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Biotransformation of uranium and other actinides in radioactive wastes

A.J. Francis*

Department of Applied Science, Brookhaven National Laboratory, Upton, NY 11973, USA

Abstract

Microorganisms affect the solubility, bioavailability, and mobility of actinides in radioactive wastes. Under appropriate conditions, actinides are solubilized or stabilized by the direct enzymatic or indirect nonenzymatic actions of microorganisms. Biotransformation of various forms of uranium (ionic, inorganic, and organic complexes) by aerobic and anaerobic microorganisms has been extensively studied, whereas limited information is available on other important actinides (Th, Np, Pu, and Am). Fundamental information on the mechanisms of biotransformation of actinides by microbes under various environmental conditions will be useful in predicting the long-term performance of waste repositories and in developing strategies for waste management and remediation of contaminated sites.

Keywords: Biotransformation; Uranium; Uranyl–citrate; Actinides; Radioactive wastes

1. Introduction

The presence of the actinides Th, U, Np, Pu, and Am in radioactive wastes, in particular in low-level and transuranic (TRU) wastes, is a major concern because of their potential for migration from the waste repositories and long-term contamination of the environment. The toxicity of the actinide elements and the long half-lives of their isotopes are the primary causes for concern. The actinides may be present in various forms, such as elemental, oxide, coprecipitates, inorganic, and organic complexes, and as naturally occurring minerals. Further, the presence of organic compounds and nitrate in low-level and TRU wastes can promote bacterial activity, which can alter the stability and mobility of the actinides. Although the physical, chemical, and geochemical processes affecting dissolution, precipitation, and mobilization of actinides are being intensively investigated, we have only limited information on such effects of microbial processes. In this paper, the state of knowledge of the microbial transformations of actinides is reviewed, emphasizing the biotransformation of uranium.

2. Mechanisms of biotransformation of the actinides

The actinides exist in various oxidation states: Th (III, IV); U (III, IV, V, VI); Np (III, IV, V, VI, VII); Pu (III, IV, V, VI, VII); and Am (III, IV, V, VI, VII); the more stable ones are III (Am, Pu, U), IV (Th, Pu, U), V (Np), and VI (Pu, U). Actinides may be present initially as soluble or insoluble forms and, after disposal, may be converted from one to the other by microorganisms [1]. Under appropriate conditions, actinides can be solubilized or precipitated by direct (enzymatic) or indirect (nonenzymatic) microbial action. These include: (i) oxidation–reduction reactions, (ii) changes in pH and Eh, (iii) chelation, or the production of specific sequestering agents, (iv) biosorption by biomass and biopolymers, (v) formation of stable minerals, and (vi) biodegradation of actinide–organic complexes. Microbial activities are influenced by electron donors and acceptors, and the extent of dissolution and precipitation could be significant, particularly under anaerobic conditions. The form of the metal (e.g. elemental, oxide, sulfide, ionic, inorganic complex, organic complex, co-precipitate), the availability of electron donors, electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, organic compounds), nutrients (nitrogen, phosphorus), and environmental factors (pH, Eh, temperature, moisture) affect the extent of microbial activity. In anaerobic environments, actinides can be reduced enzymatically from a higher oxidation state to a lower one which affects their solubility and bioavailability. For example, reduction of $U^{6+} \rightarrow U^{4+}$ decreases its solubility. Many organic compounds form stable complexes with actinides, and increase their solubilization and leaching. Likewise, microbial metabolites and the products or intermediates from waste degradation may be an important source of agents affecting the long-term solubility of actinides.

*Corresponding author.

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3. Dissolution of actinides

Actinides can be dissolved by direct and indirect microbial action. Direct action involves enzymatic oxidation–reduction; indirect action involves the production of mineral acids, organic acid metabolites, and oxidizing agents, and lowering the pH of the medium. Anaerobic microbial reduction and dissolution of iron and manganese oxides, wherein the metal is used as the terminal electron acceptor has been extensively studied [2,3]. Mostly, these redox reactions require actively metabolizing bacteria. Metal ions also can be reduced passively when they bind to reactive sites on the surface of, or within, microbial cells.

The indirect dissolution of metals is due to the production of sulfuric acid from the oxidation of sulfide minerals by autotrophic bacteria, organic acids from the metabolism of organic compounds by heterotrophic bacteria, and elaboration of metal-sequestering agents, such as siderophores. The mechanisms of uranium dissolution from minerals by autotrophic microbes have been studied [1]. An increase in heterotrophic microbial activity due to biodegradation of organic constituents of the waste can increase the solubility of the actinides. Several mechanisms have been proposed for heterotrophic microbial solubilization, including the production of organic acids, and of chelates. Often, a combined effect is important; for example, organisms may secrete organic acids which have a dual effect in increasing actinide dissolution by lowering pH, and by complexation. Microbially produced dicarboxylic acids, polyhydroxy acids, and phenolic compounds, such as protocatechuic acid and salicylic acid, are effective chelating agents. A wide variety of heterotrophic microorganisms are involved in solubilizing uranium oxide from granitic rock by producing organic–acid metabolites, such as oxalic, isocitric, citric, succinic, hydrobenzoic, and coumaric acids via their carboxylic and phenolic groups [1].

As there are chemical and biochemical similarities between Pu(IV) and Fe(III), and between Th(IV) and Pu(IV), iron-sequestering agents could be important in the complexation of Pu and other actinides, and thus increasing their solubilization and bioavailability. *Pseudomonas aeruginosa*, from a Pu-contaminated pond at Rocky Flats, that can bioaccumulate uranium, elaborated several chelating agents for thorium and uranium when grown with these metals [4]. Microorganisms grown in an iron-deficient medium elaborate specific iron chelators. For example, dissolution of plutonium dioxide was enhanced in the presence of Desferal, a polyhydroxamate chelate produced by microorganisms [5]. Desferrioximine and enterobactin isolated from *Escherichia coli* solubilized hydrous plutonium(IV) oxyhydroxide [6]. Microorganisms grown in the presence of plutonium produced complexing agents [7]; these agents may transport plutonium into the cells [8]. Anaerobic dissolution of hydrous PuO_2(s) by *Bacillus* sp. was reported, although the mechanism of action was unknown [9]. Bacteria isolated from sediments and grown with ^{241}Am in minimal medium produced exometabolites which formed soluble complexes with Am [10]. Thus, the potential exists for dissolution of actinides in wastes by microorganisms, so increasing their bioavailability and mobility.

4. Immobilization of actinides

Bioaccumulation, biosorption, precipitation, and mineral formation, the key processes involved in immobilizing actinides have received considerable attention because of their potential for bioremediating radionuclide-contaminated sites and waste streams [11,12]. However, little is known of the long-term stability of the immobilized actinides (i.e. mineralization rates and the fate of remobilized radionuclides) in the subsurface.

4.1. Bioaccumulation

Bioaccumulation is an active process wherein metals are taken up into living cells and sequestered intracellularly by complexation with specific metal-binding components or by precipitation. Intracellular accumulation of metals occurs among all classes of microorganisms, usually by an energy-dependent transport system. Localizing the metal within the cell permits its accumulation from bulk solution, although the metals cannot be easily desorbed and recovered.

4.2. Biosorption

Biosorption processes essentially are chemical ones whereby the biomass acts as a surface upon which metals bind by ligand interactions or by ion exchange. Living and dead microorganisms, possess abundant functional groups, such as carboxyl, hydroxyl and phosphate, on their surface that bind metal ions. Polymers secreted by many metabolizing microbes also immobilize metals. Desorption and recovery of the biosorbed radionuclides is easy. Radionuclide-binding to cell surfaces and polymers is a promising technology for remediating contaminated waters. The mechanisms of such metal binding, and its application in bioremediation of waste streams, were reviewed elsewhere [11–13].

Microbes biosorb or bioaccumulate Th, U, Np, Pu and Am. For example, Pu and Am was taken up by the fresh-water bacterium *Aeromonas hydrophila* [14]. Bacterial uptake of water-soluble Am appears to be primarily due to adsorption to the cell's surface; adsorption is reversible and depends upon the nutrients present, the physiological state of the cell, pH, and the release of bacterial exometabolites [10]. Np in excess of 10 mg g^{-1} dry weight of cells was taken up by *Pseudomonas aeruginosa*, *Streptomyces viridochromogenes*, *Scenedesmus ob-*

liquus, and *Micrococcus luteus* growing in solutions containing 36 mg l^{-1} of Np [15]. Francis et al. [16] evaluated the interactions and transport as biocolloids of dissolved actinides, ^{232}Th, ^{238}U, ^{237}Np ^{239}Pu, and ^{243}Am, with a pure and a mixed culture of halophilic bacteria isolated from the Waste Isolation Pilot Plant repository under anaerobic conditions. The sizes of the bacterial cells ranged from 0.54×0.48 to 7.7×0.67 μm. The association of actinides with mobile bacterial cells (10^6–10^9 cells ml^{-1}) suspended in a fluid medium containing NaCl or $MgCl_2$ brine, at various growth phases, was determined by sequential microfiltration. The amount of actinide associated with the suspended cell fraction (calculated as mol $cell^{-1}$) was very low: Th, 10^{-12}; U, 10^{-15}–10^{-18}; Np, 10^{-15}–10^{-19}; Pu, 10^{-18}–10^{-21}, and Am, 10^{-18}–10^{-19}; it varied with the bacterial culture. The differences in association were attributed to the extent of bacterial bioaccumulation and biosorption, pH, the composition of the brine, and the speciation and bioavailability of the actinides.

4.3. Biocrystallization

Biocrystallization, also called bioprecipitation or biomineralization, is the induction of metal precipitates and minerals by bacterial metabolism. Bacteria interact very strongly with metal ions and immobilize and concentrate them, eventually generating minerals. Microbial biofilms bind significant quantities of metallic ions naturally, and also serve as templates for the precipitation of insoluble mineral phases. The biochemistry of the interactions of metal ions with bacterial cell walls, extracellular biopolymers, and microfossil formations in immobilizing toxic metals was reviewed [11,12]. In nature, bacteria are constantly producing minerals in soils and sediments. *Citrobacter* sp. accumulate heavy deposits of metal phosphate, derived from an enzymatically liberated phosphate ligand. The cells have no saturation constraints and can accumulate several times their own weight of precipitated metal. For example, uranyl phosphate accumulates as polycrystalline HUO_2PO_4, at the cells' surface. The phosphatase enzyme located on the cell surface of *Citrobacter* sp. cleaves glycerol-2-phosphate liberating HPO_4^{2-}, causing precipitation of uranium [12].

4.4. Bioreduction

Reduction of an element from a higher to a lower oxidation state or to an elemental form affects its solubility, resulting in its precipitation. Reduction of hexavalent uranium to the tetravalent state occurred in axenic cultures of iron-reducing, fermentative, and sulfate-reducing bacteria, cell-free extracts of *Micrococcus lactilyticus*, and in uranium wastes by *Clostridium* sp. [17]. The reduction of uranyl ion by *Desulfovibrio vulgaris* is catalyzed by

$$U(VI)_{aq} \xrightarrow[\text{fermenters, sulfatereducers}]{\text{dissimilatory metal reducers}} U(IV)_s$$

the enzyme cytochrome c_3 but this activity is not associated with the energy yielding process regulating growth of the bacteria [2]. The enzyme(s) involved in the reduction of uranium by clostridia and other organisms are unknown.

Determining the oxidation states of uranium in natural or modified materials is important from the standpoint of its mobility or stability. Several conventional techniques used to speciate uranium involve extensive preparation of the sample, which can change its oxidation state. X-ray spectroscopic techniques (XRS) involve little manipulation of the sample, and provide more accurate information on oxidation state than do conventional methods. Speciation of uranium in microbial cultures by X-ray absorption near-edge spectroscopy (XANES) and X-ray photoelectron spectroscopy (XPS) showed that soluble U(VI) was reduced to insoluble U(IV) by the anaerobic bacterium, *Clostridium* sp., but only by living cells. Organic–acid metabolites, the extracellular components of the culture medium, and heat-killed cells, failed to reduce uranium anaerobically [17].

5. Biotransformation of actinide–organic complexes

The organic compounds in wastes consist of contaminated cellulosic materials, scintillation fluids, waste oils, decontamination agents, and compounds used in extracting and separating radionuclides. Biodegradation of selected chelating agents has been investigated, but little is known of the rate and extent of biodegradation of other organic compounds. Chelating agents are present in wastes because they are widely used for decontaminating nuclear reactors and equipment, in clean-up operations, and in separating radionuclides. The types of organic complexing agents used are carboxylic acids, such as citric, hydroxy-acetic, oxalic, and tartaric acids, and amino-carboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), and *N*-hydroxyethylenediaminetriacetic acid (HEDTA). Many of these metal chelates either are poorly biodegraded aerobically, or undergo little anaerobic biodegradation; their biodegradation should cause the precipitation of released ions as water-insoluble hydroxides, oxides, or salts, thereby retarding their migration. The relative order of degradation of several chelates in surface soil was NTA>EDTA≈DTPA [18], and in subsurface sediments NTA>DTPA>EDTA [19]. Although the biodegradation of synthetic chelating agents complexed with toxic metals has been investigated, little is known of the biotransformation of actinides complexed with natural organic compounds and chelating agents.

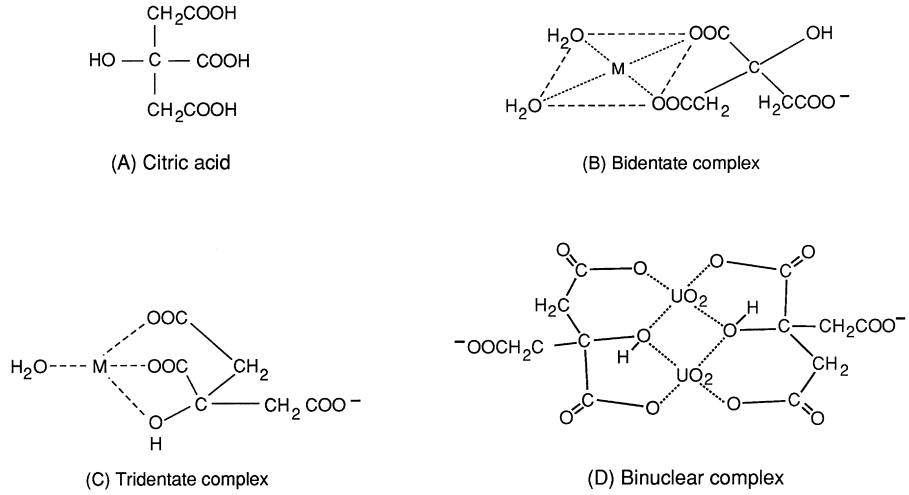

Fig. 1. Types of metal–citrate complexes.

5.1. Biotransformation of metal–citrate complexes

Citric acid, a natural compound, is a multidentate ligand and forms stable complexes with various metal ions [20]. It forms different types of complexes with transition metals and actinides and can involve formation of a bidentate, tridentate, binuclear, or polynuclear complex species (Fig. 1). Francis et al. [21] investigated the biotransformation of several toxic metals and uranium complexed with citric acid. Biodegradation depended upon the type of complex formed; bidentate complexes were readily biodegraded, whereas tridentate complexes were recalcitrant (Table 1).

5.2. Biodegradation under aerobic conditions

Pseudomonas fluorescens readily degraded bidentate complexes of Fe(III)–, Ni–, and Zn–citrate, but not those involving the hydroxyl group of citric acid, the tridentate Al–, Cd– and Cu–citrate complexes, or the binuclear U–citrate complex [22]. The presence of the free hydroxyl group of citric acid was the key determinant in effecting biodegradation of the metal complex. Lack of degradation was not due to their toxicity, but was limited by bacterial transport and/or metabolism of the complex [22]. There was no relationship between biodegradability and stability

Table 1
Biotransformation of metal–citrate complexes by *Pseudomonas fluorescens* [21–23,25]

Type of metal complex	Formula	Formation constant (log K)	Citrate degraded			^{14}C-Citrate uptake by cells (nmol min^{-1})
			Growing cells (%)	Resting cells (%)	Cell extract (μmol min^{-1} μg^{-1} protein)	
Citric acid			100	100	23±2	30±2
Bidentate						
Ca	$[Ca–cit]^-$	3.5	100	nd	nd	nd
Fe(III)	$[Fe(OH)_2–cit]^{2-}$	1.9–2.6	100	100	20±3	12±1
Mn(II)	$[Mn–cit]^-$	3.7	100	100	nd	nd
Ni(II)	$[Ni–cit]^-$	5.4	70	nd	nd (17±2)*	nd (23±3)*
Zn	$[Zn–cit]^-$	5.0	100	nd	nd (17±3)*	nd (22±2)*
Tridentate						
Al	[Al–cit]	8.1	nd	nd	-	nd
Cd	$[Cd–cit]^-$	3.8	nd	nd	nd	nd
Cu	$[Cu–cit]^{2-}$	5.9	nd	nd	nd	nd
Fe(II)	$[Fe–cit]^-$	4.4	nd	nd	nd	nd
Fe(III)	$[Fe(OH)–cit]^-$	9.4	16	nd	nd	nd
Fe(III)	[Fe–cit]	11.4	nd	nd	nd	nd
Pb	$[Pb–cit]^-$	4.1	nd	nd	nd	nd
Binuclear						
U(VI)	$[(UO_2)_2–cit_2]^{2-}$	18.9	nd	nd	12±1	nd
Mixed metal						
Fe(III)–U(IV)	$[(FeU)–cit_2]^{2-}$	?	10	nd	—	—

nd, not degraded; —, not determined; *, degradation occurred after the cells were induced by Ni or Zn.

of the complexes. The recalcitrant tridentate Fe(II)–citrate complex was readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)–citrate form, denoting a structure–function relationship in its metabolism [23].

5.3. Biodegradation under denitrifying conditions

Citrate metabolism by *Pseudomonas fluorescens* under denitrifying conditions was facilitated by the enzyme aconitase, as in aerobic metabolism, and biodegradation of various metal–citrate complexes also was similar to that observed aerobically, but was much slower. The bacterium completely degraded bidentate complexes of Fe(III)– and Zn–citrate, whereas the Ni–citrate complex was partially degraded (~59%). Ferric iron was in the colloidal form, and was not reduced to Fe(II) under denitrifying conditions. Tridentate complexes of Al–, Cd–, Cu–, and Fe(II)–citrate, and the binuclear U–citrate complex were not metabolized (Joshi-Tope and Francis, unpublished results).

5.4. Degradation of binary uranyl–citrate complex

Citric acid is metabolized intracellularly in bacteria by the enzymes, aconitase and citrate lyase. Aconitase catalyzes the isomerization of citrate to isocitrate, a key reaction in the aerobic tricarboxylic acid cycle, whereas citrate lyase drives the anaerobic metabolism of citric acid. We tested the ability of *Acinetobacter calcoaceticus*, *Citrobacter freundii*, *P. aeruginosa*, *P. fluorescens*, *P. putida*, and *P. stutzeri* and several isolates from the low-level radioactive waste sites, which can metabolize citric acid, to metabolize the uranyl–citrate complex in defined mineral salts medium. All these cultures failed to biodegrade uranyl–citrate. With uranyl ion, citric acid forms a binuclear complex with two carboxylic acid groups as well as the hydroxyl group of citric acid (Fig. 1). Growing and resting cells of *P. fluorescens* did not degrade uranyl–citrate in the defined medium at pH 6.1 (Fig. 2), nor when the pH was adjusted to 5.0, 6.0, 7.0, 8.0 and 9.0, suggesting that the complex is stable at higher pHs.

Transport studies with ^{14}C-labeled uranyl–citrate complex showed that it was not transported inside the cell nor was it degraded (Fig. 3). However, cell-free extracts of *P. fluorescens* completely degraded the binuclear U–citrate complex. Speciation of uranium and citric acid as a function of pH predicts that the amount of the U–citrate complex will decrease rapidly above pH 6.0; however, free citric acid was not evident in the biodegradation and transport studies [22]. Nunes and Gill [24] reported that cyclic 3:2 U–citrate complex was formed above pH 6.5 involving hydroxo and oxo bridges.

Adding excess citric acid to equimolar (0.52 mM) uranyl–citrate caused the metabolism of this excess citric acid; the uranyl–citrate complex was not toxic to the bacterium. In the presence of 1-, 2-, and 3-fold excess citric acid, the amount of citric acid remaining in each after biodegradation was 0.75, 0.80, and 0.83 mM, respectively (Fig. 4). The final stoichiometry of U–citric acid in all three treatments was approximately 2:3, resulting in the formation of the 2:3 U–citric acid complex.

Adding iron as $Fe(OH)_3$ or the Fe–citric acid complex did not affect the recalcitrance of 1:1 U–citric acid. In the presence of Fe–citrate a 1:1:2 U–Fe–citric acid complex was formed with the metabolism of citrate complexed with iron (Fig. 5); the released iron was in solution. Whether the iron is associated with the 1:1:2 Fe–U–citric acid complex or exists as colloidal iron is not known (Dodge, Joshi-Tope and Francis, unpublished results).

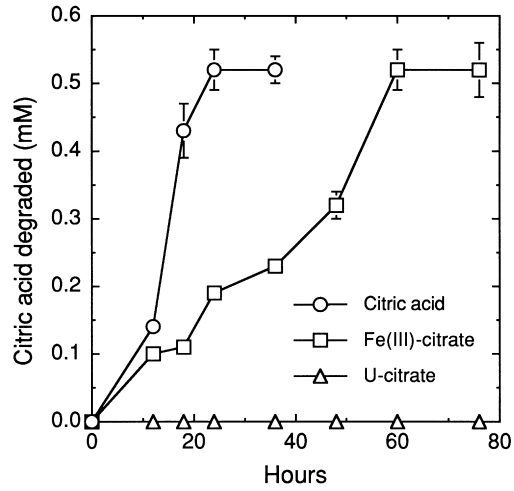

Fig. 2. Biodegradation of Fe(III)– and U(VI)–citrate complexes by *Pseudomonas fluorescens* [21–23,25].

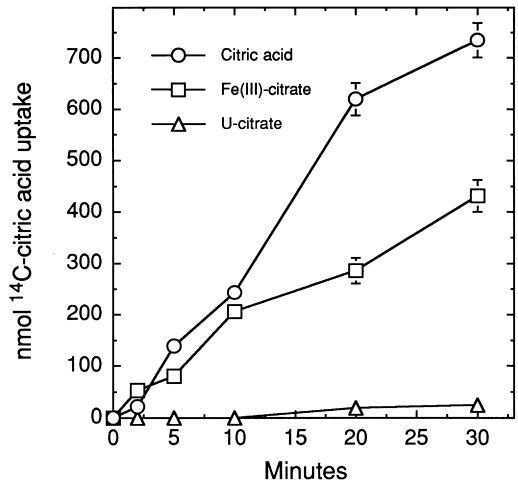

Fig. 3. Uptake of metal–citrate complexes by *Pseudomonas fluorescens* [22].

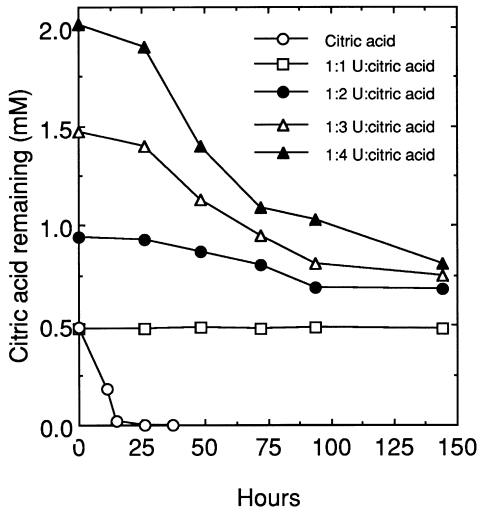

Fig. 4. Biodegradation of uranyl–citrate in the presence of excess citric acid [21,25].

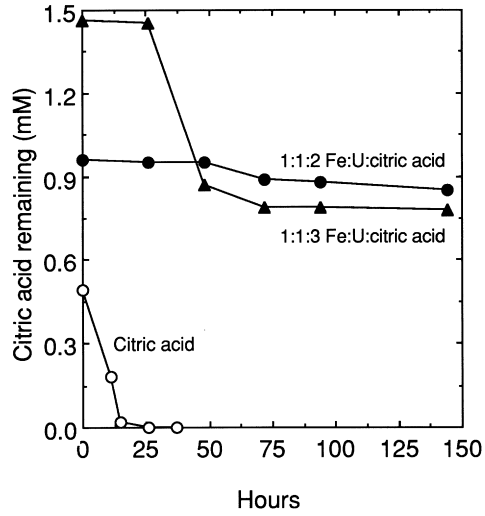

Fig. 6. Biodegradation of Fe–U–citric acid mixed-metal complex by *Pseudomonas fluorescens* [25].

5.5. Biodegradation of ternary iron– and uranium–citrate complexes

Citric acid forms ternary mixed-metal complexes with various metal ions involving its hydroxyl and carboxyl groups. The coordination of the metal to citric acid affects the biodegradation of the metal–citrate complexes and the metal's mobility. The presence of 1:1:2 Fe–U–citric acid in solution was confirmed by potentiometric titration, UV-vis spectrophotometry, gel-filtration chromatography, and extended X-ray absorption fine structure (EXAFS) analysis. Comparison of the EXAFS spectra show that the 1:1:2 Fe–U–citric acid complex is structurally similar to the 1:1 U–citric acid complex [25]. Biotransformation of Fe–U–citrate complex by *P. fluorescens* Fig. 6 revealed that ternary 1:1:2 Fe–U–citric acid complex was recalcitrant. When 1-fold excess citric acid was added to the 1:1:2 Fe–U–citric acid complex, the excess citric acid was completely degraded with no change in the stoichiometry of the complex. However, with a two-fold excess citric acid, 1:1:1 Fe–U–citric acid remained in solution after the excess citric acid was biodegraded. These results suggest that Fe–U–mixed-metal citric acid complexes resist biodegradation and may persist in the environment.

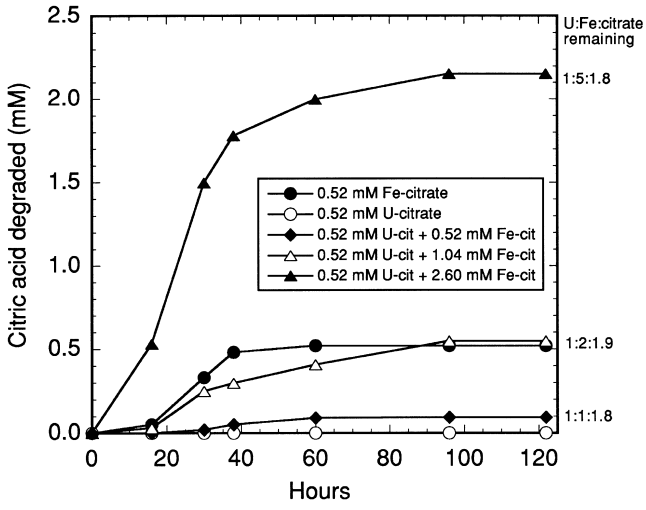

Fig. 5. Influence of addition of Fe–citrate on biodegradation of 1:1 U–citric acid complex by *Pseudomonas fluorescens*.

5.6. Biotransformation of uranyl–citrate under anaerobic conditions

Clostridium sphenoides (ATCC 19403), able to metabolize citric acid as its sole carbon source, metabolized the bidentate Fe(III)–citrate complex but not the binuclear U–citrate complex. The bacterium reduced Fe(III) to Fe(II) and concurrently metabolized citric acid. In contrast, U(VI)–citrate was reduced to U(IV)–citrate by the bacteria without metabolism of the complexed citrate, but only when supplied with the electron-donor glucose or uncomplexed citric acid (Fig. 7). Reduced U(IV)–citrate was present in solution. Citric acid complexed with U(IV) or (UVI) was not metabolized [26]. Similarly, *Clostridium* sp. (ATCC 53464) that metabolizes glucose but not citrate, reduced Fe(III)–citrate to Fe(II)–citrate only in the presence of glucose; citric acid complexed to Fe(II) and Fe(III) was not metabolized. U(VI)–citrate was reduced to U(IV)–citrate only in the presence of glucose and citric acid was not metabolized [26]. The sulfate-reducing *Desulfovibrio desulfuricans* and the facultative iron-reducing *Schwanella alga* reduced U(VI) complexed with oxalate or citrate to U(IV) anaerobically, but little uranium was precipitated [27]. These results show that complexed uranium is readily accessible for microorganisms as an electron acceptor, despite their inability to metabolize the organic ligand complexed to the actinide.

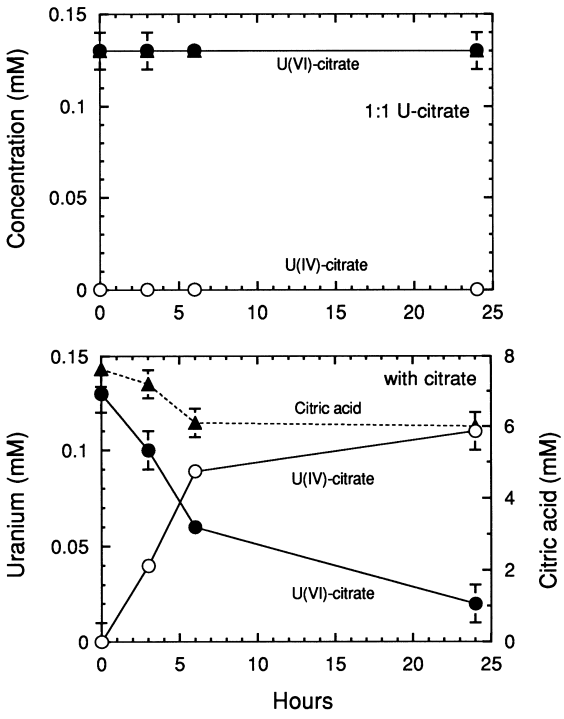

Fig. 7. Bioreduction of U(VI)–citrate to U(IV)–citrate by *Clostridium sphenoides* in the absence and presence of excess citric acid [26].

6. Summary

Microorganisms can alter the stability and mobility of actinides in radioactive wastes and in the natural environment. Such microbial transformations of uranium have been extensively studied. The direct implication of microorganisms in precipitating uranium is important because of the potential application in bioremediating contaminated sites, in pre-treating radioactive wastes, and in processes critical to nuclear waste repositories. Although a wide variety of microorganisms are present in radioactive wastes and natural radioactive mineral deposits, the extent to which they regulate the mobility of the actinides is not fully understood. Fundamental studies on the microbial transformations of actinides will be useful in evaluating the impacts on long-term storage of radioactive wastes in deep geological formations, as well as in developing novel biotechnological approaches to treating of waste streams and contaminated sites.

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