presence of phthalic acid, a common environmental organic ligand, the bioreduced uranium produced under anaerobic conditions could remain in solution as a colloid complexed with the organic ligand, thereby compromising the effectiveness of bioreductive precipitation as a remediation strategy.

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(18) Haas, J. R.; Northup, A. *Geochem. Trans.* **2004**, *5*, 41−48.
(19) Luo, W., Gu, B. *Environ. Sci. Technol.* **2009**, *43*,152−156.
(20) Goncalves, M. L. S.; Mota, A. M. *Talanta* **1987**, *34*, 839−847.
(21) Singhal, R. K.; Kumar, A.; Karpe, P. J. R.; Datta, M.; Hegde, A. G. *J. Radioanal. Nucl. Chem.* **2005**, *265*, 405−408.
(22) Shanbhag, P. M.; Choppin, G. R. *J. Inorg. Nucl. Chem.* **1981**, *43*, 3369−3372.
(23) Choppin, G. R. *Radiochim. Acta* **1988**, *44*/*45*, 23−28.
(24) Artinger, R.; Rabung, T.; Kim, J. I.; Sachs, S.; Schmeide, K.; Heise, K. H.; Bernhard, G.; Nitsche, H. *J. Contam. Hydrol.* **2002**, *58*, 1−12.
(25) Zanker, H.; Ulrich, K. U.; Opel, K.; Brendler, V. *IMWA Symposium 2007: Water in Mining Environments*; Cidu, R., Frau, F., Eds.; Cagliari, Italy; 27th −31st May 2007.
(26) Carey, G.; Martell, A. *J. Am. Chem. Soc.* **1967**, *89*, 2859−2865.