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Biodegradation of Uranium−Citrate Complexes: Implications for Extraction of Uranium from Soils

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Citrate is often used as a complexing agent to mobilize sorbed and precipitated uranium in both *in situ* and *ex situ* extraction of soils and nuclear reactor components. The biodegradability of U−citrate complexes is an important control over the potential migration of residual uranium after the extraction process is complete. In solutions buffered at pH 6−7, limited biodegradation of citrate is observed within 10 days with initial U:citrate molar ratios ranging from 1:2 to 1:8; however, over 99% of the citrate is biodegraded rapidly at pH 8−9. The increase of pH may have shifted the equilibrium speciation of uranium from $(UO_2-citrate)_2^{2-}$ to $(UO_2)_3(OH)_7^{1-}$ and, consequently, raised the bioavailability of citrate. At pH 6−7, a significant amount of uranium is also observed to associate with biomass, whereas only a negligible amount is observed at pH 8−9. Our experimental results suggest that the residual concentration of uranium−citrate complexes left in the treated soils can be reduced rapidly if the soil water pH is held between 8 and 9 after the extraction processes.

Introduction

Large volumes of unsaturated soils at DOE facilities are contaminated with uranium from decades of uranium extraction and purification. Uranium is present primarily as sorbed, complexed, and/or precipitated uranyl (UO_2^{2+}) in contaminated soils (*1*, *2*). The uranium contamination can be removed from the soils using *ex situ* extraction techniques after soils are physically removed or, alternatively, *in situ* removal techniques such as electrokinetics may be applied (*3*). The addition of proper anionic complexing agents can expedite the extraction of cationic uranyl contaminants from unsaturated soils by forming anionic complexes that are likewise repelled by most mineral surfaces. (Note though that the sorption of uranium−citrate complexes will depend upon pH with potentially greater surface sorption at low pH.) The complexing agents may also facilitate the dissolution of uranium precipitates into the pore water and therefore increase the extraction mobility of the uranium contaminants.

Citric acid is a nontoxic, tricarboxylic acid that is commonly used as a chelating agent for metal ions. Under oxidizing conditions, citrate primarily forms a binuclear complex with uranyl−$(UO_2-citrate)_2^{2-}$—and to a lesser extent, a tridentate complex, $UO_2-citrate^{1-}$ (*4*). Bench-scale electrokinetic experiments with citrate as the complexing agent showed over 70% of uranium removed within 55 days from a sediment waste (with high concentrations of calcium, copper, and magnesium) obtained from the DOE-Hanford site (*5*). For *in situ* field operations, however, it is also essential that any citrate remaining in soils (in pure or complex forms) after extraction be short-lived, hence unable to facilitate the transport of residual uranium into groundwater.

Francis et al. (*6*) observed that U−citrate complexes in a well-buffered solution (pH = 6.1) were not biodegraded by *Pseudomonas fluorescens* isolated from the leachate of a low-level radioactive waste disposal site. Nevertheless, there is little in the literature describing the biodegradability of U−citrate complexes in solutions with alkaline pH, such as are observed in our laboratory during *in situ* electrokinetic treatment of unsaturated soils. Here, we report measurements of significant biodegradation of U−citrate complexes at greater than neutral pH.

TABLE 1. Speciation of Citrate and Uranium at pH 6 and pH 9 with 1:2.4 U−Citrate Molar Ratio[a]

	pH = 6 (%)	pH = 9 (%)
Citrate Speciation		
citrate	21.3	98.1[b]
citrate−H	33.9	
citrate−H_2	1.5	
$UO_2-citrate^{1-}$	10.2	
$(UO_2-citrate)_2^{2-}$	31.5	
Uranium Speciation		
$UO_2-citrate^{1-}$	24.4	
$(UO_2-citrate)_2^{2-}$	75.5	
$UO_2(OH)_3^{1-}$		2.5
$(UO_2)_3(OH)_7^{1-}$		97

[a] Speciation was calculated using MINTEQA2 with the following parameters: citrate, 1.15 mM; uranium, 0.48 mM; 1 atm of O_2 in the headspace, 22 °C. The mineral solution composition was Ca^{2+}, $9.362e^{-3}$ mM; Fe^{2+}, $2.687e^{-3}$ mM; Mg^{2+}, $1.541e^{-2}$ mM; Mn^{2+}, $3.409e^{-3}$ mM; NH_4^+, $3.344e^{-1}$ mM; Na^+, $1.462e^{-1}$ mM; Cu^{2+}, $2.947e^{-4}$ mM; K^+, $9.602e^{-2}$ mM; Zn^{2+}, $2.865\ e^{-4}$ mM; Cl^-, $4.382e^{-1}$ mM; SO_4^{2-}, $2.341e^{-2}$ mM. [b] The remaining 1.9% of citrate was in complexation with other ions (e.g., Mg^{2+}, Ca^{2+}) in the solution.

Experimental Section

Materials. Activated sludge obtained from the Socorro Wastewater Treatment Plant (Socorro, NM) was used as inoculum to provide a wide range of citrate-metabolizing bacteria. The sludge was concentrated via centrifugation, washed twice with a mineral solution, and fed with citrate as the only substrate for at least 1 week before being used as the inoculum. The mineral solution (*7*) was designed to sustain bacterial cell synthesis yet provide a low ionic strength of 7.14×10^{-4} in order to minimize the formation of metal−citrate complexes other than U−citrate. (The composition of the mineral solution is listed in Table 1.) Phosphate and carbonate levels were kept at a minimum to prevent their complexation with uranyl ions. Glycerol 2-phosphate (0.074 mM) was used to provide phosphate for cell synthesis, and pure oxygen was sparged through the mineral solution as the source of oxygen and to minimize the concentration of carbonate in solutions. Citrate was added in the form of sodium citrate, and depleted uranium, $(NO_3)_2{}^{238}UO_2{\cdot}6H_2O$ was used as the source of uranyl ion. Piperazine-*N*,*N*′-bis(2-

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ethanesulfonic acid) (PIPES) with $pK_{a,20°C} = 6.8$ was used to buffer the pH of solutions to 6 and 7, and tris(hydroxymethyl)aminomethane (TRIS) with $pK_{a,20°C} = 8.3$ was used to maintain the pH of solutions at 8 and 9. Neither buffer solution forms complexes with the uranyl ion (*8*).

Degradation of U−Citrate Complexes at pH 6. In order to confirm the results of Francis et al. (6), experiments were first conducted to examine the biodegradation of U−citrate complexes in mineral solutions buffered at pH 6. Three to four replicates were conducted for each experimental setup in this study, and the representative results were reported. One-liter aspirator bottles, each with 700 mL of the mineral solution, were used as complete-mixed reactors. The temperature of the solution was controlled at 22 °C. The inlets and outlets of the reactors had 0.45-μm filters to prevent bacteria from entering the reactors. All of the components used in the experiments were sterilized through autoclaving or syringe filtration (0.22 μm). Experiments were conducted in the dark because both oxidation of citrate and reduction of UO_2^{2+} have been observed when uranyl citrate complexes were exposed to visible light (*9*). The initial U:citrate molar ratio in the solution was about 1:1.9 (0.42 mM U:0.78 mM citrate). The solution was maintained at pH ~6 by adding 30 mL of 0.7 M PIPES into 670 mL of the mineral solution. The initial biomass concentration was approximately 70 mg/L volatile suspended solids (VSS). Ion speciation was predicted using the geochemical equilibrium speciation model MINTEQA2 with a modified data base (*10*). Potential inhibition of uranyl ion on the cellular metabolic function was evaluated by replacing citrate with noncomplexing glucose as the substrate (U:glucose molar ratio ~ 1:1.9).

Degradation of U−Citrate Complexes in an Unbuffered Solution. In unbuffered and uranium-free mineral solutions, biodegradation of citrate with initial solution pH ~6 is observed to cause a gradual increase in pH until complete removal of citrate has been achieved. The pH increase, if it also occurs in the presence of uranyl ion, may facilitate the biodegradation of citrate as more free citrate is available in a pH 9 solution relative to the same solution at pH 6 (Table 1). In other words, uranium−citrate complexation is less important under alkaline conditions. However, it is important to note that the calculations are for equilibrium conditions and the dissociation rates of U−citrate complexes may be the rate-limiting step of citrate biodegradation.

Experiments were conducted to study the biodegradability of uranium−citrate complexes in unbuffered solutions (without the addition of any buffer such as bicarbonate, phosphate, TRIS, or PIPES). The initial citrate concentration in the bioreactor was 1.34 mM, and the initial biomass concentration was approximately 60 mg/L VSS. The specific substrate utilization rate of citrate ($U_{baseline}$) in the absence of uranium was determined over the first 12 h. About 1.6 mL of 0.182 M uranyl nitrate was added into the reactor immediately after. When the uranyl nitrate was added, the citrate concentration was 1.15 mM (U:citrate = 1:2.4), and the solution pH was approximately 7. The concentrations of dissolved oxygen (DO), pH, dissolved uranium, and citrate were measured over the duration of the experiment. The total amount of uranium sorbed onto biomass was determined at the end of the experiment in order to understand the distribution of uranium in the system.

Effects of pH and Molar Ratios on the Degradation of U−Citrate Complexes. A number of experiments were conducted under pH-buffered conditions (pH 6, 7, 8, and 9) to quantify the specific controls on degradation. Three different U:citrate molar ratios (low citrate ratios: 1/1.2−1/1.9 U/citrate; medium citrate ratios: 1/2.9−1/4 U/citrate; high citrate ratios: 1/7−1/10.4 U/citrate) were studied at each pH. The initial uranyl concentration for all cases was about 4.2 mM.

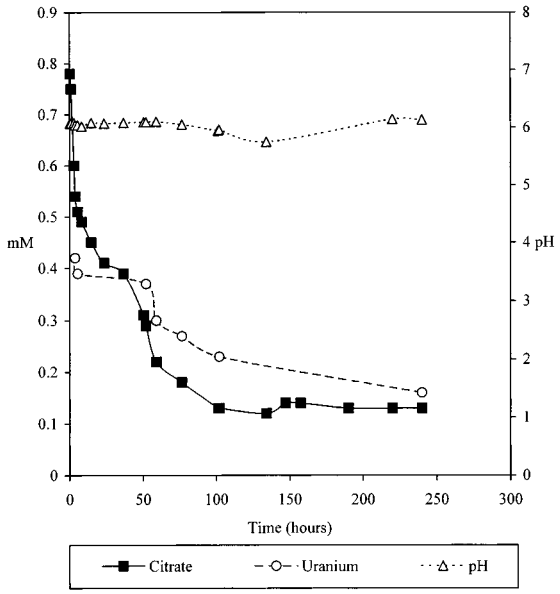

FIGURE 1. Degradation of U−citrate complexes (1:2 molar ratio) in a buffered solution (pH ≈ 6). The initial uranyl and citrate concentrations were 0.42 and 0.78 mM, respectively.

Analytical Procedures. A modified spectrophotometry method (*11*) was employed to analyze aqueous uranium concentrations. A sample of 0.5 mL was first filtered through a 0.22-μm syringe filter to remove biomass and solids in the solution. The filtrate was then mixed with 0.1 mL of oxalic acid (4%) and 0.1 mL of arsenazo (III) (0.05%) and diluted with hydrochloric acid (4 M) to a total volume of 2.5 mL before analyzing at a wavelength of 652 nm. The aqueous uranium results were confirmed using ICP−MS. The amount of uranium sorbed onto biomass was determined by the difference between the total amount (sorbed + dissolved) of uranium in a reactor and the amount of uranium in the filtrate (dissolved phase only). The total amount of uranium was determined by nitric acid digestion (*12*). Unfiltered 3-mL samples were dried overnight at 85 °C, and the residue was then digested at 80 °C for 24 h with 5 mL of nitric acid (7 M). The nitric acid was dried out at 130 °C, and the resulting solid was dissolved with deionized water to a volume of 5 mL for analysis. The amount of uranium transported through the cell membranes was also estimated by this method. The biomass was first concentrated in a test tube through centrifugation and then rinsed with three 10 mL of an EDTA solution (10 mM) and one 10 mL of deionized water before digestion. Citrate concentrations were measured using HPLC (C-18 column, 0.15 M of H_3PO_4 at pH = 2.3, isocratic, 210 nm). Biomass was estimated using the concentrations of VSS (*13*).

Results and Discussion

Confirmation of Prior Published Results. We observed a biodegradation pattern of citrate similar to that seen by Francis et al. in solutions buffered at pH ~6 (*6*). Citrate levels dropped from 0.78 mM to approximately 0.13 mM (~83% reduction) within 100 h and remained constant thereafter to about 240 h (Figure 1). No significant fluctuation in pH (<0.2 unit) was observed during the course of the experiment. Speciation calculations suggested that about 0.37 mM (47%) of the total citrate was complexed and therefore not available for biodegradation at the beginning of the experiment. The higher percent of citrate degradation was largely due to the decrease of the aqueous uranium concentration from 0.42 to 0.16 mM (~62% reduction). Biosorption, as discussed later, appeared to be the main

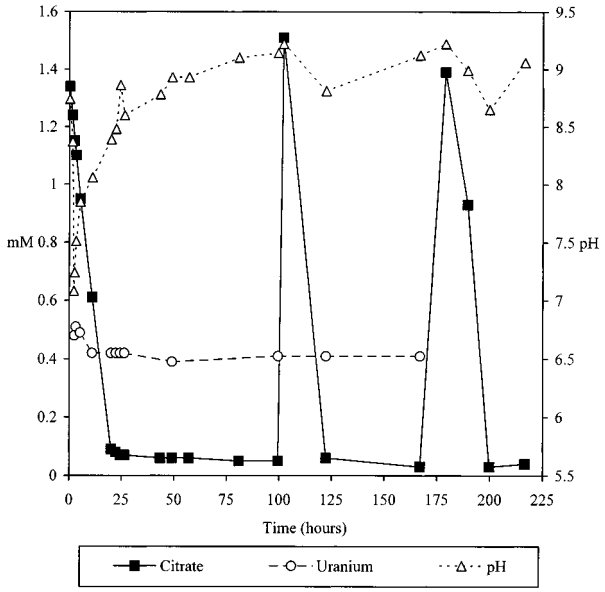

FIGURE 2. Degradation of U−citrate complexes (1:2.4 molar ratio) in an unbuffered solution. The initial uranyl and citrate concentrations were 0.48 and 1.15 mM, respectively.

removal mechanism of uranyl ions from the aqueous phase. Soon after the addition of uranium, the specific substrate utilization rate of citrate reduced significantly to about 16% of the $U_{baseline}$ (from 1×10^{-3} to 1.59×10^{-4} mmol/h·mg of VSS). The reduction of the citrate utilization rate was primarily attributed to decreased bioavailability. Negligible toxicity of uranyl ion on the bacteria was observed using glucose as the sole substrate. This result conformed to the observations of other researchers (*6*).

Effect of pH Rise on Speciation and Degradation. In the unbuffered solution, the biodegradation rate of citrate decreased by 25% upon addition of uranyl nitrate, probably due to the formation of U−citrate complexes, and an attendant decrease in free citrate. The solution pH rose from 7.08 to 9.14 in 100 h (Figure 2) during which roughly 15% of the uranyl was accumulated onto the biomass. At the same time, the citrate level decreased by 96%, to 0.046 mM. On the basis of speciation calculations, the percentage of citrate available for biodegradation rose from 66.1% to 98.1% when the pH increased from 7 to 9. At pH 9, uranyl ions are predominantly in the form of $(UO_2)_3(OH)_7^{1-}$ (~97%). Bergsma (*14*) and Willecke (*15*) suggested that the pH increase observed during citrate biodegradation may be caused by the co-transport of citrate, a divalent cation, and a proton into bacterial cells to maintain cell electroneutrality. Although U−citrate complexes themselves may not be biodegradable, as suggested by Francis (*6*), this bacterially-mediated pH increase appears to promote the formation of uranium hydroxide complexes and, consequently, the release of citrate for biodegradation. Repeated spiking of citrate at pH around 9 over 115 h showed no significant reduction in the biodegradation rate (Figure 2), indicating that the alkaline pH was not specifically retarding the metabolic function of bacteria.

Biodegradation of U−Citrate Complexes at Different pH and Molar ratios. Citrate concentrations in solutions buffered at pH 8 and pH 9 rapidly fell to nondetectable levels (<1 ppm), whereas those in solutions buffered at pH 6 and pH 7 decreased slowly, leaving between 0 and 15% of the initial citrate after 200 h (Figure 3). This trend of higher citrate degradation rate at alkaline pH correlated well with the speciation prediction of more uncomplexed citrate present at higher pH. Little influence of microbial activity was

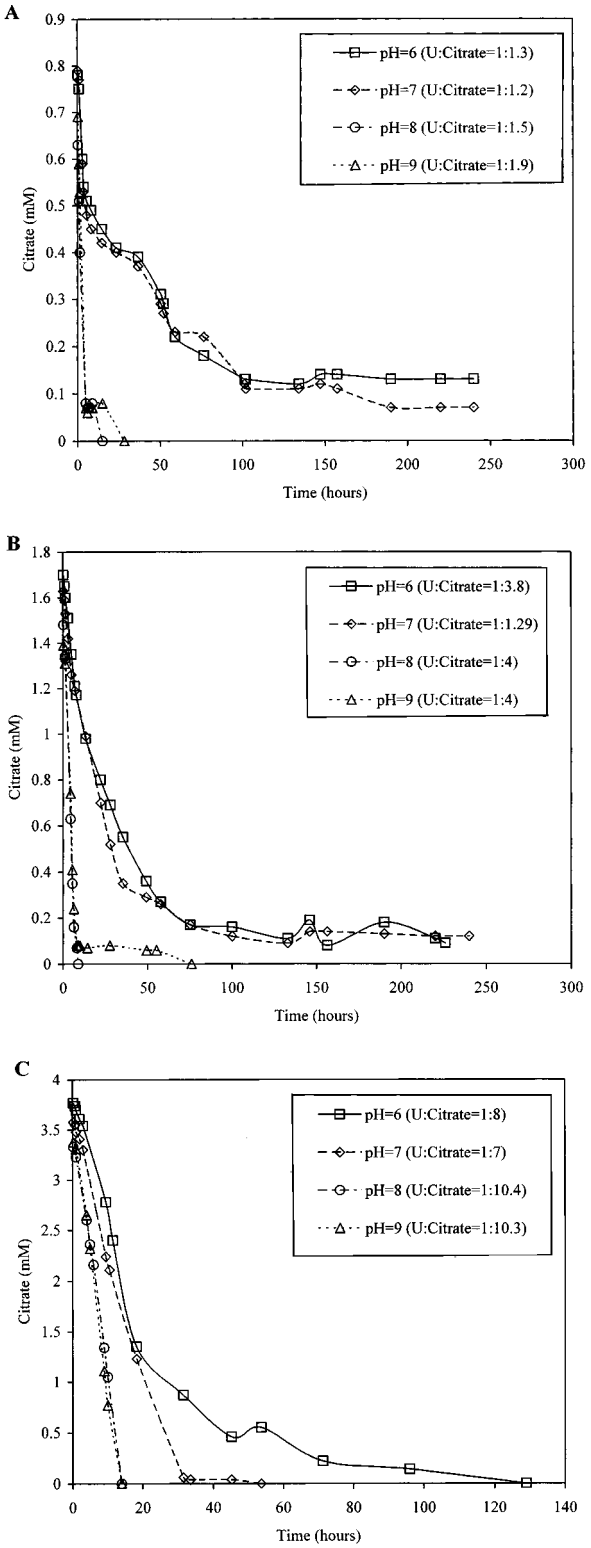

FIGURE 3. Comparisons of biodegradation potential of U−citrate complexes with different initial U:citrate molar ratios: (A) low citrate ratios; (B) medium citrate ratios; (C) high citrate ratios at pH from 6 to 9. The initial uranyl concentration for all cases was about 0.42 mM.

expected from the changing of pH alone since prior experimental results of citrate biodegradation without uranyl ion indicated that there was little changes of specific substrate utilization rate of citrate at pH between 7 and 9 (*16*). Higher average specific substrate utilization rates of citrate were

observed in solutions with high citrate ratios under all pH conditions. For example, at pH 8−9, the average specific substrate utilization rates of citrate in solutions with high citrate ratios (0.8−1.05 μmol/h·mg of VSS) were about double of those with low citrate ratios (0.3−0.48 μmol/h·mg of VSS).

Biosorption of Uranium. The uptake of uranium by biomass was markedly affected by the pH of the solutions. (Note that the uptake of uranium by biomass potentially includes sorption of uranyl ions on the surface of bacterial cells, sorption through the formation of surface precipitate with bacteria as nuclei, as well as cell internalization.) No significant accumulation of uranium by biomass was observed in solutions of alkaline pH. At pH 6 and 7, however, significant uptake of uranium by biomass was measured at about 1.28−3.84 μmol of uranium per mg of VSS with higher uptakes associated with low citrate ratios. This observed pH effect on the biosorption of uranium was similar to that observed by Horikoshi et al. (*17*) with *Actinomyces levoris* and *Streptomuces viridochromogenes*, yet the uptakes were at least 10 times higher. A negligible amount of uranium was detected after bacterial cells were washed with EDTA and digested with acid, suggesting that uranium was primarily removed from solution through extracellular adsorption. Intracellular accumulation of uranium, such as that observed by Strandberg et al. (*18*) on *Pseudomonas aeruginosa*, was apparently not significant in our study. It is not clear what the main sorption mechanisms are in this study. Phosphate and carboxyl groups on the cell surface may serve as sites of uranium complexation since the groups and uranyl ions all possess predominately negative charges at alkaline pH and positive charges at acidic pH, which should minimize surface sorption of uranium through charge repulsion.

Implications for *In Situ* Application. Our results suggest that citrate complexed with the residual uranium left in treated soils can be biodegraded rapidly if the soil water pH is kept between 8 and 9. Proper moisture content and oxygen level will also need to be maintained in order to sustain the biodegradation process. Although the transport of residual uranium through uranium−citrate complexes can be reduced by the citrate degradation process, it is not known whether uranium−hydroxyl complexes formed at alkaline pH will be less mobile.

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