

Microbes affect the speciation of various uranium compounds in wastes and soils

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Abstract. Large volumes of uranium wastes are generated by nuclear- fuel production, nuclear power plants, and by facilities producing nuclear weapons. Uranium is present in various chemical forms such as elemental, oxide, sulfide, ionic, inorganic complex, organic complex, and co-precipitated with iron-, manganese- oxides, and carbonate. The mineralogical association and the oxidation states of uranium affect solubility, stability, bioavailability, and environmental mobility. Microorganisms affect the speciation of various chemical forms of uranium present in wastes and contaminated soils. The speciation of uranium affected by microbial action such as bioreduction, biosorption, biotransformation of uranium complexed with naturally occurring organic ligands, and solubility are reviewed.

Introduction

Contamination of soils, sediments, and materials with uranium from mining and milling activities, fabricating the nuclear fuel, nuclear-power plants, nuclear accidents, disposal of nuclear wastes (low- and intermediate-level waste (LILW), transuranic (TRU) wastes), reprocessing of the spent nuclear fuel, nuclear weapons testing, phosphate mining wastes and disposal of depleted uranium is a major environmental concern. In addition to the radionuclides the LLW, ILW, and TRU wastes contain absorbed liquids, sludges, processed nitrate waste, organic compounds, cellulose, plastics, rubber materials, and chelating agents from decontamination. Unlike organic contaminants, the uranium cannot be destroyed to innocuous compounds, but must be converted to a stable form or removed for proper disposal or recycled.

Uranium in contaminated environments may be present in one or more chemical forms depending on the process and waste stream. Microbial activity could affect the chemical nature of the uranium by altering the oxidation state and speciation, the solubility and sorption properties and thus could increase or decrease the concentrations of uranium in solution. Initially uranium may be present as insoluble

ble form and may be converted to soluble form after disposal by the actions of microorganisms. The chemical form, the availability of electron donors, nutrients (nitrogen, phosphorus), the presence of electron acceptors (Fe^{3+} , Mn^{4+} , NO_3^- , SO_4^{2-} , organic compounds), and the environmental factors (pH, Eh, temperature, moisture) affect the type, rate, and extent of microbial activity, and hence, uranium transformation.

The direct implication of microorganisms in the biotransformation of various uranium compounds is of considerable interest because of its potential application in bioremediation of contaminated sites, in pre-treating radioactive wastes, and in processes critical to the performance of nuclear waste repositories. The *in-situ* or *ex-situ* bioremediation strategies of uranium contaminated soils, groundwater, and waste streams include one or more of the microbially mediated processes such as bioaccumulation, bioreduction and biodegradation of uranium-organic complexes. However, one of the major concerns is that uranium stabilized by microbial treatment is not very stable and the potential for remobilization of bioreduced uranium are very high, for example, via re-oxidation by oxygen and other oxidants or complexation with soluble organic ligands.

Chemical speciation of uranium

Uranium exists in several oxidation states U(III), U(IV), U(V), and U(VI). Environmentally relevant ones are U(IV) and U(VI). The former is relatively insoluble while the latter is highly soluble. Uranium in contaminated environments and wastes may be present as inorganic salts, oxides, coprecipitates with calcium carbonate, iron and manganese oxides, inorganic-, organic- complexes and naturally occurring minerals.

Dissolution of uranium

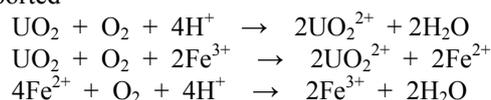
Oxidizing and reducing conditions affect the mobilization and immobilization of uranium. Inorganic and organic complexes of uranium affect its mobility in the environment. Uranium is predominantly present in the natural environment as soluble uranyl carbonate species. Many organic compounds form stable complexes with uranium, and increase its solubilization and leaching. Likewise, microbial metabolites and waste degradation products or intermediates may be an important source of agents that affect uranium solubility. Aerobic and anaerobic microorganisms are directly or indirectly involved in the mobilization and immobilization of various chemical forms of uranium.

Microbial leaching of uranium from ores. Uranium in ores is present as uraninite and pitchblende and in secondary mineral phases associated with sili-

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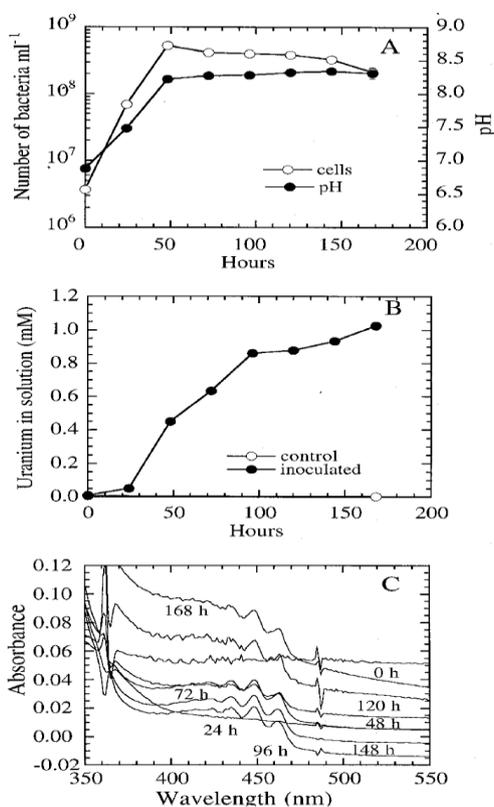
cates, phosphates, carbonates, and vanadates. The concentration of uranium can vary between 0.5 and 20%, with the highest amount occurring in Canadian ores. Mill tailings, a by-product of the mineral extraction process, contain up to 2% uranium. The residual uranium that has not been extracted may be present as a result of newly formed insoluble mineral phases (e.g. CaSO_4 , MgCO_3 , $\text{Fe}(\text{OH})_3$) which provide surface sites for uranium adsorption. Both autotrophic and heterotrophic microorganisms solubilize uranium.

Autotrophic microbial activity. The iron and sulfur oxidizing bacteria play a significant role in the solubilization of uranium from ores and in mill tailings. The biogeochemistry of uranium recovery from ores and bacterial leaching has been extensively studied. The role of autotrophic bacteria *Thiobacillus ferrooxidans* in the extraction of uranium from ore is primarily indirect action due to generation of the oxidizing agent ferric sulfate (indirect oxidation by $\text{Fe}^{2+} / \text{Fe}^{3+}$ cycle as the chemical electron carrier) and the solvent sulfuric acid from sulfur oxidation (Francis 1990). Direct oxidation of uranous sulfate and UO_2 by *T. ferrooxidans* has also been reported



Heterotrophic microbial activity. Aerobic and anaerobic microorganisms in the presence of electron donors such as metabolizable organic compounds and electron acceptors such as NO_3^- , $\text{Fe}(\text{III})$, $\text{Mn}(\text{IV})$, SO_4^{2-} , and CO_2 affect uranium oxidation state and solubility. Organic carbon is degraded under aerobic conditions to intermediary products, CO_2 and H_2O ; while under anaerobic conditions it is degraded to intermediate products which accumulate or completely degraded to CH_4 and or CO_2 , H_2 , with concomitant release of NH_3 , H_2S , PO_4^{4-} . A *Halomonas* sp when grown in WIPP brine under denitrifying conditions solubilized uranyl hydrogen phosphate precipitate to soluble uranyl carbonate due to metabolic activity (Fig 1). The organism metabolized the carbon source succinate for growth under anaerobic (denitrifying) conditions using nitrate as an alternate electron acceptor. The carbon dioxide produced from the metabolism of the carbon source accumulated in the medium causing the dissolution of uranyl hydrogen phosphate to uranyl carbonate (Francis et al. 2000).

Dissolution of uranium by organic compounds. Dissolution of uranium is due to organic acid metabolites produced by heterotrophic microorganisms as well as lowering of the pH of the medium from the metabolism of organic compounds (Berthelin and Munier-Lamy 1983; Bloomfield and Pruden, 1975). In many cases, a combined effect is important. For example, organic acids produced by microorganisms may have a dual effect in increasing U dissolution by lowering pH, and by complexation. Heterotrophic bacteria and fungi are not only known to solubilize various minerals including silicates (quartz, feldspar, mica) but also release



(A), Growth of bacteria and changes in pH of the medium.

(B), Dissolution of UO₂HPO₄·2H₂O to uranyl dicarbonate as function of bacterial activity.

(C), UV-vis spectra of the culture medium showed the formation of uranyl carbonate as a function of bacterial growth.

Fig 1. Dissolution of uranyl-hydrogen phosphate precipitate by *Halomonas* sp. (Francis et al. 2000).

metals associated with them, including Cu and Ni from copper-nickel concentrates, Cu from low-grade copper ore, uranium from granites, and potassium from leucite.

A wide variety of heterotrophic microorganisms, such as *Bacillus* sp., *B. luteus*, *B. subtilis*, *B. cereus*, *B. pumilis*, *Pseudomonas striata*, *P. viscosa*, *P. perolens*, *P. choloroaphis*, *Achromobacter xerosis*, *A. stoloniferum*, and *A. healii* may be involved in solubilizing uranium from granitic rock where uranium is generally present as an oxide. Such solubilization is due to the production of organic-acid metabolites, such as oxalic, isocitric, citric, succinic, hydroxybenzoic, and coumaric acids via their carboxylic and phenolic groups (Bertelin and Munier-Lamy, 1983). When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators, such as siderophores (Brainard et al. 1992). Iron-sequestering agents such as siderophores elaborated by microorganisms could play an important role in the complexation of radionuclides and so increase their solubility. *Pseudomonas aeruginosa*, grown in the presence of uranium elaborated several metabolic products which complexed uranium (Premuzic et al. 1985). Microbially produced dicarboxylic acids, oxalic, isocitric, citric, succinic, ketoglu-

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conic acid, polyhydroxy acids, and phenolic compounds such as protocatechuic acid, and salicylic acid are effective chelating agents but their ability to extract uranium from ores has not been fully explored.

The complexation of uranium with organic ligands such as ketogluconic, oxalic, malic, citric, protocatechuic, salicylic, phthalic, and fulvic acids and catechol were investigated (Dodge, Vasquez, and Francis, unpublished results). Potentiometric titration of uranium with the organic ligands confirmed complex formation. EXAFS analysis and electrospray ionization-mass spectrometry (ESI-MS) showed that ketogluconic acid formed a mononuclear complex with uranium involving the carboxylate group, while malic acid, citric acid, and catechol formed binuclear complexes. Phthalic acid formed a bidentate complex involving the two carboxylate groups, while catechol bonded to uranium through the two hydroxyl groups. The hydroxycarboxylic acids were bound in a tridentate fashion to uranium through two carboxylates and the hydroxyl group.

Immobilization of uranium

The immobilization of uranium is brought about by bioaccumulation, bioreduction and bioprecipitation reactions. Uranium is reduced by a wide variety of facultative and strict anaerobic bacteria under anaerobic conditions in the presence of suitable electron donor. Consequently, the potential exists for the use of anaerobic bacteria to concentrate, contain and stabilize uranium in contaminated groundwaters and in waste with concurrent reduction in waste volume. However, the long-term stability of bacterially reduced uranium in the natural environment is not known.

Biosorption and bioaccumulation of uranium. Biosorption and bioaccumulation of uranium has been observed in a wide range of microorganism (Francis et al. 1998, 2004; Gillow et al.2001). It is one of the intensely investigated areas of research because of the potential use of biomass to remove uranium from waste

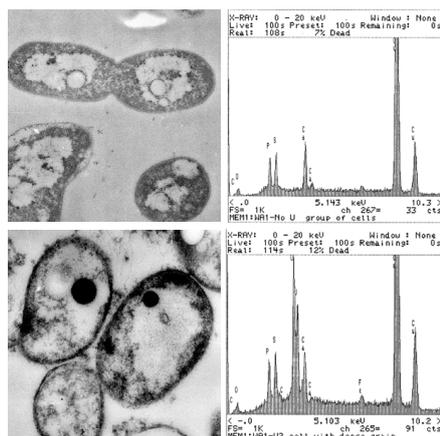


Fig 2. Intra- and extra- cellular accumulation of uranium by *Halomonas* sp. (Francis et al 2004).

EDS shows U and P as the major constituents of the intracellular granules.

- (A) *Halomonas* sp. whole cells (not exposed to U) shows poly beta hydroxybutyric acid (PHB) and polyphosphate granules; EDS shows almost equal amounts of Ca and P.
- (B) After exposure, U is accumulated in the polyphosphate granules.

streams. Bacterial cell walls, exopolymers, proteins, and lipids contain carboxylate, phosphate, amino, and hydroxyl functional groups which bind to uranium. Extracellular and intracellular association of uranium with bacteria was observed but the extent of its accumulation differs greatly with the species of bacteria. Extracellular association of uranium with bacterial cell surfaces is primarily due to

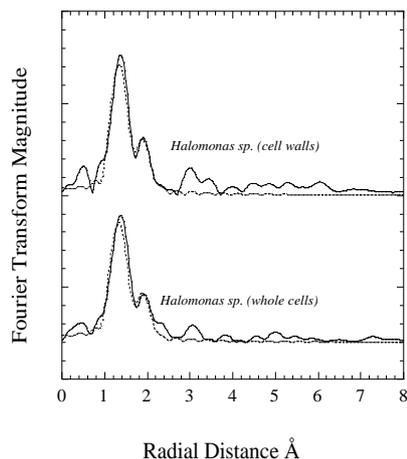


Fig 3. EXAFS analysis show uranium is complexed to diphosphate with whole cells; whereas the lysed cells show bidentate carboxylate bonding in addition to uranyl hydroxide (Francis et al 2004).

physical- and chemical- interactions involving adsorption, ion exchange, and complexation and does not depend on metabolism. Intracellular accumulation is due to binding to anionic sites or precipitating as dense deposits. Intracellular accumulation involves transporting the metal across the cell membrane, which depends on the cell's metabolism. The intracellular uranium transport system into the cell has not yet been unidentified.

Nuclear magnetic resonance spectroscopy (NMR), time resolved laser fluorescence spectroscopy (TRLFS), and extended X-ray absorption fine structure (EXAFS) have been used to determine the functional groups involved in the complexation of uranium with bacteria. In *Halomonas sp.* uranium accumulated as electron-dense intracellular granules and was also bound to the cell surface (Fig 2). EXAFS analysis of the association of U with halophilic bacteria showed that it was associated predominantly with phosphate as uranyl hydrogen phosphate and additional forms of phosphate such as hydroxophosphato or polyphosphate complexes as well as other ligands such as carboxyl species (Figs 3 and 4) (Francis et al. 2004). These studies demonstrate that phosphate, including the polyphosphates, bind significant amounts of uranium in bacteria. Uranium associated with the bacteria is not very stable, as it was desorbed completely by Na_2CO_3 , NaHCO_3 or 0.1M EDTA.

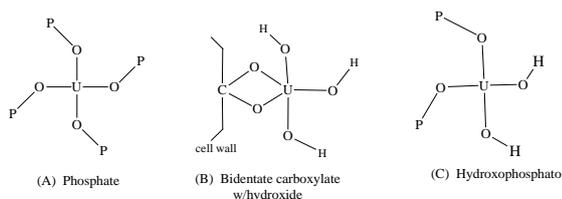
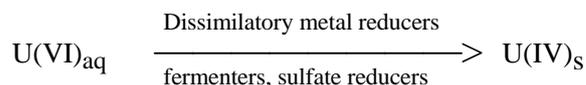


Fig 4. Proposed structures showing uranium association with bacteria (Francis et al 2004).

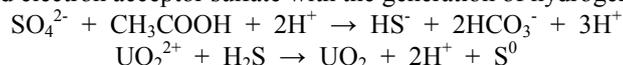
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Reductive precipitation of uranium. In anaerobic environments, certain metals can be reduced enzymatically from a higher to a lower oxidation state which affects their solubility and bioavailability. For example, reduction of Fe(III) → Fe(II), Mn(IV) → Mn(II) increases the solubility, while reduction of Cr(VI) → Cr(III), U(VI) → U(IV) decreases the solubility. The reduction of uranium was reported in axenic cultures of iron-reducing bacteria, fermentative bacteria, sulfate-reducing bacteria, and cell-free extracts of *Micrococcus lactilyticus*, and in uranium wastes by clostridia.

Bioreduction is the reduction of uranium from higher to lower oxidation state resulting in its precipitation. Although it is a two electrons transfer process, however, it has been proposed that it occurs as one electron transfer reaction with the reduction of U(VI) to U(V) by bacteria and disproportionation of U(V) to U(IV) and U(VI). A wide variety of facultative and strict anaerobic bacteria reduced uranyl-nitrate or uranyl carbonate to U(IV) under anaerobic conditions.



Sulfate-reducing bacteria catalyze uranium reduction in the presence of electron donor and electron acceptor sulfate with the generation of hydrogen sulfide.



Enzymatic reduction is due to reductases and that cytochrome C_3 is involved in the reduction of U(VI) by *Desulfovibrio vulgaris*. The mechanisms of uranium reduction by various microbes are not fully understood (Wall and Krumholz, 2006).

Uranium reduction by clostridia. Clostridia are strict anaerobic spore-forming fermentative bacteria ubiquitous in soils, sediments, and wastes, reduce uranium from higher to lower oxidation state (Fig 5). The bacterium also reduces iron, manganese, technetium, and plutonium. Reactive barrier technology is based on the activities of the anaerobic bacteria. Reduction of soluble uranyl carbonate ($\text{UO}_2(\text{CO}_3)_2^{2-}$), uranyl nitrate ($\text{UO}_2 \text{NO}_3)_2$, uranyl acetate to insoluble U(IV) by *Clostridium sp.* in culture medium was confirmed by x-ray absorption near edge spectroscopy (XANES) (Fig 6).

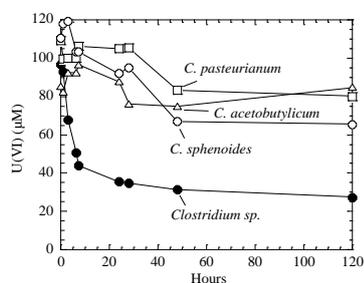


Fig 5. Reduction of U(VI) by Clostridia (Gao and Francis, 2008).

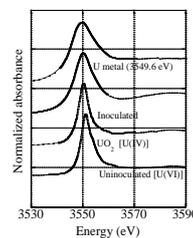


Fig 6. XANES spectra of U reduction by *Clostridium sp.* (Francis et al 1994).

Analysis of uranium contaminated sediment and sludge from Y-12 plant, Oak Ridge, TN showed that the uranium was predominantly in the hexavalent form. Anaerobic bacterial treatment of the sludge and sediment sample showed that uranium was reduced to the tetravalent form. XANES analyses of the samples treated with *Clostridium* sp showed a shift (decrease) in the position of the absorption peak maximum indicating reduction of U(VI) to U(IV) (Fig 7). Complete reduction of U(VI) to U(IV) in the sludge and only partial reduction in sediment were observed. The lack of complete reduction is due to the nature of the mineralogical association of uranium in the sediment (Francis, 1994, 1999).

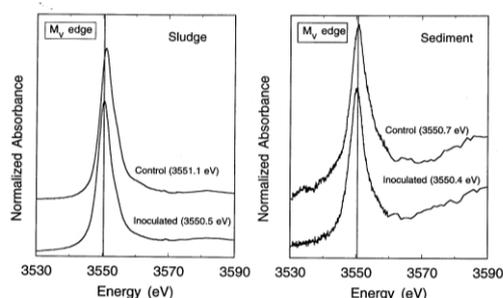


Fig 7. Comparison of XANES spectra of uranium contaminated sludge and sediment before and after anaerobic microbial treatment show reduction of U(VI) to U(IV) (Dodge et al. 1966).

The bioreduced uranium is not stable over the long-term because it can be readily reoxidized by the oxidants such as O₂, Fe(III), hydrogen peroxide or it can be mobilized if soluble organic ligands are present. Therefore it is important that the bioreduced uranium is converted to a more stable form by some additives.

Biotransformation of uranium associated with organic ligands

Naturally occurring soluble organic complexing agents present at the uranium-contaminated sites may not only affect the mobility of uranium but also affect the microbial transformation and reductive precipitation of uranium. Biotransformation of the complexed uranium should result in its precipitation and retard migration. There is a paucity of information on the mechanisms of microbial transformations of uranium complexed with naturally occurring low molecular weight soluble organic ligands.

Complexation and biotransformation of uranyl citrate under aerobic conditions. Citric acid is a naturally occurring, multidentate ligand which forms stable complexes with various metal ions. It forms stable complexes with transition metals and actinides and can involve formation of bidentate, tridentate, binuclear, or polynuclear complex species (Fig 8). Calcium, ferric iron and nickel formed bidentate, mononuclear complexes with two carboxylic acid groups of the citric acid molecule. Copper, ferrous iron, cadmium and lead formed tridentate, mononuclear complexes with citric acid involving two carboxylic acid groups and the hydroxyl group. Uranium has been shown to form a predominantly binuclear complex with two uranyl ions and two citric acid molecules involving four

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carboxylic groups and two hydroxyl groups. However, the proposed basic structure can vary as a result of changes in solution pH, the ratio of uranium to citrate, temperature and presence of other metals.

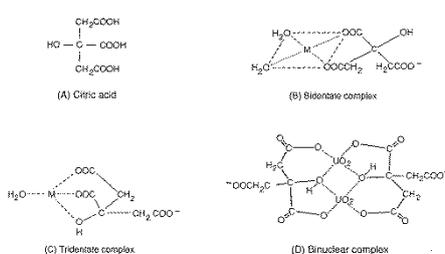


Fig 8. Citric acid forms different types of complexes with metals (Francis et al 1992).

The type of complex formed plays an important role in determining its biodegradability (Francis et al. 1992, Joshi-Tope and Francis 1995). The rate and extent of biodegradation of several metal-citrate complexes by microorganisms varies. For example, *Pseudomonas pseudoalcaligenes* degraded Mg-citrate at a much lower rate than Ca-, Fe(III)-, and Al(III)-citrate. Studies with a *Klebsiella sp.* showed that citric acid and Mg-citrate were readily degraded, whereas Cd-, Cu-, and Zn-citrate were resistant. Both studies also showed that metal toxicity was not responsible for the lack of or the lower rate of degradation of certain metal-citrate complexes but gave no other explanation. Biodegradation studies with *Pseudomonas fluorescens* showed that bidentate complexes of Fe(III)-, Ni-, and Zn-citrate were readily biodegraded, whereas complexes involving the hydroxyl group of citric acid, the tridentate Al-, Cd- and Cu-citrate complexes, and the binuclear U-citrate complex were not (Fig 9). The presence of the free hydroxyl group of citric acid is the key determinant in effecting biodegradation of the metal complex. The lack of degradation was not due to their toxicity, but was limited by the transport and/or metabolism of the complex by the bacteria (Francis et al. 1992, Joshi-Tope and Francis 1995). No relationship was observed between biodegradability and stability of the complexes. The tridentate Fe(II)-citrate complex, although recalcitrant, was readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)-citrate form, denoting a structure-function relationship in the metabolism of the complex (Francis and Dodge 1993). Biodegradation of Fe(III)-citrate complex resulted in the formation of carbon dioxide and ferrihydrite. Uranyl-citrate however, is not biodegraded and remains in solution relatively in a pure form which can be further processed by photodegradation.

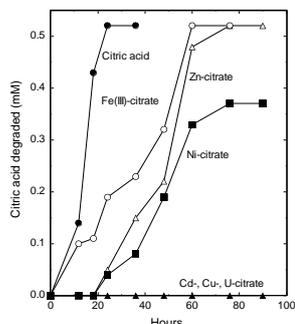


Fig 9. Biodegradation of metal citrates by *Pseudomonas fluorescens* (Francis et al. 1992, Joshi-Tope and Francis 1995).

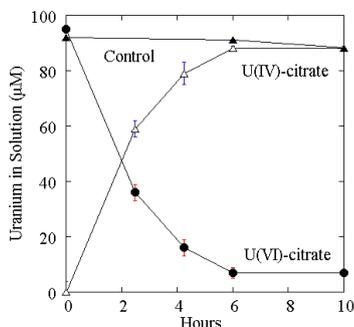


Fig 10. Bioreduction of U(VI)-citrate complex by Clostridia (Francis and Dodge 2008).

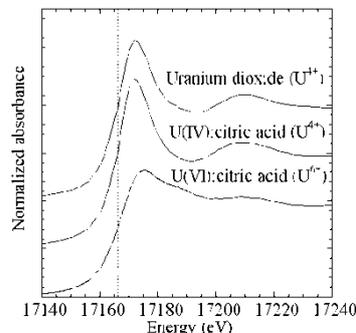


Fig. 11. XANES spectra of U(VI)-citrate before and after bacterial reduction (Francis and Dodge 2008).

Biotransformation of uranyl citrate under anaerobic conditions. The presence of organic ligands affected the extent of precipitation of reduced uranium under anaerobic conditions. Gu et al. (2005) reported that, in the presence of humic materials, the bioreduction of U(VI) did not result in its precipitation; but, the uranium remained in the solution phase as the U(IV)-humic acid complex. The sulfate-reducing bacteria *Desulfovibrio desulfuricans* and the facultative iron-reducing bacteria *Shewanella halotolerans* reduced U(VI) complexed with oxalate or citrate to U(IV) under anaerobic conditions with little precipitation of uranium (Ganesh et al. 1997). Studies with anaerobic bacteria *Clostridium* sp. (ATCC 53464) and *C. sphenoides* (ATCC 53464) showed U(VI) complexed with organic ligands was reduced to U(IV) under anaerobic conditions with little precipitation of uranium (Francis and Dodge 2008). The reduction of U(VI)-citrate to U(IV)-citrate occurred only when supplied with an electron donor glucose or citrate (Fig 10). The bacteria did not metabolize the citrate complexed to the uranium. XANES analysis showed that the reduced form of uranium was present in solution (Fig 11), while EXAFS analysis (Fig 12) showed that the U(IV) was bonded to citric acid as a mononuclear biligand complex (Fig 13).

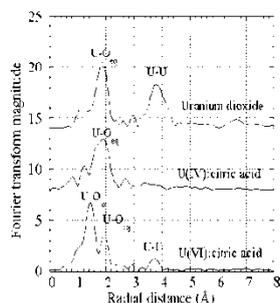


Fig 12. EXAFS analysis of U-citrate complexes before and after bacterial action (Francis and Dodge 2008).

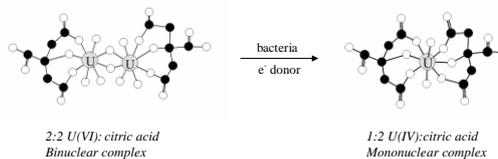


Fig 13. Reduction of biligand U(VI)-citrate to monoligand U(IV)-citrate complex (Francis and Dodge 2008).

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Biotransformation of U(VI)-phthalate. Phthalic acid, a common environmental organic ligand, forms strong complexes with the uranyl ion. It formed with uranyl ion bidentate-, mononuclear-, and biligand complexes involving both carboxylate groups. Anaerobic bacterium *Clostridium* species reduced U(VI)-phthalate to a U(IV)-phthalate under anaerobic conditions. EXAFS analysis revealed the association of the reduced uranium with the phthalic acid as a biligand 1:2 U(IV):phthalic acid and finally existing as a repeating biligand 1:2 U(IV):phthalic acid colloidal polymeric U(IV)-phthalate biligand complex (Vazquez, et al. 2009).

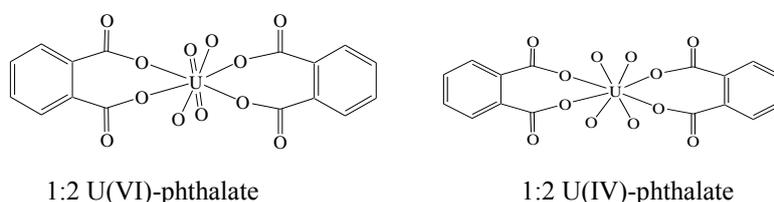


Fig 14. Reduction of U(VI)-phthalate to U(IV)-phthalate complex by *Clostridium* sp under anaerobic conditions (Vazquez, et al. 2009).

Although the bioreduction and the precipitation of uranium is an attractive strategy to immobilize uranium in contaminated environments, the presence of naturally occurring organic ligands such as, oxalic, citric, phthalic, and humic acids might prevent the precipitation of the bioreduced uranium under anaerobic conditions and enhance its mobility as uranium organic complexes or colloids (Ganesh et al. 1997; Gu et al. 2005; Francis and Dodge, 2008; Vazquez et al. 2009). These results show that the complexed uranyl ion is readily accessible as an electron acceptor despite the inability of the bacterium to metabolize the organic ligand. These results also suggest that reduced uranium, when complexed with an organic ligand, can remain in solution; and this finding is contrary to the conventional belief that reduced uranium will precipitate from solution.

Summary

Microorganisms play a major role in the transformations of uranium and regulate its mobility and stability in the environment. The key microbial processes of interest include oxidation-reduction reactions, dissolution, precipitation, biosorption and colloid formation. Fundamental understanding of the mechanisms of microbial transformations of several chemical forms of the uranium in the presence of electron donors and acceptors under various environmental conditions such as aerobic and anaerobic (denitrifying, fermentative, sulfate-reducing, and methanogenic) will be useful in assessing the microbial impact on the long-term behavior of uranium in contaminated environments.

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