BNL-82098-2009-CP

BROOKHAVEN NATIONAL LABORATORY

Microbial Transformation of Actinides and Other Radionuclides

A.J. Francis and C.J. Dodge

Submitted to the 9th Biennial DAE-BRNS Symposium on Nuclear and Radiochemistry (NUCAR02009)

SVKM's Mithibai College, Vile Parie, Mumbai
January 7-10, 2009

Environmental Sciences Department/Environmental Research & Technology Division

Brookhaven National Laboratory
P.O. Box 5000
Upton, NY 11973-5000
www.bnl.gov

Microbial Transformations of Actinides and Other Radionuclides

A.J. FRANCIS and C.J. DODGE
Environmental Sciences Department, Brookhaven National Laboratory, Upton, New York 11973, USA

Abstract

Microorganisms can affect the stability and mobility of the actinides and other radionuclides released from nuclear fuel cycle and from nuclear fuel reprocessing plants. Under appropriate conditions, microorganisms can alter the chemical speciation, solubility and sorption properties and thus could increase or decrease the concentrations of radionuclides in solution in the environment and the bioavailability. Dissolution or immobilization of radionuclides is brought about by direct enzymatic action or indirect non-enzymatic action of microorganisms. Although the physical, chemical, and geochemical processes affecting dissolution, precipitation, and mobilization of radionuclides have been extensively investigated, we have only limited information on the effects of microbial processes and biochemical mechanisms which affect the stability and mobility of radionuclides. The mechanisms of microbial transformations of the major and minor actinides U, Pu, Cm, Am, Np, the fission products and other radionuclides such as Ra, Tc, I, Cs, Sr, under aerobic and anaerobic conditions in the presence of electron donors and acceptors are reviewed.

INTRODUCTION

Radionuclides released from nuclear fuel cycle and from nuclear fuel processing plants of concern are the actinides major (U,Pu) and minor (Am, Np, Cm), and the fission products (I, Cs, Sr, Tc). The radionuclides released may be present in various forms such as elemental, oxide, coprecipitates, ionic, inorganic-, and organic-complexes. Microbial activity could affect the chemical nature of these radionuclides by altering the speciation, solubility and sorption properties and thus could increase or decrease the concentrations of radionuclides in solution and their bioavailability. For example, under appropriate conditions, dissolution or immobilization of radionuclides is brought about by direct enzymatic or indirect non-enzymatic actions of microorganisms [13]. These include (i) oxidation-reduction reactions, (ii) changes in pH and Eh, (iii) chelation or production of specific sequestering agents, (iv) bioaccumulation by biomass, (v) biocolloid formation, (vi) bioprecipitation, (vi) biotransformation of radionuclide-organic and -inorganic complexes, and (vii) production of volatile compounds by biomethylation. In this paper our understanding of the various mechanisms of biotransformation of radionuclides outlined above are highlighted with selected aerobic and anaerobic microorganisms with particular emphasis by Clostridia under anaerobic conditions.

BIOTRANSFORMATION OF URANIUM

Uranium exists as U(III), U(IV), U(V), and U(VI) oxidation states, of which U(IV) and U(VI) are the predominant forms found in the environment. Both aerobic and anaerobic microorganisms are directly or indirectly involved in the mobilization and immobilization of various chemical forms of uranium in the environment. The direct implication of microorganisms in the biotransformation of uranium 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.

Microbial Leaching of Uranium from Ores. Uranium in ores is present as uraninite and pitchblende and in secondary mineral phases associated with silicates, 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. $CaSO_4$, $MgCO_3$, $Fe(OH)_3$) which provide surface sites for uranium adsorption. However, there is lack of information on the mineralogical and chemical characterization of uranium in various ores.

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 [13]. 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 and the solvent sulfuric acid.

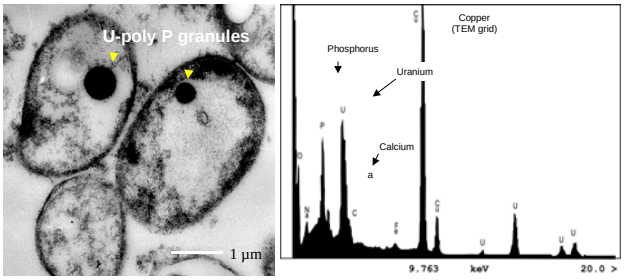
Heterotrophic Microbial Activity. Dissolution of radionuclides by heterotrophic microorganisms is due to production of organic acid metabolites, as well as lowering of the pH of the medium from the metabolism of organic compounds. In many cases, a combined effect is important.

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 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. Microbially produced dicarboxylic acids, oxalic, isocitric, citric, succinic, ketogluconic acid, polyhydroxy acids, and phenolic compounds such as protocatechuic acid, and salicylic acid are effective chelating agents however their ability to extract uranium from ores has not been fully explored [2,3].

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. When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators, such as siderophores. 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 or thorium, elaborated several metabolic products which complexed both elements [44].

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 fully understood.

Biosorption and Bioaccumulation of Uranium. Biosorption and bioaccumulation of uranium has been observed in a wide range of microorganism [43, 45]. It still is one of the intensely investigated areas of research because of the potential use of biomass to remove uranium from waste 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 U 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



TEM of thin section **EDS of granule**

Fig 1. Intra- and extra- cellular accumulation of uranium by *Halomonas* sp. EDS shows U and P as the major constituents of the intracellular granules [11].

primarily due to physical- and chemical- interactions involving adsorption, ion exchange, and complexation and does not depend on metabolism. Intracellularly, uranium binds 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 transport of the U into the cell involves an as-yet unidentified transport system.

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 U with bacteria.

In *Halomonas* sp. U accumulated as electron-dense intracellular granules and was also bound to the cell surface (Figure 1). EXAFS analysis of the association of U with halophilic and non-halophilic bacterial cells 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 [11]. These results demonstrate that phosphate, including the polyphosphates, bind significant amounts of uranium in bacteria.

Polyphosphates are widely distributed throughout the bacterial cell. Numerous and varied biological functions are performed by polyphosphate including phosphate storage in the cell, a reservoir of energy for cellular functions, a chelator of metals (e.g., Mn^{2+} and Ca^{2+}), a pH buffer, a capsule for bacteria, and in physiological adjustments to growth, development, stress, and deprivation. In particular, the polyphosphates play a vital role in the dynamics of metabolic adjustments of cells to stationary phase and their survival in response to a variety of nutritional limitations and environmental stresses. The amount of polyphosphate that is stored by cells varies between bacterial species, and is determined in part by the rate at which it can be degraded for example, in response to the presence of metals, and the amount of inorganic phosphate secreted into the medium. In as much as all of uranium exposure studies reported were conducted with cells in the stationary phase, the cells are responding to

heavy metal stress by releasing phosphate from the mineralization of cellular polyphosphate. In some studies reported in the literature the cells were in fact incubated with uranium from several hours to days. Under these conditions, the cells undergo lysis and release inorganic phosphate ($H_2PO_4^-$) with the precipitation of uranium as uranyl phosphate [$UO_2(H_2PO_4)_2$].

Uranium associated with the bacteria is not very stable, as it was removed completely by Na_2HCO_3 from *Halomonas* sp. from an *Arthrobacter* sp. by 0.1 sodium hydrogen carbonate, 0.1M EDTA and Na_2CO_3 and from Bacillus strains by EDTA. Although bacteria possess a variety of functional groups, studies suggest that cellular phosphate is the predominant functional group complexes with U. Inorganic phosphate generated inside cells during starvation or under stress bind U and other cations.

Bioreduction of Uranium. A wide variety of facultative and strict anaerobic bacteria reduced U(VI) added as uranyl-nitrate or uranyl carbonate to U(IV) under anaerobic conditions. These include axenic cultures of iron-reducing, fermentative, and sulfate-reducing bacteria. Mixed cultures of bacteria in uranium contaminated ground waters and in wastes also reduced uranium.

$$U(VI)_{aq} \xrightarrow[\text{fermenters, sulfate reducers}]{\text{Dissimilatory metal reducers}} U(IV)_S$$

Desulfovibrio, *Geobacter*, and *Shewanella*, can couple the oxidation of organic compounds to the reduction of U(VI), and thus reductively precipitate uranium. However, the mechanisms of microbial uranium reduction are not fully understood [50].

Clostridia are strict anaerobic spore-forming fermentative bacteria ubiquitous in soils, sediments, and wastes catalyze the reduction of uranium from higher to lower oxidation state [Figure 2]. Reduction of soluble U(VI) to insoluble U(IV) by *Clostridium sp.* in culture medium was confirmed by x-ray absorption near edge spectroscopy (XANES) [Figure 3].

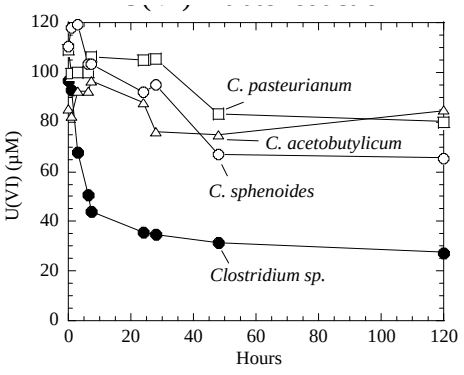


Fig 2. Reduction of U(VI) by Clostridia [24].

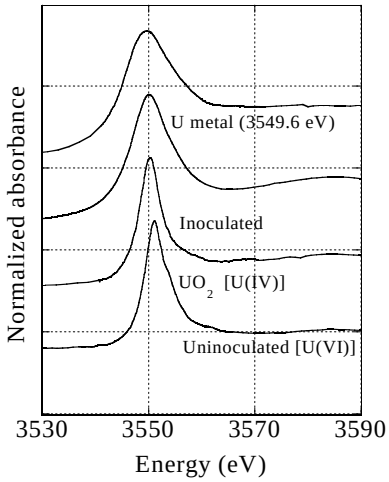


Fig 3. XANES spectra of U reduction by *Clostridium* sp. at the M_V absorption edge [21].

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.

We investigated the mechanisms of complexation and biotransformation of uranium with organic ligands ketogluconic, oxalic, malic, citric, protocatechuic, salicylic, phthalic, and fulvic acids and catechol. 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.

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 [Figure 4]. Calcium, ferric iron and nickel formed bidendate, mononuclear complexes with two carboxylic acid groups of the citric acid molecule. Copper, ferrous

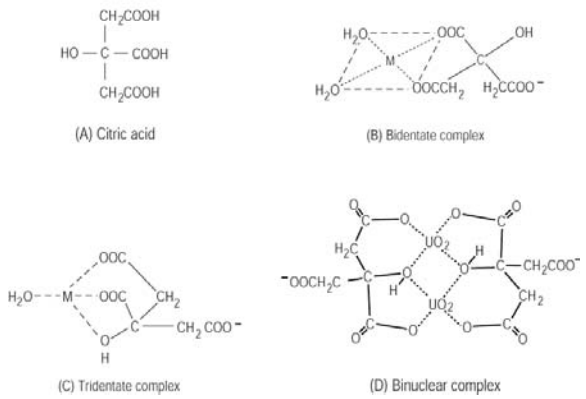


Fig 4. Citric acid, a hydroxytricarboxylic acid forms different types of complexes with metals [14].

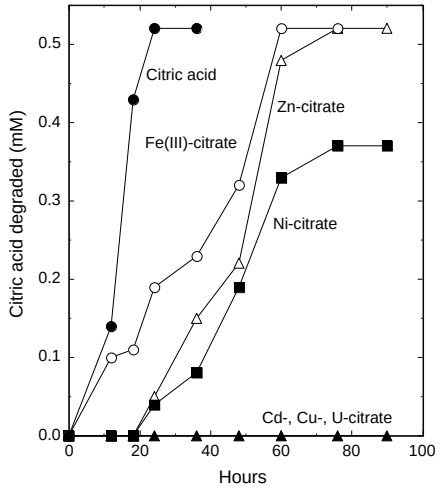


Fig 5. Biodegradation of metal citrates by *Pseudomonas fluorescens* [15].

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 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.

Biotransformation of Uranyl Citrate Under Aerobic Conditions. The type of complex formed plays an important role in determining its biodegradability [14,15]. 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 [Figure 5]. 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 [14,15]. 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 [15]. 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.

Biotransformation of Uranyl Citrate Under Anaerobic Conditions. The presence of organic ligands affected the extent of precipitation of reduced uranium under anaerobic conditions. For example, *Clostridium* sp. (ATCC 53464) which ferments glucose but not citrate reduced U(VI)-citrate [16] or U(VI)-phthalate [Vazquez et al., in preparation]only when supplied with glucose. Also 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 [23]. Studies with anaerobic bacteria *Clostridium* sp. (ATCC 53464) and *C. sphenoides* (ATCC 53464) showed U(VI) complexed with organic

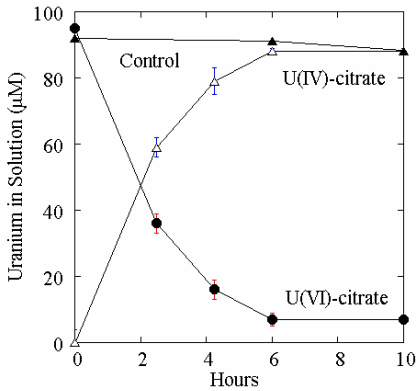


Fig 6. Bioreduction of U(VI)-citrate complex by Clostridia [16].

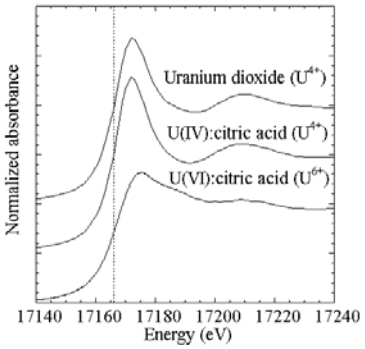


Fig. 7. XANES spectra of uranium citrate before and after bacterial reduction of U(VI) [16].

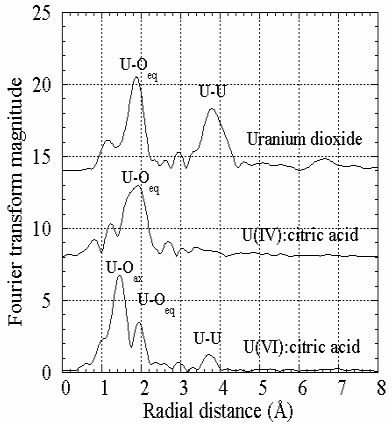


Fig 8. EXAFS analysis of U-citrate complexes before and after bacterial action [16].

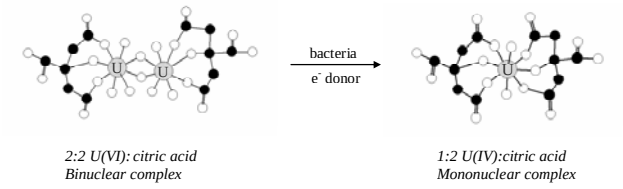


Fig 9. Reduction of biligand U(VI)-citrate to monoligand U(IV)-citrate complex [16].

ligands was reduced to U(IV) under anaerobic conditions with little precipitation of uranium. The reduction of U(VI)-citrate to U(IV)-citrate occurred only when supplied with an electron donor glucose or citrate [Figure 6]. The bacteria did not metabolize the citrate complexed to the uranium. XANES analysis showed that the reduced form of uranium was present in solution [Figure 7], while EXAFS analysis [Figure 8] showed that the U(IV) was bonded to citric acid as a mononuclear biligand complex [Figure 9].

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; this finding is contrary to the conventional belief that reduced uranium will precipitate from solution. The persistence of reduced uranium complexed with chelating agents in subsurface environments is a major concern because of the potential for increasing the transport of the radionuclide.

BIOTRANSFORMATION OF PLUTONIUM

Plutonium can exist in several oxidation states (III, IV, V, VI, VII) and the solution chemistry is very complex. Plutonium (IV) is the predominant form found in contaminated soils. Plutonium can simultaneously coexist as Pu(IV), Pu(V), and Pu(VI) in oxic environments. Plutonium has a high ionic charge, and tends to undergo hydrolysis, leading to the formation of polymers in systems with pH>2. Pu generally is considered to be relatively immobile; however, the transport of Pu, albeit at very low concentrations, was observed at several DOE sites (i.e., Rocky Flats, LANL, and NTS). Soil pH, its organic-matter content, redox conditions, and mineralogy affect the chemical speciation of Pu. Chemical characterization of Pu at contaminated sites shows that its environmental form varies according to site, and depends on the waste stream. For example, at Rocky Flats, CO, the predominant form appears to be PuO_2(s); at the NTS, Pu was found to be associated with mineral colloids [7,28]. Recent studies show that Pu is associated with organic degradation product in a colloidal from at the Rocky Flats site [56].

Microorganisms may directly or indirectly affect the oxidation and reduction of Pu [Figure 10]. For example, a slight increase in microbial activity (respiration) can alter the oxidation state of Pu(VI) to Pu(IV) because of the very small differences in the reduction potential between Pu(VI), Pu(V), and Pu(IV). The direct enzymatic reduction of Pu(VI) and Pu(V) to Pu(IV) by bacterial cell suspension of *Shewanella putrefaciens*, *S. oneidensis*, and *Geobacter metallireducens* have been reported [37].

Reductive Dissolution of Pu(IV) by Anaerobic Bacteria. Bioreduction of Pu(IV) to.Pu(III) by *Bacillus* sp. has been inferred [46]. Recently, reductive dissolution of Pu(IV) to Pu(III) by the strict anaerobic bacterium

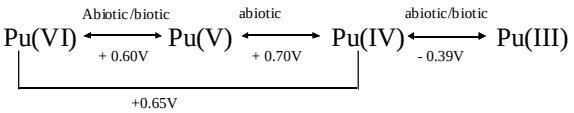


Fig. 10. Biotic and abiotic reduction of Pu(VI) to Pu(III) [6].

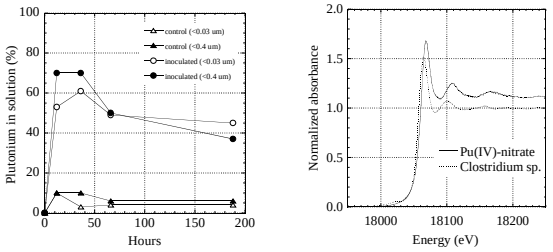


Fig 11. Reduction of Pu (IV) to Pu(III) by *Clostridium* sp. [20].

Clostridium sp was shown by Francis et al [20] and the Pu(III) oxidation state was confirmed by XANES. The potential exists for the microbial oxidative dissolution of the more stable and environmentally predominant form of Pu(IV) to the more soluble and bioavailable form of Pu(V) and Pu(VI). The microbes may indirectly affect the oxidation state and solubility of Pu by changing the Eh and pH of the medium, as well as by producing sequestering agents [5,9,27,36,37].

Sorption studies of Pu(VI) with bacterial biomass have shown that the interaction with bacteria can cause changes in the oxidation state [38,40] and most of the Pu(VI) was reduced to Pu(IV) and bound to the phosphate groups on the cell surface [41]. Knowledge of plutonium complexes formed with bacterial cells and metabolic products are important in assessing the migration behavior and transport of Pu from contaminated environments to humans.

Biotransformation of Plutonium Complexed with Organic Compounds. Chelating agents are present in TRU and mixed wastes due to decontamination of nuclear reactors and equipment, cleanup operations, and chemical separation of radionuclides. Plutonium forms very strong complexes with a variety of organic ligands. Naturally occurring organic complexing agents, such as humic and fulvic acids, and microbially produced complexing agents, such as citrate, and siderophores, as well as synthetic chelating agents can affect the mobility of Pu in the environment. The presence of organic ligands in radioactive wastes is a major concern because of the potential for increasing the transport of radionuclides from disposal sites. Biotransformation of the radionuclide-organic complex should precipitate the radionuclide from solution thereby retarding its mobility in the environment. Chelating agents, such as citric acid, are present in TRU and mixed wastes. Citric acid forms a strong complex with Pu(IV) and has been used in chelation therapy and in extracting ^{239}Pu from contaminated soil.

Analyses of Th(IV)-citrate by potentiometric titration and conductimetric measurements showed the formation of $[Th_2cit_3]^{4+}$.Tetravalent plutonium forms a 1:1 Pu:citric acid ($K=10^{15}$) complex and a 1:2 Pu:citric acid complex ($K=10^{30}$). In the presence of excess citric acid, Pu(IV) oxidation state is stable. At pH 6.5, we identified a monomeric [Pucit] species, two forms of biligand [$Pucit_2$] species, and a dimeric [Pu_2cit_2] species of tetravalent plutonium with citric acid. Speciation calculations showed that the biligand complex is the predominant form. In addition, analysis of the complex over 100-h demonstrated that citric acid inhibits polymer formation. We determined the complexation of Pu-citrate by ESI-MS; and XANES and EXAFS to determine the oxidation state of the Pu and the nature of Pu complexation with citric acid [17]. XANES analysis confirmed the Pu was present as tetravalent form. Based upon this analysis the mononuclear biligand complex is the predominant form and the mononuclear monoligand and binuclear biligand complex were also present (Figure 12).

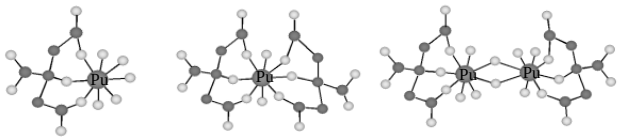


Fig 12. Proposed structures at pH 6 for the monoligand 1:1 Pu:citric acid complex (A); biligand 1:2 Pu:citric-acid complex $[PuO(cit)_2]^{4-}$ (B);and dimeric 2:2 Pu:citric acid complex (C). The open circles represent oxygen and the filled circles represent carbon atoms [17].

Biodegradation of Citric Acid and Pu(IV)-citrate Complexes. Figure 13 depicts the rate and extent of citrate degradation in samples containing 10^{-6} M and 10^{-8} M Pu present as Pu-citrate complexes at an ionic strength of 0.18 M. Citric acid (10^{-4} M) in the absence of Pu was metabolized completely at a rate of 4.9 μM/h. With 10^{-6} and 10^{-8} M Pu present as the Pu-citrate complex we observed a slight decline on the rate and extent of citrate degradation in comparison to the sample lacking Pu. In

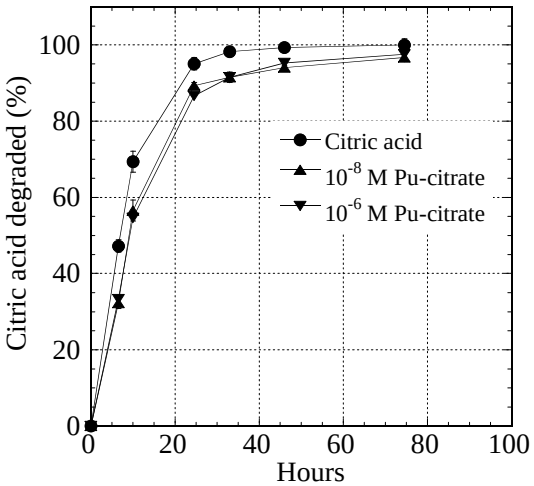


Figure 13. Effect of 10^{-6} and 10^{-8} M ^{242}Pu on citrate metabolism. Citric-acid concentration is 10^{-4} M in all treatments [17].

both samples, citrate was degraded >96%, and at the rate of 4.0 μM/h and 3.8 μM/h, respectively. In the presence of 10^{-6} M Pu, 20% was retained on the 0.4 μm filter, and 27% on the 0.03 μm filter; similar results were found with 10^{-8} M Pu, at 43% and 57%, respectively (data not shown). Comparison of the 10^{-6} M Pu samples inoculated with bacteria with that of the control samples (uninoculated sample) showed that there is greater retention on the >0.4 μm filter in the former.

These data suggest that the soluble 10^{-6} and 10^{-8} M Pu-citrate complexes underwent significant biodegradation with Pu released as a particle-reactive species that adsorbed to the cells (>0.4 μm), and possibly as a colloidal species, (>0.03 μm). Although the Pu(IV)-citrate complex is stable in the absence of bacteria, adding bacteria to the 1:100 and 1:10000 Pu(IV)-citrate complexes resulted in the retention of Pu species at 20-43% by the 0.4 μm filter (biomass associated), and 27-57% by the 0.03 μm filter (colloid fraction).

BIOTRANSFORMATION OF NEPTUNIUM

Neptunium is one of the minor actinides generated from the reprocessing of nuclear fuels. It exists in solution primarily as the Np^{5+} species that forms stable carbonate complexes. In general, the pentavalent species of all actinides are unstable, except for Np(V) which is the common form in some natural waters. The chemical characteristics of pentavalent actinides, for example NpO^{2+}, are similar to those of simple monovalent cations: i.e., low ligand-complexing abilities with a high environmental mobility. Neptunyl species (NpO_2^+) which is mobile, non-sorptive can be biologically reduced to insoluble Np^{4+} under anaerobic conditions [31,35,37,45]. Complexation of Np(IV) by fermentation intermediate products prevented its precipitation [45]. Although Np^{4+} is easily oxidized in solution, it is stabilized in the presence of complexing ligands. For example, *Shewanella putrefaciens* reduced Np^{5+} to Np^{4+} that then was precipitated from solution as Np^{4+} phosphate [31]. Reduction of Np(V) to Np(IV) by cell suspension of *S. putrefaciens* MR-1 [37] and by the sulphate-reducing bacteria *Desulfovibrio desulfuricans* [35] have been reported. Neptunyl (NpO_2^+), which is generally thought to be non-sorptive showed significant sorption by *Pseudomonas fluorescens* cells [48]. XANES analysis of Np associated with the cells showed no reduction Np(V). This is in contrast to previous studies which showed negligible uptake of Np by *P. aeruginosa*, S*treptomyces viridochromogenes*, *Scenedesmus obliquus*, and *Micrococcus luteus* [49].

BIOTRANSFORMATION OF AMERICIUM

Americium can exist in multiple oxidation states Am (III, IV, V, VI, VII); but under environmental conditions, Am(III) is the most stable and predominant form. The principle oxidation state of Am is 3+, with the $Am(OH)_3$ species being extremely surface-reactive. The presence of soluble carbonate complexes, specifically $AmCO_3^+$, $Am(CO_3)_2^-$,and $Am(CO_3)_3^-$ is well established. Microbial activity may convert $Am(OH)^{2+}$ species to Am(III)-carbonate complexes due to carbon dioxide production; likewise, biodegradation of Am(III)-organic complexes may precipitate Am as the hydroxide species. Sorption of Am to biomass has been reported. Bacteria isolated from sediments and grown with ^{241}Am in minimal medium produced exometabolites, which formed soluble complexes with Am [25]. Thus, the potential exists for dissolution of Am in wastes by microorganisms, thereby increasing its bioavailability and mobility.

BIOTRANSFORMATION OF CURIUM

Biosorption of Cm(III) on the surface of microorganisms *Chlorella vulgaris*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Halomonas* sp., *Halobacterium salinarum* and *Halobacterium halobium* [39] and sulfate reducing bacteria *Desulfovibrioaspensis* [34] have been reported. No incorporation of Cm(III) into the bacterial cells was found and that the presence of organic compounds in the exudates of microorganisms affected the biosorption and coordination of Cm with the bacterial cells.

BIOTRANSFORMATION OF TECHNETIUM

Technetium is produced in large quantities by the fission of ^{235}U during nuclear power generation and defense related activities including nuclear testing and reactor operations. At present there are 19 known isotopes with mass numbers from 92 to 107 with half-lives ranging from a few seconds to several hundred thousand years (2.1×10^5 years). Tc can exist in oxidation states 0, +3, +4, +5, +6, and +7; however, the predominant chemistry concerns only the stable heptavalent pertechnetate ion (TcO_4^-) and the quadrivalent Tc(IV) ion. Its chemical behavior resembles that of rhenium. Technetium is readily reduced and oxidized. Uncomplexed Tc(IV,III) undergoes hydrolysis and precipitates from solution. Tc(V) and Tc(VI) undergoes disproportionation reactions to form Tc(IV) and Tc(VII). The very soluble pertechnetate ion is precipitated from dilute hydrochloric acid (up to 5 M) by hydrogen sulfide and the insoluble reduced form is oxidized to the soluble pertechnetate anion by hydrogen peroxide in alkaline solution.

Microorganisms affect the dissolution or precipitation of Tc by oxidation-reduction reactions and by complexation with organic by-products and macromolecules. Chemolithotrophic, haloalkaliphilic, aerobic, facultative, and anaerobic (fermentative and sulfate-reducing) bacteria reduced pertechnetate ion to an

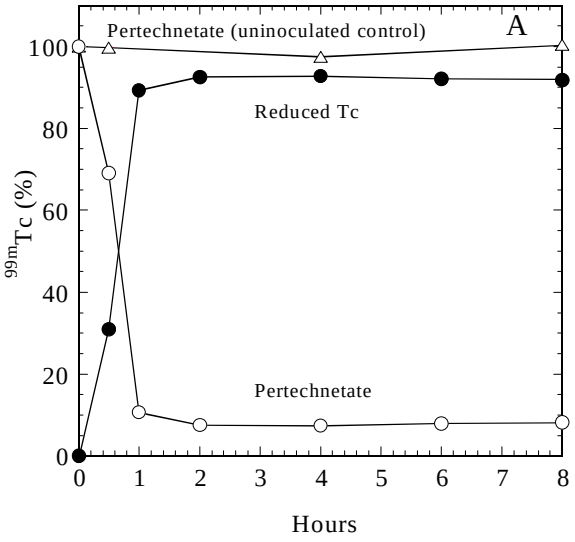


Fig. 14. Reduction of pertechnetate by *Clostridium sphenoides*[10].

insoluble form [10,26,29,30,32,43,51,55]. For example, *Clostridium sphenoides* capable of citrate and glucose metabolism and *Clostridium* sp. capable of fermenting glucose but not citric acid reduced Tc(VII) to Tc(IV) (Figure 14). The reduced Tc was predominantly associated with the cell biomass. It also was present in solution complexed with bacterial metabolic products (MW>5000). Addition of diethylenetriamine pentaacetic acid (DTPA) to *Clostridium* sp. resulted in the formation of a soluble Tc(IV)-DTPA complex [10]. Technetium absorption by soil has been attributed to soil microbial activity. It has been found that 98% of technetium is absorbed within 2 to 5 weeks by eight of the eleven soils and sterilization of the soil eliminated this absorption. Peretrukhin et al. [42] reported technetium sorption by bottom sediments of a lake in Russia due to microbial sulfate reduction. Biogenic hydrogen sulfide converts the initial readily soluble sodium pertechnetate to poorly soluble technetium (VII) and technetium (IV) sulfides.

$$\mathbf{Tc(VII)_{aq}} \xrightarrow[\text{fermenters, sulfate reducres}]{\text{Dissimilatory metal reducers}} \mathbf{Tc(IV)_S}$$

Speciation of microbially reduced Tc. The predominant reduced Tc species identified include TcO_2, $Tc(OH)_4$, TcS_2 depending on the type of microorganism involved. For example, the sulfate reducers generate H_2S, which results in the reduction and precipitation of Tc as TcS, whereas with non-sulfate reducers it is present as Tc oxide and hydroxide species. However there is a significant amount of Tc bound to the cells and also with macromolecules, which could affect the long-term stability and mobility of reduced Tc complexed with organic ligands. We found that a substantial amount of reduced Tc is associated with bacterial cells and with bacterial macromolecule most probably as an organic complex. The potential exist for colloidal transport of the reduced Tc-organic complexes. The nature and stability of the Tc-organic complexes is not known. Tc may be present as insoluble or soluble form or as colloids, depending on the type and extent of bacterial activity in subsurface environments and therefore, the potential exists for the transport of reduced Tc in these forms.

BIOTRANSFORMATION OF RADIUM

The effects of sulfur-oxidizing bacteria on the release of $^{226}Ra^{2+}$ from uranium mine tailings containing 0.72% pyrite showed that the bacterial oxidation of the pyrite increased the amount of sulfate and decreased the amount of $^{226}Ra^{2+}$ in the effluent. At many uranium mining and milling sites, soluble radium is removed as a coprecipitate with $BaSO_4$ by the addition of $BaCl_2$ to sulfate-rich tailing effluents. The resulting $(Ba,Ra)SO_4$ precipitate is allowed to settle, yielding a supernatant which is sufficiently low in $^{226}Ra^{2+}$ for discharge to the environment and a radioactive sludge. The disposal of radioactive sludges must ensure that $^{226}Ra^{2+}$ does not leach into groundwater because the stabilized radioactive waste may be transformed into mobile compounds due to microbial activity. For example, radium coprecipitated with barium sulfate was solubilized by sulfate reducing bacteria *Desulfovibrio vulgaris* under anaerobic conditions in the presence of useable carbon source with the reduction of $SO_4^{=}$ to H_2S and concurrent release of Ba^{2+} and $^{226}Ra^{2+}$[33].

$$(Ba,Ra)SO_4 \xrightarrow{\textit{sulfate – reducing bacteria}} {}^{226}Ra^{2+} + Ba^{2+} + H_2S$$

These results suggest that ultimate disposal of these waste should ensure that they are maintained under aerobic conditions to minimize the activity of sulfate reducing bacteria.

BIOTRANSFORMATION OF CESIUM AND STRONTIUM

Bioaccumulation of Sr and Cs (structural analogues for Ca and K, respectively), has been reported for several microorganisms [8,52]. Consequently, there is considerable interest in using microorganisms to remove radioactive Sr and Cs from waste streams and contaminated sites. Mixed cultures of bacteria, isolated from low-level radioactive waste leachates preferentially accumulated ^{85}Sr in mineral salts medium containing a mixture of radionuclides [13]. The bacteria accumulated $^{85}Sr > {}^{60}Co > {}^{137}Cs$. Similarly, ^{137}Cs and ^{226}Ra are concentrated less than uranium by microbial cultures [50]. Sr-binding activity in *Micrococcus luteus* is localized on the cell envelope and is sensitive to pretreatment. Bound Sr can be displaced by chelating agents, divalent cations or

H^+ (other monovalent cations are less effective at displacing Sr). Sr binding in *M. luteus* is reversible, though both ion exchange, mediated by acidic cell surface components and intracellular uptake may be involved [8].

Cesium-accumulating bacteria isolated from soil [52] display the rod-coccus growth cycle and contain mesodiaminopimelic acid, mycolic acids, and tuberculostearic acids. Cs uptake is optimal at pH 8.5 and cannot be modeled as simple sorption. Potassium and rubidium inhibit Cs accumulation suggesting that Cs is taken up through the potassium transport system [53]. Nevertheless, the nature of Cs association with the cells (extra or intra cellular) remains unclear, as does the long-term fate of bioassociated Cs.

In soils ^{90}Sr and ^{137}Cs is likely to be present as an exchange form or bound to soil organic matter, iron (hydr)oxides, or insoluble carbonate or phosphate. Microorganisms can affect the association of above mentioned forms of Sr and Cs in soils in the following way: (i) dissolution of carbonate and phosphate phases, clays, and other minerals due to production of organic acids and sequestering agents [12]; (ii) reductive dissolution of iron and the release of Sr and Cs coprecipitated or associated with the iron oxides [12], (iii) biodegradation of the organic carbon associated Sr and Cs fractions; (iv) immobilization due to precipitation reactions i.e., formation of strontium carbonate, microbial formation of strontium calcite phase and by biomass/exopolymers. The nature of the association of Sr and Cs in soil and the mechanisms of remobilization due to aerobic and anaerobic microbial activity is not fully understood.

BIOTRANSFORMATION OF IODINE

Iodine–129 (half life 1.57 $x10^7$ y) is one of the persistent radionuclides released into the environment from nuclear fuel processing activities. The predominant aqueous chemical forms of iodine (I_2), iodide (I^-) and iodate (IO_3^-) are highly soluble and mobile in the environment. Microorganisms are known to affect the chemical behavior of iodine through processes such as volatilization (CH_3I), oxidation of I^- to I_2, reduction of IO_3^- to I^-, and bioaccumulation by bacterial cells both intracellularly and extracelluarly [Figure 15]. Microbial volatilization of organic iodine was observed in soil slurries and seawater samples by aerobic bacteria through methylation of iodide (I^-) to form methyl iodide (CH_3I). The volatilization of iodide was also found in iodide-rich natural brine water. In addition to the organic iodine compounds, a significant amount of molecular iodine (I_2) was produced. Axenic cultures of bacteria are known to produce diiodomethane (CH_2I_2) and chloroiodomethane (CH_2ClI). Iodide-oxidizing bacteria, which oxidize I^- to I_2 were isolated from seawater and natural brine water. Sulfate-reducing bacteria *Desulfovibrio desulfuricans* and metal reducing bacteria *Shewanella putrefaciens* have been shown to reduce iodate to iodide. Iodate (IO_3^-) is electrochemically or biologically reduced to I^- prior to uptake by rice plants. Changes in iodine redox states could have important effects on the mobility of iodine in natural systems. Conditions that are known to influence microbial activity and survival of microorganisms affected iodine sorption. Incubation of soil samples with varying levels of biomass, oxygen concentration, and soil water content showed the participation of soil microflora in iodine immobilization. Pure cultures of soil bacteria and fungi incorporated radioiodine [4]. Radioiodine (^{125}I) adsorption by soil treated with nutrient showed elevated levels of microbial biomass with increased adsorption of radioiodine [4]. Anaerobiosis during the incubation period lowered adsorption. Migration of radioiodine in water saturated soil columns was influenced by the quantity of microorganisms present. Soils high in organic substance and soil biomass exhibited higher radioiodine sorption compared to clay minerals; and the iodine sorption process was predominantly irreversible [4].

In terrestrial environments iodine concentrations accumulated in soils average of 5 mg kg^{-1} world-wide which is much higher than those of their parent materials such as rocks and plants (0.05 to 0.5 mg kg^{-1}). Similarly, iodine concentrations in certain marine sediments are high (100 to 2000 mg kg^{-1}) compared with that in seawater (0.06 mg L^{-1}). Such high iodine accumulation in soils and

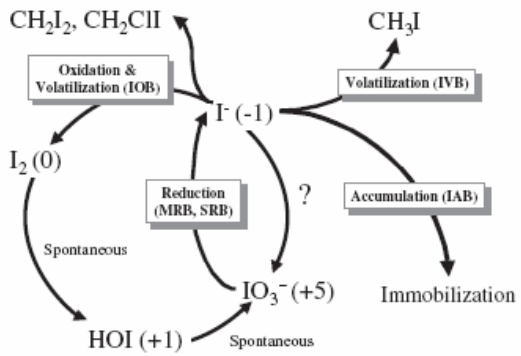


Fig. 15. Biotransformation and biogeochemical cycling of iodine [1].

sediments has been attributed to at least in part to microbial effects, although the mechanism of accumulation process is not fully understood. One possible explanation for this accumulation of iodine is that the iodide ion (I^-) is actively transported into the bacteria isolated from the marine sediment which accumulated iodide >5000-fold [1]. Iodide adsorption by the Gram-positive soil bacterium *Bacillus subtilis* showed that positively charged single sites on the cell wall were responsible for iodide sorption onto the surface of *B. subtilis* with a concentration of 3.54± 3.80 µmol iodide g^{-1} bacteria. Uptake and accumulation of iodide in washed cell suspensions of marine bacteria increased with the addition of glucose, while iodate was

not accumulated by the bacteria [1]. Although a wide variety of terrestrial and marine bacteria has the potential for fixation of iodine in the environment there is very little information on the chemical speciation of the bioaccumulated iodine in bacteria and also the long term-stability of such species.

SUMMARY

Microorganisms play a major role in the transformations of radionuclides generated from nuclear fuel cycle and regulate the mobility and stability of the radionuculides in the environment. The key microbial processes involved in the mobilization or immobilization of selected radionuclides of the interest are summarized in Table 1.

Table 1. Summary of Microbial Transformations of Actinides and Other Radionuclides

Process	U	Np	Pu	Am	Tc	I	Cs	Sr
Oxidation[1]	++	ND	ND	NA	+	++	NA	NA
Reduction[2]	++++	+?	+?	NA	+++	++	NA	NA
Dissolution[3]	+++	?	+	?	+++	++	+	+
Precipitation	+++	+	++	?	+++	++	?	++
Biosorption	++++	+	++	+	?	++	++	++
Biomethylation	NA	NA	NA	NA	NA	+++	NA	NA
Biocolloid[4]	++	+	+	+	?	?	?	?

NA- not applicable; ND- not determined.
[1]Dissolution due to oxidation from lower to higher valence state.
[2]Reductive precipitation due to enzymatic reduction from higher to lower valence state.
[3]Dissolution due to oxidation from lower to higher valence state, changes in pH, production of organic acids and sequestering agents.
[4]Association of radionuclides with suspended bacteria, which can be transported as biocolloids.

Fundamental understanding of the mechanisms of microbial transformations of several chemical forms of the radionuclides 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 radionuclides released from nuclear fuel reprocessing plants and in developing appropriate management and remediation strategies for contaminated sites.

ACKNOWLEDGMENTS

This research was supported by the Environmental Remediation Sciences Program, Office of Biological and Environmental Research, Office of Science, U.S. Department of Energy, under contract No. DE-AC02-98CH10886.

REFERENCES

1. Amachi, S., T. Fuji, H. Shinoyama and Y. Muramatsu. 2005. Microbial influences on the mobility and transformation of radioactive iodine in the environment. J. Nucl. Radiochem. Sci. 6:21-24.
2. Berthelin, J. and C. Munier-Lamy. 1983. Microbial mobilization and preconcentration of uranium from various rock materials by fungi. *In* R. Hallberg, (ed.) Environmental Biogeochemistry. Ecol. Bull. Stockholm 35:395-401.
3. Bloomfield, C. and G. Pruden. 1975. The effects of aerobic and anaerobic incubation on the extractabilities of heavy metals in digested sewage sludge. Environ. Pollut. 8:217-232.
4. Bors, J. and R. Martens. 1992. The contribution of microbial biomass to the adsorption of radioiodide in soils. J. Environ. Radioact. 15:35-49.
5. Brainard, J. R., B. A. Strietelmeier, P. H. Smith, P. J. Langston-Unkefer, M. E. Barr and R. R. Ryan. 1992. Actinide binding and solubilization by microbial siderophores. Radiochim. Acta 58-59:357-363.
6. Choppin, G.R. (1999). Utility of oxidation state analogs in the study of plutonium behavior. Radiochim. Acta 85:89-95.
7. Clark, D.L., D.R. Janecky and L.J. Lane. 2006. Science-based cleanup of Rocky Flats. Phys. Today 5934-40.
8. Faison, B.D., C.A. Cancel, S.N. Lewis and H.I. Adler. 1990. Binding of dissolved strontium by *Micrococcus luteus*. Appl. Environ. Microbiol. 56:3649-3656.
9. Francis A. J. 2001. Microbial transformation of Pu and implications for its mobility: in *Plutonium in environment* Kudo, A., Ed; Elseveir Sci., Ltd. Amsterdam, 201-219.
10. Francis A.J, C.J Dodge and G.E Meinken. 2002. Biotransformation of pertechnetate by Clostridia. Radiochim. Acta 90:791-797.
11. Francis A.J, J.B Gillow, C.J Dodge, R Harris, T.J Beveridge and H.W Papenguth. 2004. Association of uranium with halophilic and nonhalophilic bacteria and archaea. Radiochim. Acta 92:481-488.
12. Francis, A. J. and C. J. Dodge. 1990. Anaerobic microbial remobilization of toxic metals coprecipitated with iron oxide. Environ. Sci. Technol. 24:373-378.

13. Francis, A.J. 1990. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. Experientia 46:840-851.
14. Francis, A.J., C.J. Dodge and J.B. Gillow. 1992. Biodegradation of metal-citrate complexes and implication of for toxic metal mobility. Nature 356:14-142.
15. Francis, A.J. 1994. Biotransformation of uranium and other actinides in radioactive wastes. J. Alloys Compds. 271/273:78-84.
16. Francis, A.J. and C.J. Dodge. 2008. Bioreduction of uranium (VI) complexed with citric acid by Clostridia affects its structure and solubility. Environ. Sci. Technol. 42:8277-8282.
17. Francis, A.J., C.J. Dodge and J.B. Gillow. 2006. Biotransformation of plutonium complexed with citric acid. Radiochim. Acta 94:731-737.
18. Francis, A.J. 2007. Microbial mobilization and immobilization of plutonium. J. Alloys Cmpds. 444-445:500-505
19. Francis, A.J., C.J. Dodge and T. Ohnuki. 2007. Microbial transformations of plutonium. J. Nucl. Radiochem. Sci. 8:121-126.
20. Francis, A.J., C.J. Dodge and J.B. Gillow. 2008. Reductive dissolution of Pu(IV) by *Clostridium* sp. under anaerobic conditions. Environ. Sci. Technol. 42:2355-2360.
21. Francis, A.J., C.J. Dodge, F. Lu G. Halada and C.R. Clayton. 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp. Environ. Sci. Technol. 28:636-639.
22. Francis, A.J., J. B. Gillow, C.J. Dodge, M. Dunn, K. Mantione, B.A.Strietelmeier, M.E. Pansoy-Hjelvik and H.W. Papenguth. 1998. Role of microbes as biocolloids in the transport of actinides from a deep underground radioactive waste repository. Radiochim. Acta 82:347-354.
23. Ganesh, R., K.G. Robinson, G.R. Reed and G. S. Saylor. 1997. Reduction of hexavlaent uranium from organic complexes by sulfate- and iron-reducing bacteria. Appl. Environ. Microbiol. 63:4385-4391.
24. Gao, W. and A.J. Francis. 2008. Reduction of uranium (VI) to uranium (IV) by *Clostridia*. Appl. Environ. Microbiol. 74:4580-4584.
25. Giesy, J. P., Paine, D. and Hersloff, L. W. 1977. Effect of naturally occurring organics on plutonium-237 uptake by algae and bacteria. In Transuranics in Natural Environments. (M. G. White and P. B. Dunaway (Eds.). Nevada Applied Ecology Group, US Energy Research and Development Administration, Las Vegas, NV. NVO-178 UC-1. pp 531-543.
26. Henrot, J. 1989. Bioaccumulation and chemical modification of Tc by soil bacteria. Health Phys. 57:239- 245.
27. John S. G., C.E. Ruggiero, L.E. Hersman, C.S. Tung and M. P. Neu. 2001. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). Environ. Sci. Technol. 35:2942-1948.
28. Kersting, A.B. D.W. Efurd, D.L. Finnegan, D.K. Rokop, D.K. Smith and J.L. Thomson. 1999. Migration of plutonium in groundwater at the Nevada Test Site. Nature 397:56-59.
29. Khijniak, T.V., N. N. Medvedeva-lyalikova and M. Simonoff. 2003. Reduction of pertechnetate by haloalkaliphilic strains of *Halomonas*. FEMS Microbiology Ecology 44:109-115.
30. Lloyd J.R. and L.E. Macaskie, 1996. A novel phosphorImager based technique for monitoring the microbial reduction of technetium. Appl. Environ. Microbiol. 62:578-582.
31. Lloyd, J.R., P. Yong and L.E. Macaskie. 2000. Biological reduction and removal of Np(V) by two microorganisms. Environ. Sci. Technol. 34: 1297-1301.
32. Lyalikova, N.N. and T.V. Khizhnyak. 1996. Reduction of heptavalent technetium by acidophilic bacteria of the genus *Thiobacillus*. Microbiologia 65:468-473.
33. McCready, R.G.L., C.J. Bland and D.E. Gonzales. 1980. Preliminary studies on the chemical, physical and biological stability of Ba/Ra-SO_4 precipitates. Hydrometallurgy 5:109-116.
34. Moll H. T. Stumpf, M. Merroun, A. Rossberg, S. Selenska-Pobell and G. Bernhard. 2004. Time-resolved laser fluorescence spectroscopy study on the interaction of curium(III) with *Desulfovibrio aspoensis* DSM 10631T. Environ. Sci. Technol. 38:1455-1459.
35. Nagaoka, T. 2005. Microbially mediated removal of Np(V) by *Desulfovibrio desulfuricans* – Implications of microbial immobilization at the radioactive waste repository. J. Nucl. Radiochem. Sci. 6:85-86.
36. Neu, M.P. C.E. Ruggiero and A.J. Francis. Bioinorganic Chemistry of Plutonium and Interactions of Plutonium with microorganisms and Plants. In "*Advances in Plutonium Chemistry 1967-2000"* D. Hoffman (Ed), 2002 pp 169-211. ANS, La Grange Park Illinois and University Research Alliance, Amarillo, Texas
37. Neu, M.P., G.A. Icopini, and H. Boukhalfa. 2005. Plutonium speciation affected by environmental bacteria. Radiochim. Acta 93:705-714.
38. Ohnuki, T., T. Yoshida, T. Ozaki, N. Kozai, F. Sakamoto, T. Nankawa, Y. Suzuki and A.J. Francis. 2007. Chemical speciation and association of plutonium with bacteria, kaolinite clay, and their mixture. Environ. Sci. Technol. 41:3134-3139.
39. Ozaki, T., J.B. Gillow, T. Kimura, T. Ohnuki, Z. Yoshida and A.J. Francis. 2004. Sorption behavior of europium(III) and curium(III) on the cell surfaces of microorganisms. Radiochim. Acta 92:741-748.

40. Panak, P. J. and H Nitsche. 2001. Interaction of aerobic soil bacteria with plutonium(VI). Radiochim. Acta 89: 499-504.
41. Panak, P.J., Booth, C.H., Caulder, D.L., Bucher, J.J., Shuh, D.K. and Nitsche, H. 2002. X-ray absorption fine structure spectroscopy of plutonium complexes with *Bacillus sphaericus*. Radiochim. Acta 90:315-321.
42. Peretrukhin, V.F., T.V. Khizhnyak, N.N. Lyalikova and K.E. German. 1996. Biosorption of technetium-99 and some actinides by bottom sediments of Lake Belsso Kosino of the Moscow region. Radiochem. 38:440-443.
43. Pignolet, L. F. Auvray, K. Fosny, F. Capot and Z. Moureau. 1989. Role of various microorganisms on Tc behavior in sediments. Health Phys. 57:791-800.
44. Premuzic, E.T., A.J. Francis, M. Lin and J. Schubert. 1985. Induced formation of chelating agents by *Pseudomonas aeruginosa* grown in presence of thorium and uranium. Arch. Environ. Contam. Toxicol. 14:759-768.
45. Rittmann, B.E., J.E. Banazak and D.T. Reed. 2002. Reduction of Np(V) and precipitation of Np(IV) by an anaerobic microbial consortium. Biodegradation, 13:329-342.
46. Rusin, P. A., Quintana, L., Brainard, J. R., Strietelmeier, B. A., Tait, C. D., Ekberg, S. A., Palmer, P. D., Newton, T. W. and Clark, D. L. 1994. Solubilization of plutonium hydrous oxide by iron-reducing bacteria. Environ. Sci. Technol. 28:1686-1690.
47. Sakaguchi, T. 1996. *Bioaccumulation of Uranium*. Kyushu University Press, Hukuoka, Japan.
48. Songakasiri, K., D.T. Reed and B.E. Rittmann. 2002. Biosorption of neptunium(V) by *Pseudomonas fluorescens*. Radiochim. Acta 90:785-789.
49. Stranberg, G. and W.D. Arnold. 1988. Microbial accumulation of neptunium. J. Ind. Microbiol. 3:329-331.
50. Strandberg, G.W., S.E. Shumate II and J. R. Parrot Jr. 1981. Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. Appl. Environ. Microbiol.41:237-245.
51. Tagami, K. and S. Uchida. 1996. Microbial role in the immobilization of technetium soil under waterlogged conditions. Chemosphere 33:217- 225.
52. Tomioka, N., H. Uchiyama and O. Yagi. 1992. Isolation and characterization of cesium-accumulating bacteria. Appl. Environ. Microbiol. 1019-1023.
53. Tomioka, N., H. Uchiyama and O. Yagi. 1994. Cesium accumulation and growth characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. Strain CS402. Appl. Environ. Microbiol. 60:2227-2231.
54. Wall, J. D. and L. R. Krumholz. 2006. Uranium reduction. Ann. Rev. Microbiol. 60:149-166.
55. Wildung, R. E., Y.A. Gorby, K.M. Krupka, N.J. Hess, S.W. Li, A.E. Plymale, J.P. McKinley and J.K. Fredrickson. 2000. Effect of electron donor and solution chemistry on products of dissimilatory reduction of technetium by *Shewanella putrefaciens*. Appl. Environ. Microbiol. 66:2451-2460.
56. Xu, C., P. Santschi, J. Zhong, P. Hatcher, A.J. Francis, C.J. Dodge, K. Roberts, C-C. Hung and B. Honeyman. Colloidal cutin-like substances cross-linked to siderophore decomposition products mobilizing plutonium from contaminated soils. Environ. Sci. Technol. 42:8211-8217.