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Relative Reactivity of Biogenic and Chemogenic Uraninite and Biogenic Noncrystalline U(IV)

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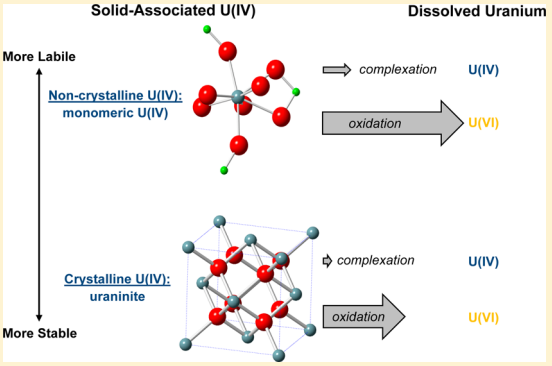
Supporting Information

ABSTRACT: Aqueous chemical extractions and X-ray absorption spectroscopy (XAS) analyses were conducted to investigate the reactivity of chemogenic uraninite, nanoparticulate biogenic uraninite, and biogenic monomeric U(IV) species. The analyses were conducted in systems containing a total U concentration that ranged from 1.48 to 2.10 mM. Less than 0.02% of the total U was released to solution in extractions that targeted water-soluble and ion exchangeable fractions. Less than 5% of the total U was solubilized via complexation with a 0.1 M solution of NaF. Greater than 90% of the total U was extracted from biogenic uraninite and monomeric U(IV) after 6 h of reaction in an oxidizing solution of 50 mM $K_2S_2O_8$. Additional oxidation experiments with lower concentrations (2 mM and 10 mM) of $K_2S_2O_8$ and 8.2 mg L^{-1} dissolved oxygen suggested that monomeric U(IV) species are more labile than biogenic uraninite; chemogenic uraninite was much less susceptible to oxidation than either form of biogenic U(IV). These results suggest that noncrystalline forms of U(IV) may be more labile than uraninite in subsurface environments. This work helps fill critical gaps in our understanding of the behavior of solid-associated U(IV) species in bioremediated sites and natural uranium ore deposits.



INTRODUCTION

The chemical stability of U(IV) is of central importance to the effectiveness of bioremediation techniques to mitigate subsurface uranium (U) contamination and to the management of *in situ* leach uranium mining.[1,2] Knowledge of the molecular-scale speciation and reactivity of reduced U(IV) in subsurface environments is crucial to optimizing the long-term performance of *in situ* bioremediation strategies. The application of *in situ* uranium bioremediation, which stimulates the growth of microbes and results in the direct and indirect enzymatic reduction of soluble U(VI) to less soluble U(IV) species, was proposed in 1992,[3,4] and has been tested at the field scale over the past decades.[5−7] However, the relative stability of U(IV) products in uranium-contaminated field sites remains poorly understood.

Although uraninite was long assumed to be the sole product of *in situ* bioremediation,[6,8] recent studies have shown that other poorly ordered or noncrystalline U(IV) products can form.[2,8−12] These include U(IV) bound to biomass or precipitated as poorly ordered solids [most likely bound to carboxylate, carbonate, or phosphate ligands[9−11,13]]. Monomeric U(IV) is a dominant product of U(VI) bioreduction in shallow aquifer sediments [2] and also has been observed in flow-through column experiments with aquifer sediments performed to evaluate uranium immobilization and speciation following biostimulation.[14] Monomeric U(IV) can be formed as a product of U(VI) reduction in the presence of Fe(II) in solids, biogenic vivianite, magnetite presorbed with phosphate, and by direct microbial reduction of U(VI);[11,14−16] the presence of phosphate hinders uraninite formation and instead causes the formation of monomeric U(IV) when U(VI) is reduced by phosphate-reacted biogenic magnetite and by bacteria.[11,15] Because uranium is not bound directly to other U atoms via shared atoms in these species, they have been referred to as "monomeric" or "mononuclear" U(IV).[12] However, in many U(IV)-phosphates, the ligand probably occurs as a polymeric network, a motif likely to appear in bioreduced samples. Such complexes have been referred to as "coordination polymers".[2] To account for the range of U(IV) coordination possibilities, we will use the term "monomeric U(IV)" here to include U(IV)

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atoms coordinated to polymeric networks and in truly monomeric complexes.

Several geochemical factors impact the stability of U(IV) in subsurface environments. For instance, uraninite dissolution rates increase with increasing dissolved oxygen and decreasing pH.[17−19] The presence of carbonate promotes the dissolution of uraninite at circumneutral pH by facilitating the detachment of surface-associated U under oxidizing and reducing conditions,[20] and carbonate can also solubilize monomeric U(IV) species from solids.[10] Groundwater cations such as Ca^{2+}, Zn^{2+}, and SiO_4^{4-} have been shown to inhibit uraninite dissolution.[21,22] Thus, a wide range of aqueous chemistry conditions should be considered when evaluating the reactivity of U(IV) species in subsurface environments. The lability of solid U(IV) species due to reaction with complexants and oxidants can result in soluble U products that could be more susceptible to mobilization by advective and diffusive transport processes in the environment.

Little is known about the relative reactivity of nonuraninite products of biological U(VI) reduction. Recent experiments showed that monomeric U(IV) produced by biomass, biogenic vivianite, or phosphate-reacted magnetite, or in a field sediment from Rifle, CO, were more labile than uraninite when exposed to an anoxic 1 M $NaHCO_3$ solution at pH 8.7.[12] However, the lability of monomeric U(IV) species at field-relevant bicarbonate concentrations has not been tested. Although anecdotal evidence suggests that monomeric U(IV) is more labile than uraninite under oxidizing conditions, limited experimental data exist to support this assumption. It is important to understand the differences in lability among U(IV) species under oxidizing conditions, as knowledge of their reoxidation potential directly bears on the feasibility of using *in situ* bioremediation to immobilize uranium contamination.[23,24] Understanding the differences in reactivity between U(IV) species is also relevant to understand ore genesis and uranium mobility in reduced sediments of contaminated aquifers.[2]

Chemical extractions can be used to assess the reactivity of different U(IV) and U(VI) species. Data obtained from chemical extractions are usually complementary to spectroscopic data and can also be used to help interpret spectroscopic results. For example, bicarbonate extractions have been used to quantitatively distinguish monomeric U(IV) from uraninite; monomeric U(IV) can be extracted from biogenic uraninite after reaction with an anoxic 1 M $NaHCO_3$ solution at pH 8.7.[12] Additionally, applying lower concentrations of sodium bicarbonate (~50−100 mM $NaHCO_3$) at pH 8.3 can serve as a pretreatment step to remove traces of U(VI).[12] A recent study developed a sequential extraction method for determining iron and uranium redox speciation in reactions of U(VI) with Fe(II)-bearing clays; the method involved the extraction of all U from the solid in strong acid followed by a U(VI)-specific analytical method.[25]

The objective of this study was to evaluate the release of U from uraninite (chemogenic and biogenic) and biomass-associated monomeric U(IV) species under aqueous chemical conditions targeting U(IV) fractions that are water-soluble, ion exchangeable, amenable to complexation by a ligand, and oxidizable. Uranium L_{III}-edge X-ray absorption spectroscopy (XAS) was employed to characterize differences in the local molecular structure of U between unreacted control samples and those exposed to the various chemical extractions. The results obtained from this investigation, performed under controlled laboratory conditions, serve as a foundation toward understanding the reactivity of these U(IV) species in natural and bioremediated sediments.

MATERIALS AND METHODS

Materials. All reagents used in this study were certified analytical grade or better and used without further purification. Chemogenic uraninite was synthesized using the protocol outlined in Ulrich et al.[19] Briefly, studtite was precipitated by reaction of uranyl nitrate with hydrogen peroxide, dialyzed against ultrapure water, dried, and reduced to uraninite by heating to 400 °C with H_2(g). X-ray diffraction analysis and scanning electron microscopy measurements confirmed that this synthesis yielded crystalline UO_2 particles with mean diameters of about 100−200 nm.[19] Uraninite preparation and anoxic experiments presented in this study were conducted in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, Michigan) containing a gas mixture of 95% (volume/volume, v/v) N_2(g) and 5% (v/v) H_2(g) circulated through a Pd catalyst and silica gel.

Biomass-associated uraninite and monomeric U(IV) species were produced via microbial U(VI) reduction according to the methods outlined in Bernier-Latmani et al.[10] Briefly, *Shewanella oneidenis* MR-1 was cultured and grown in Luria−Bertani (LB) medium as described in a previous study [26] and then harvested by centrifugation at 8000*g* for 10 min during the late exponential growth phase. Cells were washed in an anoxic solution containing 30 mM $NaHCO_3$ and 20 mM PIPES buffer set to pH 6.8 (BP medium) to remove any remaining growth medium. Following the washing step, cells were resuspended to an optical density (OD_{600}) of 1.0 in an anaerobic chamber (97% N_2/3% H_2) in either BP medium to favor the production of uraninite or in Widdel Low Phosphate (WLP) medium (Supporting Information [SI], Table S1) to favor the production of monomeric U(IV). Biogenic uraninite particles had mean diameters of approximately 3.5 nm as noted in previous studies.[8,19] To initiate uranium reduction, media were amended with 20 mM L(+)-lactic acid and 1 mM uranyl acetate. Following reduction, cell suspensions were collected by centrifugation, resuspended in a small volume of anoxic water, placed in serum bottles with a 3% H_2/97% N_2 headspace, sealed within Mylar bags purged with N_2, and shipped from EPFL to Washington University using an overnight delivery service. The final concentration of uranium in the cell slurries was approximately 6780 mg L^{-1}. Alessi et al. [12] found that the system favoring uraninite production (BP medium) produces a mixture of approximately 65% biogenic uraninite and 35% monomeric U(IV), whereas the monomeric U(IV) favoring system (WLP medium) produces approximately 90% monomeric U(IV) and 10% biogenic uraninite. These contents of monomeric U(IV) and biogenic uraninite for the materials used in this study were verified by the bicarbonate extraction method proposed by Alessi et al.;[12] experiments were performed both at EPFL and Washington University, obtaining reproducible results.

Procedure for Chemical Extractions. Before beginning extraction experiments, each U(IV) material had its initial total U content precisely determined by analysis following digestion. For the acid digestion, 20 mg of chemogenic uraninite, or 1.095 mL of monomeric U(IV) or biogenic uraninite slurries were loaded into 50 mL digestion tubes. The tubes were then filled with 8 mL of concentrated HNO_3 (67−70% by mass), 2 mL concentrated HCl (34−37% by mass), and 40 mL of deionized water, and placed in a heated digestion block held at 100 °C for

Table 1. Summary of the Samples and Chemical Extractants Used in This Study

sample name	sample description	chemical extractant(s)
Mono U(IV)	90% monomeric U(IV) and 10% biogenic uraninite	deionized water, NH_4NO_3, NaF, $K_2S_2O_8$, and DO
Bio UO_2	biogenic uraninite not washed with HCO_3^-, [which contains 65% biogenic uraninite and ~35% monomeric U(IV)]	deionized water, NH_4NO_3, NaF, $K_2S_2O_8$, and DO
Chem UO_2	100% chemogenic uraninite	deionized water, NH_4NO_3, NaF, $K_2S_2O_8$, and DO
Mono-Chem Mix Anoxic	50% monomeric U(IV) + 50% chemogenic uraninite (anoxic control)	HCO_3^-
Mono-Chem Mix Oxidized	50% monomeric U(IV) + 50% chemogenic uraninite	$K_2S_2O_8$
washed Bio UO_2	biogenic uraninite washed with 1 M HCO_3^- [to remove monomeric U(IV)]	DO

4 h to achieve complete digestion. An aliquot of the resulting digest was then filtered through a 0.22 μm pore-size filter membrane (PTFE, Millipore) and diluted as necessary for ICP-MS analysis. This filtration step was effective for removing uraninite particles; stable colloids were not noticed. Each digestion was performed in duplicate. The stock slurries of biogenic uraninite and monomeric U(IV) were found to have total U concentrations of 28.5 mM. Prior to reaction with any specific extractant, chemogenic uraninite samples were reacted with 50 mM $NaHCO_3$ at pH 8.3 for 1 h to remove traces of U(VI) present in the sample. This step was shown by Alessi et al. [12] to selectively mobilize U(VI). The samples were then washed in anoxic deionized water buffered with 0.01 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) at pH 7 for 1 h to remove excess bicarbonate and U(VI).

Chemical extractions were conducted to evaluate the relative reactivity of chemogenic and biogenic uraninite, and monomeric U(IV). For each extraction, 20 mg of chemogenic uraninite, or 1.095 mL of monomeric U(IV) or biogenic uraninite slurries, respectively, were reacted in 20 mL (for biogenic U(IV) samples) or 50 mL (for chemogenic uraninite) of solution to achieve a target U loading ranging from 0.35 − 0.5 g L^{-1} (1.48 − 2.10 mM U). Water-soluble extractions were performed for 24 h using deionized water at pH 7 buffered with 0.01 M MOPS. Ion exchangeable extractions were conducted for 24 h using a 1 M NH_4NO_3 solution set to pH 7 and buffered with 0.01 M MOPS. Ligand extractable experiments were performed for 24 h using 0.1 M NaF buffered to pH 6 with 0.01 M 2-(*N*-morpholino)ethanesulfonic acid (MES). Fluoride was chosen as the complexant based on chemical equilibrium modeling (illustrated in Figure S1 of the SI) that indicated that it had a stronger affinity for U(IV) at pH 6 than would 1 M bicarbonate at pH 8.7; 0.1 M F^- is calculated to fully dissolve a 1.48 mM loading of uraninite at pH 6.0 whereas 1 M bicarbonate could not fully dissolve the uraninite at any pH, which was also confirmed by the experimental studies of Alessi et al.[10] Oxidizable fractions of U were determined using a solution of 50 mM potassium persulfate ($K_2S_2O_8$) and 100 mM $NaHCO_3$ (6 h of reaction time). Persulfate was selected because of its oxidizing strength, and 100 mM $NaHCO_3$ was included to mobilize the U(VI) produced by oxidation. Additional experiments with dissolved oxygen (DO), a milder oxidant than persulfate and a reactant of major environmental relevance, as well as at lower concentrations of persulfate were also conducted (see next subsection). Samples were filtered through 0.22 μm pore-size filter membranes (PTFE, Millipore). After extractions, selected solid samples were preserved in the anaerobic chamber prior to XAS analyses for further characterization.

Additional Oxidation Experiments. The effects of $K_2S_2O_8$ and DO on the oxidation of chemogenic uraninite, biogenic uraninite, and monomeric U(IV) were studied as a function of time and oxidant concentration. Persulfate extraction solutions consisted of 100 mM $NaHCO_3$ and $K_2S_2O_8$ added at concentrations of 2, 10, and 50 mM. Experiments with dissolved oxygen were conducted using 100 mM $NaHCO_3$ and 8.2 mg L^{-1} DO (0.26 mM O_2), which corresponds to the concentration in equilibrium with air at 25 °C. Mixtures of chemogenic uraninite, biogenic uraninite, and monomeric U(IV) were prepared to evaluate the selectivity of persulfate and oxygen to extract specific U(IV) species. Mixtures consisting of 50% monomeric U(IV) and 50% chemogenic uraninite by mass were prepared in a fixed volume of 50 mL (target U loading of 0.5 g L^{-1}) of anoxic deionized water inside an anaerobic chamber. As previously stated, the biogenic uraninite consists of approximately 65% uraninite and 35% monomeric U(IV) associated to biomass.[12] As such, two sets of biogenic uraninite samples were prepared for selected experiments. In one set, the monomeric U(IV) fraction was removed from these samples with a 1 M $NaHCO_3$ solution as previously described [10] prior to the oxidation experiments so that only the biogenic uraninite fraction remained. The other set was extracted without removing the monomeric U(IV). The samples and chemical extractants used in this study are summarized in Table 1. Selected solid samples from these experiments were saved for XAS analyses for further characterization.

Solution Chemistry Analyses. Total dissolved U was measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce). Aqueous samples were diluted in a solution of 2% HNO_3. The U detection limit was less than 0.01 μg L^{-1} for a 95% confidence level based on comparing the statistical difference between the standard deviations obtained from measuring the blank and a 0.05 μg L^{-1} U standard seven times.[27] The pH measurements were taken using a pH electrode (Accumet) and meter (Accumet AB15 pH meter).

X-ray Absorption Spectroscopy. XAS measurements were performed at the Stanford Synchrotron Radiation Lightsource (SSRL). Uranium L_{III}-edge spectra were obtained at beamline 4-1 using a Si(220) monochromator calibrated using Y foil (Y K edge energy 17038.4 eV). Prior to XAS analyses, samples were stored and loaded into holders in an anaerobic chamber (described above) prior to and after being shipped to SSRL in a hermetically sealed stainless steel shipping can (Schuett-biotec GmbH, Göttingen, Germany). Samples were either dried and homogenized with boron nitride or filtered from suspensions onto 0.2 μm nitrocellulose membranes; samples were mounted in aluminum holders covered

with Kapton tape. The spectra were measured in fluorescence and transmission mode using a 13-element germanium detector or a Lytle-type ionization chamber and N_2-filled ionization chambers, respectively. For each sample the results of 2−6 scans were averaged. Postbackground subtraction and data normalization to the subtracted EXAFS were performed by standard methods using IFFEFIT and the Horae program suite.[28] Data were Fourier transformed for the k range of 3−10.2 $Å^{-1}$ using a Kaiser-Bessel window with a dk = 3; the upper limit of the Fourier transform range was chosen to avoid multielectron excitations observed at $k \approx 10.5$ $Å^{-1}$ in monomeric U(IV).[29]

Chemical Equilibrium and Kinetic Modeling. MINEQL + v 4.6 [30] was used to calculate equilibrium solubility. The default database was updated to include the equilibrium constants and reactions included in the latest critically revised thermodynamic databases;[31,32] the full set of reactions is presented in Table S2 SI.

The rates of U(IV) oxidation were analyzed by interpreting the rate of soluble U(VI) production as a first-order process with respect to the amount of U(IV) species present (eq 1). Given the constraint that the total uranium in the system was constant (eq 2), this first-order rate model yields an expression for the concentration of soluble U(VI) (mol L^{-1}) as a function of time (eq 3),

$$\frac{d[U(VI)]}{dt} = k[U(VI)] \quad (1)$$

$$[U(IV)] + [U(VI)] = \text{constant} = [U(IV)]_0 \quad (2)$$

$$[U(VI)] = [U(IV)]_0(1 - e^{-kt}) \quad (3)$$

where $[U(IV)]_0$ is the initial concentration of U(IV) species (mol L^{-1}) and k is the first-order rate constant (s^{-1} or mol U(VI) · mol $(IV)^{-1}$ · s^{-1}). Rate constants can then be converted to forms that express the U(VI) production relative to the mass of U(IV) species (mol U(VI) · g $U(IV)^{-1}$ · s^{-1}). In these units, which are useful for comparison with previously reported uraninite dissolution rates, the rate constant is also equal to the initial dissolution rate (R_{init}) by rearrangement of eq 1.

$$k = R_{init} = \frac{\left(\frac{d[U(VI)]}{dt}\right)_{init}}{[U(IV)]_0} \quad (4)$$

Because the samples of monomeric U(IV) and biogenic uraninite were not completely pure, the release of U(VI) for these systems was interpreted as the sum of the release from each form of U(IV) (eq 5).

$$[U(VI)] = [U(IV)]_{mono,0}(1 - e^{-k_{mono}t}) + [U(IV)]_{bio\text{-}UO_2,0}(1 - e^{-k_{bio\text{-}UO_2}t}) \quad (5)$$

The data sets for the biogenic uraninite and monomeric U(IV) extractions in water in equilibrium with atmospheric oxygen were used simultaneously to find the optimal rate constants to fit the experimental data. Additional details of the optimization procedure are presented in the Supporting Information. For chemogenic uraninite, the extent of dissolution was so low that the concentration of U(IV) was essentially constant over the duration of the experiment and the rate constant could be determined by linear regression of the U(VI) concentration versus time.

RESULTS AND DISCUSSION

Experiments Targeting U Fractions That Are Water Soluble, Ion Exchangeable, Amenable to Complexation, or Oxidizable. Negligible fractions of total U, i.e., less than 0.02%, were extracted during the reaction of deionized water (water-soluble) and NH_4NO_3 (ion exchangeable) with the three U(IV) species included in this study (Figure 1). Higher U

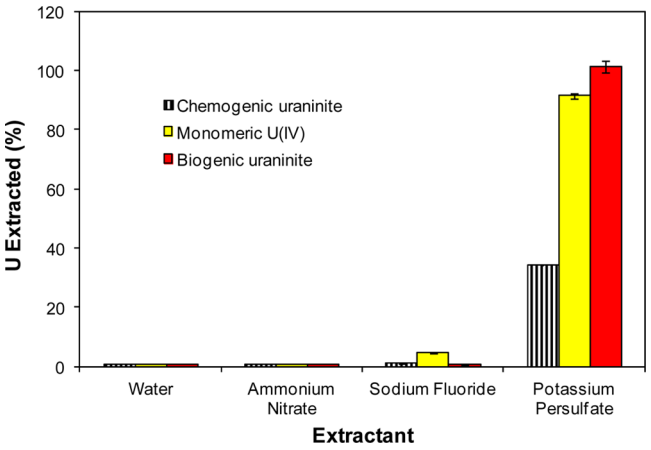

Figure 1. U extracted (%) from water-soluble, ion exchangeable, ligand extractable, and oxidizable extraction experiments with chemogenic uraninite, monomeric U(IV), and biogenic uraninite (not washed with HCO_3^-). The total U concentration for these experiments ranged from 1.48 to 2.10 mM. Less than 0.02% of the total amounts were extracted from the reaction with deionized water (water-soluble) and NH_4NO_3 (ion exchangeable). Potassium persulfate extractions presented in this figure were conducted with 50 mM $K_2S_2O_8$ and 6 h of reaction.

release was obtained from monomeric U(IV) as compared to biogenic and chemogenic uraninite for the NaF extractions that mobilized U(IV) by complexation. This result is consistent with the study of Alessi et al.,[12] which reported that monomeric U(IV) is more labile than biogenic uraninite when reacted with 1 M bicarbonate. However, the reaction with 0.1 M NaF still resulted in extraction of only 5% of the U from biomass-associated monomeric U(IV) species, 0.3% of the U in biogenic uraninite, and 1% of the U in chemogenic uraninite. The equilibrium calculations shown in Figure S1 in SI indicate that all of the uraninite (1.48 mM) would dissolve if the system were allowed to reach equilibrium with 0.1 M NaF, suggesting that the release of U(IV) to solution is controlled by kinetics and that a reaction time longer than 24 h would be necessary to observe higher U release.

The highest fractions of U released were observed from oxidation experiments with 50 mM $K_2S_2O_8$ after 6 h of reaction time. These extractions mobilized 35% of total U in chemogenic uraninite and 90% and 100% of total U in monomeric U(IV) and biogenic uraninite, respectively. Additional experiments were pursued to further investigate the reactivity of monomeric U(IV), biogenic uraninite, and chemogenic uraninite under oxidizing conditions.

Oxidation Experiments with Potassium Persulfate and Dissolved Oxygen. Because 50 mM $K_2S_2O_8$ could oxidize more than 90% of the biogenic U(IV) species after 6 h of reaction, further investigation of the susceptibility of U(IV) species to oxidative mobilization focused on the rates of release and examined a wider range of conditions. Experiments using

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50 mM $K_2S_2O_8$ and sampled at hourly intervals showed that ~97% of the biogenic uraninite was oxidized within the first hour (SI, Figure S2). Thus, the kinetics of oxidation of U(IV) species were studied by obtaining solution samples at hourly intervals and at lower concentrations of potassium persulfate (2 mM and 10 mM $K_2S_2O_8$). It is worth noting that $K_2S_2O_8$ was in excess of U(IV) [added as 1.48 mM U], even at concentrations of 2 mM and 10 mM, based on the 1:1 stoichiometry of the redox reaction between U(IV) and persulfate:

$$U(IV) + S_2O_8^{2-} \rightarrow U(VI) + 2SO_4^{2-} \quad (6)$$

We also evaluated the effect of dissolved oxygen (DO), which is a milder oxidant and a reactant of major environmental relevance, in the oxidation of the three forms of U(IV).

Chemogenic uraninite was more resistant to oxidation than either form of biogenic U(IV) (Figure 2 and Table 2). The differences in U released between the different U(IV) species are particularly noticeable during the first 3 h of these oxidation experiments. Previous studies have provided a detailed characterization of the structure and nanoparticulate nature of biogenic uraninite.[8,33] It is likely that nanoparticles of biogenic uraninite (mean particle diameter ~3.5 nm) are more reactive than chemogenic uraninite particles (mean particle diameter ~100−200 nm) due to the difference in size and reactive surface area. The specific surface area of chemogenic uraninite using this synthesis method was previously measured as 5.9 m^2/g and that of a biogenic uraninite washed with NaOH was 50.1 m^2/g from BET-N_2 measurement;[20] the surface area of the biogenic uraninite is lower than would be predicted for well-dispersed uraninite nanoparticles, and precise quantification of reactive surface area is not possible.[19] Another investigation reported higher mass-normalized dissolution rates for biogenic uraninite than chemogenic uraninite under moderately oxidizing conditions in the presence of 1 mM dissolved inorganic carbon at a pH range of 7.6−8.2.[20] This is consistent with the higher fraction of U oxidized in the presence of $K_2S_2O_8$ and $NaHCO_3$ from biogenic uraninite when compared to that of chemogenic uraninite that was observed in this study. For example, for the reaction with 8.2 mg L^{-1} DO and 100 mM $NaHCO_3$, the rate constant for biogenic uraninite oxidation was 6.2×10^{-7} mol · g U^{1-} · s^{-1} while that for chemogenic uraninite was 1.0×10^{-9} mol · g U^{1-} · s^{-1}. Although the rate constants and associated rates from this study were obtained from batch experiments, the dissolution rate for chemogenic uraninite, now expressed per unit mass of UO_2, (8.9×10^{-10} mol · g UO_2^{-1} · s^{-1}) is just over an order of magnitude lower than a rate reported in a previous study (3.0×10^{-8} mol · g UO_2^{-1} · s^{-1}) performed using continuous flow tank reactors under oxidizing conditions.[20] This may be due to differences in the reactor types (batch vs continuous-flow) that affect the accumulation of U(VI) products in the system. The rate constant of dissolution for biogenic uraninite reported in this study (5.5×10^{-7} mol · g UO_2^{-1} · s^{-1}), which is equivalent to the initial dissolution rate (eq 4), was also an order of magnitude higher than that obtained by Ulrich et al. [20] under oxidizing conditions (($5-7$) $\times 10^{-8}$ mol · g UO_2^{-1} · s^{-1}). Another difference is that the biogenic uraninite used in this study was associated with biomass; the biogenic uraninite used by Ulrich et al. [20] was treated with 1 M NaOH to isolate the material from biomass. The treatment with NaOH could have removed monomeric U(IV) as well as biomass from the biogenic uraninite sample used by Ulrich et al.[20]

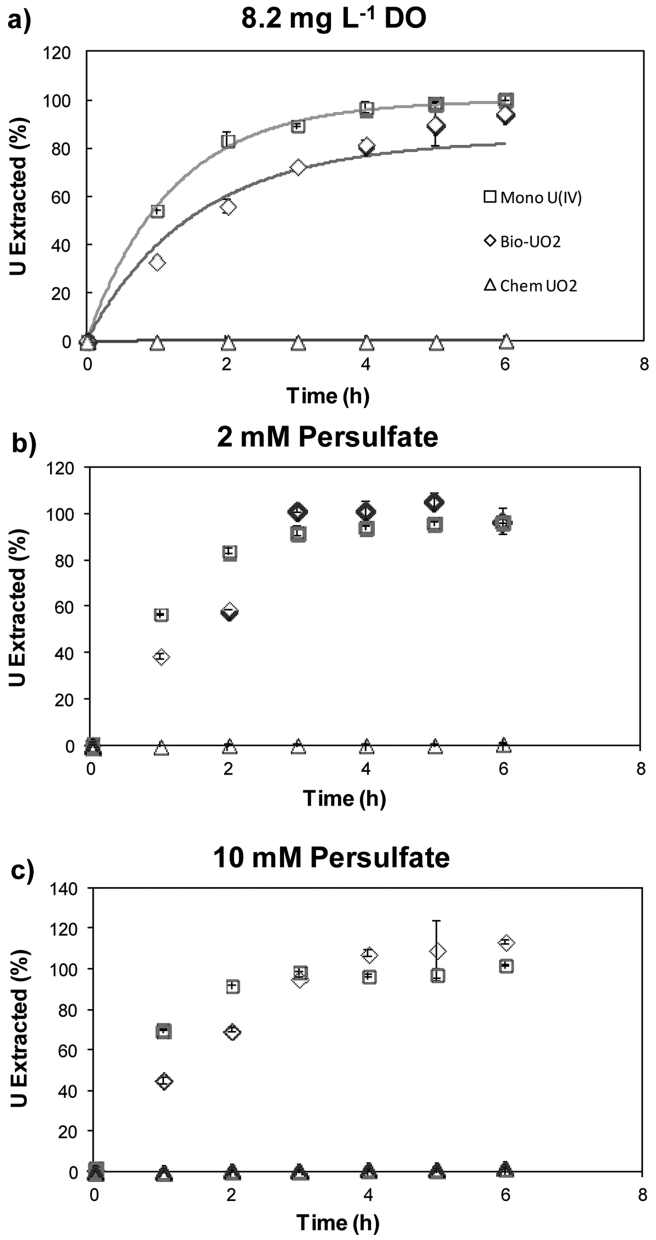

Figure 2. Oxidation of monomeric U(IV), biogenic uraninite not washed with HCO_3^- (Bio UO_2), and chemogenic uraninite (Chem UO_2) with 100 mM HCO_3^- under the following conditions: (a) 8.2 mg L^{-1} dissolved oxygen (DO), (b) 2 mM potassium persulfate ($K_2S_2O_8$); and (c) 10 mM $K_2S_2O_8$. The error bars on most of the data points were smaller than the size of the points. In panel a, the data are shown together with simulations (lines) based on the oxidation rate interpretation using eq 1-5.

Monomeric U(IV) species bound to biomass were more susceptible than biogenic uraninite to oxidation by DO (Figure 2a). Both monomeric U(IV) and biogenic uraninite follow similar trends of U released over time when reacted with 2 mM and 10 mM $K_2S_2O_8$ and 100 mM $NaHCO_3$ (b and c of Figure 2), confirming the susceptibility of these biogenic U(IV) species to oxidation with persulfate. Since DO is a milder oxidant than persulfate, the differences in U released from biogenic uraninite and monomericU(IV) over time were more noticeable in the presence of DO. A concentration of 8.2 mg L^{-1} DO was sufficient to oxidize all of the biogenic uraninite and monomeric U(IV) after 6 h of reaction (Figure 2a). In

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Table 2. U Extracted from Individual Forms and Different Mixtures of Chemogenic Uraninite, Biogenic Uraninite, and Monomeric U(IV) Samples Reacted with HCO_3^-, $K_2S_2O_8$, and DO and Analyzed with X-ray Absorption Spectroscopy

sample name	sample description	reactants in d.i. H_2O	reaction time (h)	% U extracted
Mono-Chem Mix Anoxic	50% monomeric U(IV) + 50% chemogenic uraninite (anoxic control)	100 mM HCO_3^-	6	1
Chem UO_2 + $K_2S_2O_8$	100% chemogenic uraninite	100 mM HCO_3^- + 50 mM $K_2S_2O_8$	6	30
Mono U(IV) + $K_2S_2O_8$	90% monomeric U(IV) and 10% biogenic uraninite	100 mM HCO_3^- + 50 mM $K_2S_2O_8$	6	100
Mono-Chem Mix Oxidized	50% monomeric U(IV) + 50% chemogenic uraninite	100 mM HCO_3^- + 50 mM $K_2S_2O_8$	6	59
Bio UO_2 + $K_2S_2O_8$	biogenic uraninite not washed with HCO_3^-, [which contains 65% biogenic uraninite and ~35% monomeric U(IV)]	100 mM HCO_3^- + 50 mM $K_2S_2O_8$	6	100
Bio UO_2 + DO	biogenic uraninite not washed with HCO_3^-, [which contains 65% biogenic uraninite and ~35% monomeric U(IV)]	100 mM HCO_3^- + 8.2 mg L^{-1} DO	2	92
Washed Bio UO_2 + DO	biogenic uraninite washed with 1 M HCO_3^- [to remove monomeric U(IV)]	100 mM HCO_3^- + 8.2 mg L^{-1} DO	2	52

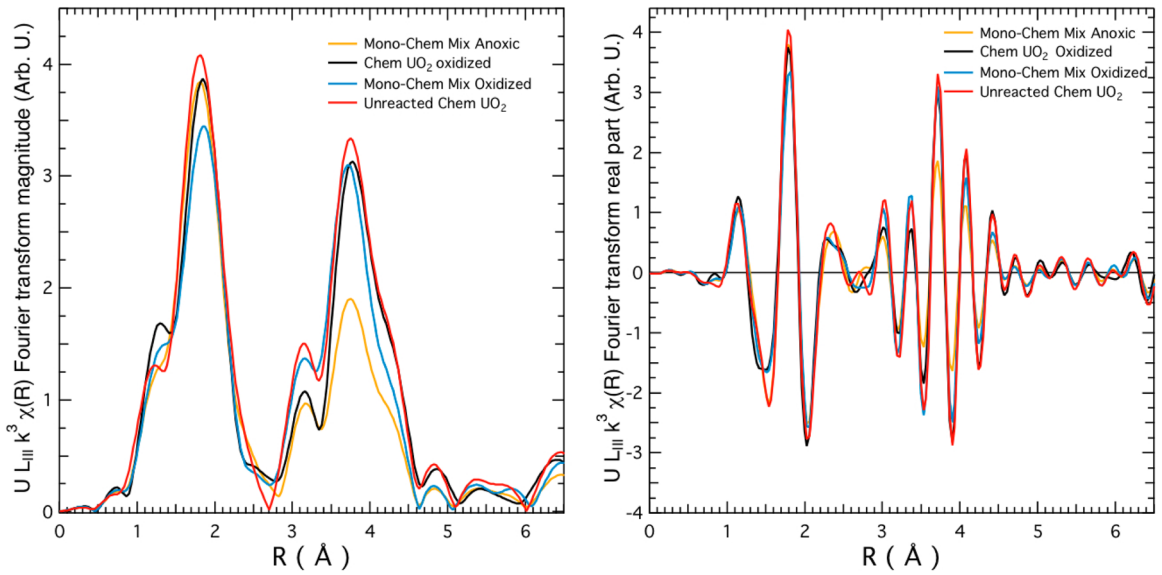

Figure 3. U L_{III}-edge Fourier-transformed EXAFS spectra (Left: Magnitude, Right: Real-part) for a 50% chemogenic UO_2 and 50% monomeric U(IV) mixture exposed to 100 mM HCO_3^- both with an oxidant (Mono-Chem Mix Oxidized with 50 mM persulfate) and without (Mono-Chemic Mix Anoxic, control). Chemogenic UO_2 (Chem UO_2) reacted with 50 mM persulfate and an unreacted chemogenic UO_2 material are included for comparison.

terms of the oxidation rate constants, the value determined for monomeric U(IV) (9.9×10^{-7} mol · g U^{1-} · s^{-1}) was only slightly higher than that determined for biogenic uraninite (6.2×10^{-7} mol · g U^{1-} · s^{-1}).

Oxidation Experiments with Mixtures of U(IV)-Containing Materials. We performed additional experiments to evaluate the selectivity of persulfate and oxygen to extract specific U(IV) species. X-ray absorption spectroscopy (XAS) analyses were conducted to further investigate the effect of oxidation reactions on mixtures of U(IV)-containing materials. The amounts of U extracted from individual forms and mixtures of U(IV) samples exposed under specific oxidizing conditions are presented in Table 2. Only 1% of the total U was solubilized from a mixture consisting of 50% monomeric U(IV) and 50% chemogenic uraninite (Mono-Chem Mix Anoxic, control) when reacted under anoxic conditions with 100 mM $NaHCO_3$ at pH 8.3 for 6 h; the small fraction of U extracted from this mixture was likely surface-associated U(VI). This conjecture was supported by the XANES spectra (Figure S3 in the Supporting Information) which show that the peak for this anoxic sample is slightly wider than that of a $UO_{2.00}$ reference material due to a small fraction of U(VI) present in the mixture of 50% monomeric U(IV) and 50% chemogenic uraninite. There are no detectable differences between the XANES spectra for the anoxic sample and the others exposed to oxidizing conditions. It is likely that the 100 mM $NaHCO_3$ used for these experiments solubilized U(VI) from the oxidized samples so that essentially no U(VI) remained with the solid phase to be detected in the XANES spectra. The fraction of U extracted from all other samples exposed to oxidizing conditions was equal to or greater than 30%. Solids recovered from these experiments were further characterized with EXAFS to investigate the oxidation of the specific U(IV) species included in each of the mixtures tested.

The Fourier transforms of the EXAFS spectra (Figure 3) suggest that monomeric U(IV) was more readily extracted from the mixed samples due to its oxidation by persulfate. This can be confirmed by the increase in the amplitude of the peak near 3.85 Å (associated with the U−U shell observed in uraninite), indicating that the relative fraction of uraninite in this sample

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had increased after persulfate oxidation of the sample. The amplitude of the U−U shell of the sample mixtures oxidized with persulfate (Mono-Chem Mix Oxidized) is similar to that of the unreacted chemogenic uraninite sample (anoxic control). Note that the Fourier transform of the spectrum of the sample with a mixture of 50% monomeric U(IV) and 50% chemogenic uraninite under anoxic conditions (Mono-Chem Mix Anoxic) has the lowest amplitude of the U−U shell; the low amplitude of the U−U shell in this mixture is due to the contribution of noncrystalline monomeric U(IV) as observed by Alessi et al.[12] Monomeric U(IV) does not have a U−U shell and, thus, its presence interferes with the signal of uraninite in the mixed sample. When monomeric U(IV) is oxidized this interference effect disappears, causing an increase in the amplitude of the U−U shell due to the contribution of the remaining chemogenic uraninite.

Although the oxidative dissolution of chemogenic uraninite by persulfate is thermodynamically favorable, the prevalence of the bulk chemogenic uraninite material can be observed in the Fourier transform of the EXAFS spectra even after 6 h of reaction time with 50 mM $K_2S_2O_8$ and 100 mM $NaHCO_3$. This is consistent with the results shown in Table 2 that indicate incomplete (30% U) extraction from the chemogenic uraninite sample after 6 h of reaction with 50 mM $K_2S_2O_8$ and 100 mM $NaHCO_3$. Thus, more than 6 h would be required for complete oxidation of chemogenic uraninite to occur.

Environmental Implications. Recent investigations have reported that a mixture of biogenic uraninite and monomeric U(IV) is present in bioreduced sediments.[2,12,14] Thus, characterizing the stability of these U(IV) products is necessary to understand the fate and transport of uranium in subsurface environments. The results of this study indicate that monomeric U(IV) is slightly more susceptible to oxidation than biogenic uraninite in the presence of dissolved oxygen. These results could at least partially explain the often rapid reoxidation of uranium reported in other bioreduction experiments.[23,24] Monomeric U(IV) bound to biomass, biogenic vivianite and magnetite, and a field sediment from Rifle, CO, are more labile than uraninite when exposed to bicarbonate.[12] More ligand-extractable U was released from monomeric U(IV) as compared to biogenic and chemogenic uraninite in this study. Chemogenic uraninite was more stable than monomeric U(IV) and biogenic uraninite when reacted with the chemical extractants used in this study.

It should be noted that the present study focused on understanding the relative rates of U release from biogenic and chemogenic uraninite and monomeric U(IV) when reacted under controlled experimental conditions. Future research can couple this information on the rates of chemical processes with advective and diffusive transport processes through the use of reactive transport models. Our results suggest that exposure to oxidants (e.g., dissolved oxygen) is more important than the actual form of biogenic U(IV), given the major differences in U release in the presence and absence of oxidants and the minor differences observed in the oxidation rate constants of biogenic uraninite and monomeric U(IV). Consequently, the transport processes that control the exposure of biogenic U(IV) species to dissolved oxygen will be critical to assessing the long-term fate of uranium in actual subsurface environments. The importance of diffusion-limited microsites for sequestering U in natural systems has been reported in other studies.[34−36] Another aspect to consider is that the specific surface area of biogenic uraninite will vary depending on the particle size, and the accessibility of monomeric U(IV) to oxygen can be affected by the nature of the biomass with which it is associated. Thus, reactive transport models applied to the context of bioremediated and natural subsurface environments should take into account diffusive transport processes and the physical characteristics of the U(IV) species when incorporating the reaction rate constants presented in this study.

Bioremediation strategies should aim to minimize the contribution of monomeric U(IV) to the total U(IV) produced during microbial in situ reduction of U(VI), and methods that result in the most crystalline and lowest surface area forms of uraninite will be most resistant to oxidative remobilization of the uranium. Understanding the factors that control the stability of U(IV) species in subsurface environments is of fundamental importance toward the long-term optimization of in situ bioremediation strategies.

■ ASSOCIATED CONTENT

Supporting Information

Two additional tables (S1 and S2), six additional figures (S1−S6), and discussion of the oxidation rate analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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