

Impacts of microorganisms on radionuclides in contaminated environments and waste materials

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Abstract: Microorganisms affect the solubility and stability of the radionuclides in nuclear wastes disposed of in the subsurface and deep geological formations, and contaminated environments. A wide variety of microorganisms are present in transuranic (TRU) wastes, Pu-contaminated soils, low-level radioactive wastes, backfill materials, natural analog sites, and waste-repository sites slated for disposal of high-level wastes. Under the appropriate conditions, microorganisms can alter the chemical speciation, solubility and sorption properties of the radionuclides and thus increase or decrease their concentrations in solution and affect their environmental mobility and bioavailability. Dissolution or immobilization of radionuclides is brought about by direct enzymatic action of the microorganisms or their indirect non-enzymatic. In this review, I discuss the mechanisms of microbial transformations of the actinides, the fission and activation products and other radionuclides that are of concern under relevant environmental and microbial process conditions in the presence of electron donors and acceptors.

Key words: microorganisms, radionuclides, oxidation, reduction, solubilization, immobilization, bioaccumulation, biomethylation, biotransformation, radionuclide-organic complexes, radioactive wastes, gas generation.

6.1 Introduction

A variety of radionuclides are generated from uranium mining and milling operations, nuclear power plants, reprocessing of spent nuclear fuel, weapons testing, and nuclear accidents. Of particular concern are the actinides (Th, U, Np, Pu, Am), fission products (^{137}Cs , ^{90}Sr , ^{99}Tc and ^{129}I), and activation products (^{60}Co , ^{63}Ni , ^{14}C and ^3H). The radionuclides may be present in various forms such as elemental, oxide, coprecipitates, ionic, inorganic- and organic-complexes, and naturally occurring minerals depending on source and the process stream. In addition, low-level waste (LLW), intermediate-level waste (ILW) and transuranic (TRU) wastes contain a variety of organic and inorganic compounds (cellulose, chelating agents, plastics, nitrate, and sulfate)

1 which can support microbial growth and activity. The presence of these
2 compounds causes a major concern because of their potential for migration
3 from the waste repositories and contaminated sites and the contamination
4 of the environment.

5 Microorganisms have been detected in low-level radioactive wastes,
6 transuranic wastes, Pu-contaminated soils, and in waste-repository sites under
7 consideration for the disposal of high level nuclear waste (HLW) (Francis 1990,
8 2001; Anderson *et al.* 2011). The presence of active microbial populations
9 in deep subsurface environments suggests that, under appropriate conditions,
10 these microbes could play a significant role in the transformation and transport
11 of radionuclides in the subsurface environments. The radionuclides in LLW,
12 ILW, HLW, TRU and mixed wastes are present as various chemical species
13 and oxidation states and complicate assessing their environmental behavior.
14 The radiation and chemical toxicity of the radioactive elements and the long
15 half-lives of their isotopes are the primary causes for concern. Significant
16 microbial activity is expected in LLW, ILW, and TRU waste because of
17 the presence of organic carbon and nitrogen-containing compounds such as
18 nitrate which serve as carbon and nitrogen sources for microbial growth. In
19 the absence of oxygen, nitrate and sulfate present in the waste can be used
20 as electron acceptors. Biodegradation of organic constituents of the waste
21 results in the production of gas and pressurization of waste containers and
22 thus compromises the integrity of the waste within the repository. Further,
23 microbial reactions may affect metal corrosion and therefore the integrity
24 of the waste form.

25 Microbial activities also affect the chemical nature of the radionuclides
26 by altering the speciation, solubilities and sorption properties and thus could
27 increase or decrease the concentrations of radionuclides in solution and their
28 bioavailability. Under appropriate conditions, dissolution or immobilization
29 of radionuclides is brought about by direct enzymatic (oxidation–reduction
30 reactions which affect the valence state) or indirect non-enzymatic actions
31 of microorganisms. Dissolution of radionuclides by microorganisms is due
32 to changes in the Eh and pH of the local environment, by their production
33 of organic acids, extracellular metabolites, dissolved carbonate species and
34 production of chelating or sequestering agents such as oxalic acid, citric
35 acid and siderophores. Immobilization or precipitation of radionuclides
36 is due to changes in the Eh of the environment, enzymatic reductive
37 precipitation (reduction from higher to lower oxidation state), biosorption,
38 bioaccumulation, bioprecipitation reactions, and biotransformation of
39 radionuclide-organic and radionuclide-inorganic complexes. Microbes are
40 also involved in biomethylation reactions in the presence of a suitable methyl
41 donor, resulting in the volatilization of radionuclides, and mobilization by
42 biocolloid formation.

43 The direct implication of microorganisms in the biotransformation of

radionuclides 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. In this chapter the solution chemistry and microbiology of the mechanisms of biotransformation of radionuclides by microorganisms under various microbial process and environmental conditions are reviewed.

6.1.1 Microbial activity and its impact on radionuclide chemistry

Microbes are ubiquitous in the environment and play a major role in the biogeochemical cycling of various elements. Under appropriate conditions, various microbial populations with diverse metabolic activity and growth can be stimulated by the presence of organic and inorganic nutrients, electron donors and acceptors, and environmental factors such as moisture, temperature, pH, and Eh. Microbes are versatile and easily adapt to a new environment; in fact, the environment selects the organisms. Specific groups of microorganisms are found in abundance where both electron donors and specific electron acceptors are present. The metabolic pathway that a given microorganism will use depends on the energy source, light or chemical, and thus a microorganism is described as either a phototroph or a chemotroph, respectively. Phototrophs (photolithotrophs) are autotrophic organisms which derive energy from sunlight and use CO₂ for cell carbon. Chemotrophs, depending upon where they obtain their energy and carbon, are divided into autotrophs and heterotrophs. Autotrophs (chemoautotrophs or chemolithotrophs) derive energy from the oxidation of inorganic compounds such as ammonium, iron, sulfur, and cell carbon from CO₂. Examples of chemoautotrophs include iron-oxidizing bacteria involved in the oxidation of elemental and ferrous iron ($\text{Fe}^0 + 2\text{H}^+ \rightarrow \text{Fe}^{2+} + \text{H}_2$; $\text{Fe}^{2+} + \text{H}^+ + \frac{1}{4}\text{O}_2 \rightarrow \text{Fe}^{3+} + \frac{1}{2}\text{H}_2\text{O}$); sulfur-oxidizing bacteria catalyze the oxidation of elemental sulfur to sulfuric acid ($\text{S} + 1\frac{1}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4$); and nitrifying bacteria which oxidize ammonia to nitrite ($\text{NH}_4^+ + 1\frac{1}{2}\text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$) by *Nitrosomonas* sp.; and oxidation of nitrite to nitrate ($\text{NO}_2^- \rightarrow \text{NO}_3^-$) by *Nitrobacter* sp.

Heterotrophs use organic carbon as a source of energy, hence it is the dominant process in the environment. Under aerobic conditions organic carbon is completely mineralized (oxidized) to CO₂ and H₂O and oxygen is used as terminal electron acceptor. If N, S, and P are present in the original organic compound, they are converted to NO₃⁻, SO₄²⁻, and PO₄³⁻ respectively. Under anaerobic conditions organic carbon is incompletely degraded to intermediate products which accumulate or are completely metabolized to CH₄ and/or CO₂, with the release of H₂, NH₃, and H₂S. Instead of oxygen, NO₃⁻, Fe³⁺,

1 Mn^{4+} , SO_4^{2-} , organic compounds, CO_2 and some metals serve as terminal
2 or alternate electron acceptors.

3 The primary pathways of organic carbon oxidation tend to occur in a
4 predictable sequence. The most energetically favorable is aerobic respiration,
5 followed, in order of decreasing free energy yield, by denitrification,
6 Mn(IV) reduction, Fe(III) reduction, fermentation, sulfate reduction, and
7 methanogenesis. The common electron acceptors are O_2 , NO_3^- , MnO_2 ,
8 Fe(OH)_3 , SO_4^{2-} , and CO_2 . Fermentation does not rely on an external electron
9 acceptor; instead, it partially oxidizes and reduces an organic substrate. The
10 final fermentation products include CO_2 , H_2 , alcohols and organic acids.

11 Microbial activities affect solution chemistry (e.g., pH, Eh, and ΣCO_2) and
12 impact radionuclide chemistry in several ways. Dissolution of radionuclides by
13 the activities of autotrophs in mining and mill tailing wastes, and mobilization
14 or immobilization by heterotrophs in wastes containing organics, could be
15 significant. The autotrophic iron and sulfur oxidizing bacteria play a significant
16 role in the solubilization of uranium from ores and mill tailings. Microbial
17 leaching of pyretic uranium ore is primarily indirect, and confined to the
18 generation of the oxidizing agent ferric sulfate, and the solvent sulfuric
19 acid.

20 An increase in heterotrophic microbial activity due to biodegradation of
21 organic compounds can affect the solubility of radionuclides and metals.
22 Microbes may be directly involved in redox reactions of multivalent
23 radionuclides such as U and Pu, whose solubilities highly depend on oxidation
24 states. Bacterial groups are known to use many redox pairs to derive their
25 energy. Many facultative and strict anaerobic bacteria that are involved in
26 the reductive dissolution of metals such as Fe^{3+} to Fe^{2+} and Mn^{4+} to Mn^{2+}
27 from higher to lower oxidation state are also known to catalyze the reductive
28 precipitation of U(VI) to U(IV) , Pu(VI) to Pu(IV) , Np(V) to Np(IV) and
29 Tc(VII) to Tc(IV) , and the reductive dissolution of Pu(IV) to Pu(III) .

30 Heterotrophic bacteria and fungi are able to release metals from various
31 materials, copper–nickel concentrates, low-grade copper ore, uranium from
32 granites and manganese ore. Several mechanisms for heterotrophic aerobic
33 microbial solubilization of insoluble metal have been proposed. These include
34 organic acid production, formation of chelating agents, and metabolism of
35 metal-associated anions. Leaching by heterotrophic organisms is entirely due
36 to chemical reaction of excreted microbial metabolites and decomposition
37 products. Microorganisms release low molecular weight organic acids which
38 are able to complex with radionuclides. Biodegradation of radionuclide-
39 organic complexes may eliminate the organic ligands and precipitate the
40 radionuclides.

41 Microbes play an important role in the generation (formation) and
42 destabilization of both intrinsic and pseudo-colloids. Free-living bacteria
43 suspended in the groundwater fall within the colloidal size range and may

have strong radionuclide sorbing capacity (bioaccumulate radionuclides intracellularly or extracellularly), giving them the potential to transport radionuclides in the subsurface (Francis *et al.* 1998). Growing bacteria are several micrometers long with volumes of several cubic microns, while non-growing bacteria, under oligotrophic conditions, at a minimum may be as small as 0.2–0.3 μm with a volume not less than 0.05 μm^3 . In addition, biomass accumulated on surfaces (biofilms) can become detached and generate biocolloids. Microorganisms are important sources of organic colloids, as are their metabolic by-products and exocellular polymers. Upon cell lysis the biomass-associated actinides are released as organic and inorganic colloidal particles.

6.1.2 Microbial transformations of actinides

The actinides exist in various oxidation states and the ones of concern are III (Am, Pu, U), IV (Th, Pu, U), V (Np, Pu), and VI (Pu, U). Microbial activities are influenced by electron donors and acceptors and their presence could significantly affect the extent of dissolution and precipitation of actinides, particularly under anaerobic conditions. Actinides may be present initially as soluble or insoluble forms and, after disposal, may be converted from one to the other by the activities of microorganisms. Microbial transformations of various uranium compounds have been extensively studied (Lovely 1993; Francis 1998; Wall and Krumholz 2006) whereas very limited studies have been conducted with other actinides such as Th, Np, Pu, Am and Cm.

6.2 Biotransformation of uranium

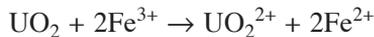
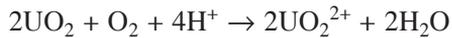
Uranium exists as U(III), U(IV), U(V), and U(VI) oxidation states, of which U(IV) and U(VI) are the predominant forms found in the environment. Both aerobic and anaerobic microorganisms are directly or indirectly involved in the dissolution and immobilization of various chemical forms of uranium in the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

6.2.1 Dissolution of uranium from ores

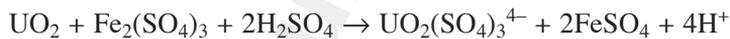
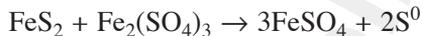
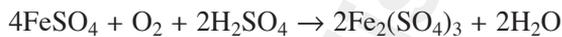
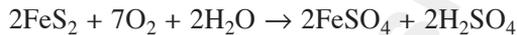
Uranium in ores is present as uraninite and pitchblende and in secondary mineral phases associated with silicates, phosphates, carbonates, and vanadates. The concentration of uranium can vary between 0.5 and 20%, with the highest amount occurring in Canadian ores. Mill tailings, a by-product of the mineral 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 , $\text{Fe}(\text{OH})_3$) which provide surface sites for uranium adsorption.

6.2.2 Autotrophic microbial activity

Iron and sulfur oxidizing bacteria (*Thiobacillus ferrooxidans* and *T. thiooxidans*) were isolated from several uranium ores (Bhurat *et al.* 1973; Kulshrestha *et al.* 1973). The iron and sulfur oxidizing bacteria play a significant role in the solubilization of uranium from ores and in mill tailings. The role of autotrophic bacteria *T. ferrooxidans* in the extraction of uranium from ore is primarily indirect action due to (1) involvement of $\text{Fe}^{2+}/\text{Fe}^{3+}$ in the process of cyclically mediating the oxidation of the insoluble uranium oxide:



and (2) generation of the oxidizing agent ferric sulfate and the solvent sulfuric acid from sulfur oxidation (Munoz *et al.* 1993). The following equations describe the 'direct' and 'indirect' mechanisms for the oxidation of pyrite and dissolution of uraninite:



An important reaction mediated by *T. ferrooxidans* is the generation of ferric sulfate. Ferric sulfate is a strong oxidizing agent capable of dissolving a wide range of metal sulfide minerals. Leaching brought about by ferric sulfate is termed indirect leaching and proceeds in the absence of both oxygen and viable bacteria. This mode is responsible for leaching several minerals. *Thiobacillus ferrooxidans* can also directly oxidize reduced compounds of uranium (uranous sulfate and UO_2) to their hexavalent form without the involvement of extraneous $\text{Fe}^{3+}/\text{Fe}^{2+}$ complexes as the chemical electron carrier (DiSpirito and Tuovinen 1981).

6.2.3 Heterotrophic microbial activity

Dissolution of uranium by heterotrophic microorganisms is due to indirect action resulting from the production of CO_2 and 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. Fungal-derived, low molecular weight carboxylic acids with strong chelating properties (e.g. oxalic acid) are involved in uranium mineral dissolution. Ligand-promoted dissolution of uranium oxides UO_3 (uranium trioxide) and U_3O_8 (triuranium octaoxide) is much more significant than proton-promoted acidification (Fomina *et al.* 2007).

Heterotrophic bacteria and fungi are known not only to solubilize various minerals including silicates (quartz, feldspar, mica) but also to 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, and ketogluconic acid, polyhydroxy acids, 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.

Several fungal strains of *Cladosporium oxysporum*, *Aspergillus flavus* and *Curvularia clavata* isolated from uranium mines solubilized uranium from low grade uranium ore (Mishra *et al.* 2009). Saprotrophic, ericoid and ectomycorrhizal fungi exhibit a high uranium oxide tolerance, and possess the ability to solubilize UO_3 and U_3O_8 and to accumulate uranium within the mycelium (Fomina *et al.* 2007). Uranium oxide solubilization by fungi *Beauveria caledonica*, *Rhizopogon rubescens*, *Penicillium simplicissimum*, *Serpula himantoides* and *Hymenoscyphus ericae* and accumulation of mobilized uranium from uranium oxides correlated with the amount of excreted oxalate. The fungi also produced gluconic, malic, succinic, and formic acids which might have enhanced uranium dissolution (Gadd and Fomina 2011).

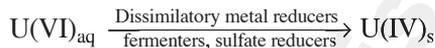
A wide variety of heterotrophic bacteria, such as *Bacillus* sp., *B. luteus*, *B. subtilis*, *B. cereus*, *B. pumilis*, *Pseudomonas striata*, *P. viscosa*, *P. perolens*, *P. chloroaphis*, *Achromobacter xerosis*, *A. stoloniferum*, and *A. healii* are involved in solubilizing uranium from granitic 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 (Bloomfield and Pruden 1975; Berthelin and Munier-Lamy 1983). When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators, such as siderophores which are known to form complexes with uranium and increase their solubility (Ruggiero *et al.* 2002). For example, *Pseudomonas aeruginosa*, grown in the presence of uranium, elaborated several metabolic products which formed complexes with uranium (Premuzic *et al.* 1985). Likewise, pyoviridin-type siderophores secreted by *P. fluorescens* formed complexes with U (Moll *et al.* 2008).

Bacterial activity can enhance the dissolution of uranium by forming

1 uranyl carbonate species. Halophilic bacteria *Halamonas* sp., isolated from
 2 the Waste Isolation Pilot Plant (WIPP) site, solubilized $K(UO_2)_5(PO_4)_3(OH)_2 \cdot$
 3 nH_2O precipitate in culture medium under denitrifying (anaerobic) conditions.
 4 Dissolution of uranium precipitate was concomitant with the growth of the
 5 bacteria. The UV-vis spectra of the culture medium during growth showed
 6 that a uranyl dicarbonate complex $UO_2(CO_3)_2^{2-}$ was formed due to CO_2
 7 production from the metabolism of carbon source succinate (Fig. 6.1) (Francis
 8 *et al.* 2000).
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10 6.2.4 Reductive precipitation of uranium

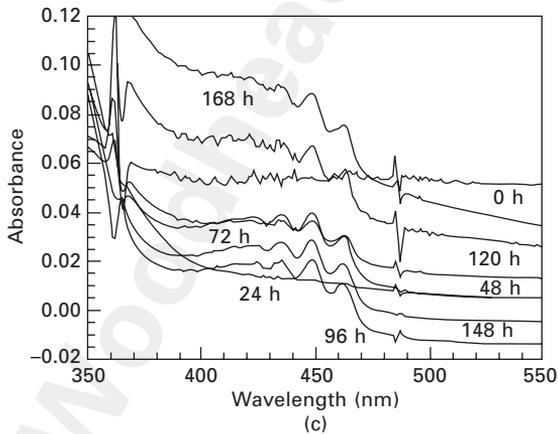
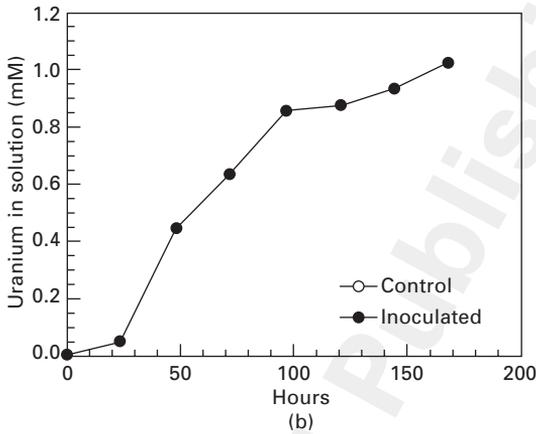
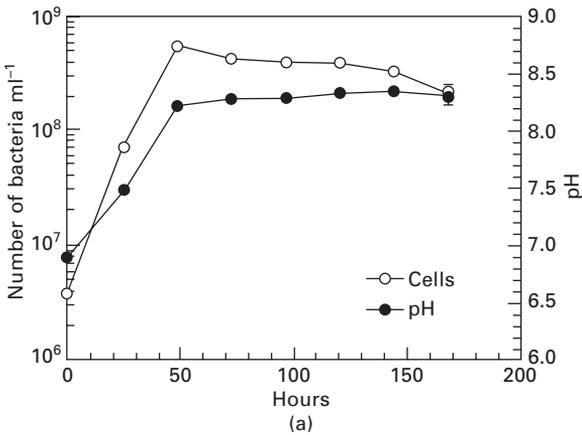
11
 12 A wide variety of bacteria present in radioactive wastes, soils and sediments
 13 are involved in the reductive precipitation of uranium. They include
 14 phylogenetically diverse organisms like hyperthermophilic archaeons,
 15 thermophilic bacteria, mesophilic iron- and sulfate-reducing bacteria, and
 16 fermentative bacteria (Wall and Krumholz 2006). Facultative and strict
 17 anaerobic bacteria reduced U(VI) added as uranyl nitrate or uranyl carbonate
 18 to U(IV) under anaerobic conditions.
 19



21
 22 These include axenic cultures of iron-reducing, fermentative, and sulfate-
 23 reducing bacteria. Mixed cultures of bacteria in uranium-contaminated
 24 groundwaters and in wastes also reduced uranium. However, the mechanisms of
 25 microbial uranium reduction and the enzymes involved are not fully understood
 26 (Wall and Krumholz 2006). *Geobacter*, *Shewanella*, and *Desulfovibrio*
 27 can couple the oxidation of organic compounds to the reduction of U(VI),
 28 and it was shown that a periplasmic hydrogenase in combination with the
 29 cytochrome *c3* was necessary for U(VI) reduction. Reductases specific for
 30 U(VI) have not been identified, suggesting the involvement of multiple low-
 31 redox-potential electron carriers in reducing uranium. It has been proposed
 32 that U(VI) is reduced to U(V) through one electron transfer reaction and that
 33 the unstable U(V) disproportionate to U(IV) and U(VI) or U(V) is further
 34 enzymatically reduced to U(IV) (Renshaw *et al.* 2005).
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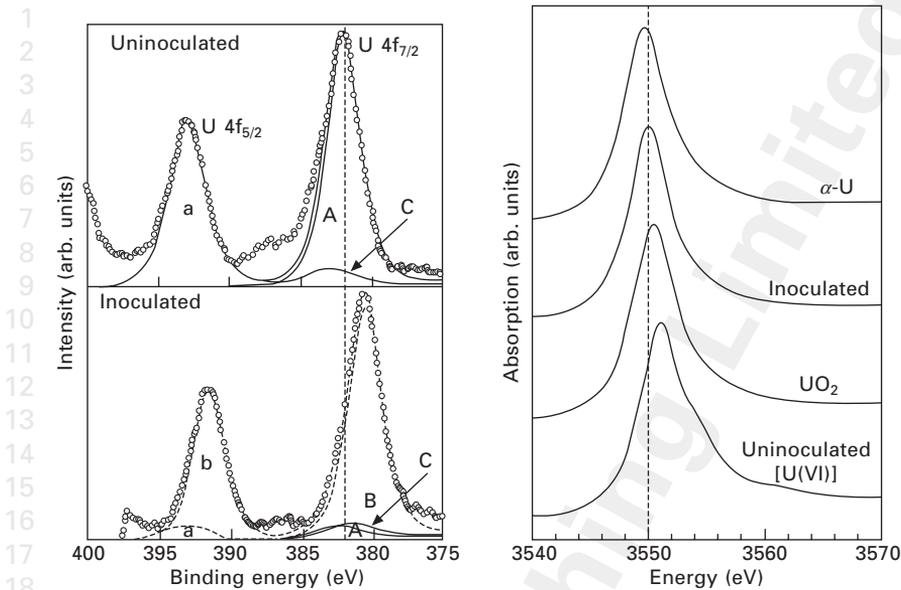
36 Clostridia are strictly anaerobic spore-forming fermentative bacteria
 37 ubiquitous in soils, sediments, and wastes that catalyze the reduction of
 38 uranium from a higher to a lower oxidation state. Reduction of soluble U(VI)
 39 to insoluble U(IV) by *Clostridium* sp. in culture medium was confirmed by
 40 X-ray photoelectron spectroscopy (XPS) and X-ray absorption near edge
 41 spectroscopy (XANES) (Fig. 6.2).
 42

43 Sulfate-reducing bacteria play a key role in the immobilization of uranium
 (Mohagheghi *et al.* 1985; Lovley and Phillips 1992). Microbial reduction of
 sulfate in waste containing toxic metals and radionuclides may result in the



6.1 Dissolution of uranium by *Halomonas* sp. under anaerobic (denitrifying) conditions. Addition of uranyl nitrate to the bacterial growth medium resulted in the precipitation of uranium as uranyl hydroxophosphato species $[K(UO_2)_5(PO_4)_3(OH)_2 \cdot nH_2O]$. (a) Growth of bacteria and changes in the pH of the medium; (b) dissolution of uranium precipitate; (c) UV-vis spectra of the culture solution showing the formation of uranyl carbonate as function of bacterial growth (Francis *et al.* 2000).

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6.2 XPS and XANES spectra of uranium(VI) reduction to U(IV) by *Clostridium* sp. XPS analysis of the uranyl nitrate-treated sample shows a 1.4 eV decrease in binding energy to 380.6 eV compared to uranyl ion (382.0 eV); XANES spectra at the M_V absorption edge for U metal, U(IV), U(VI) and sample inoculated with *Clostridium* sp. A shift in sample absorption peak from 3551.1 eV for U(VI) to 3550.1 eV shows reduction to U(IV). These complementary techniques confirm bacterial reduction of uranyl ion to U(IV) (Francis *et al.* 1994).

formation of metal sulfides. In general most of the metal sulfides exhibit low solubility in aqueous medium. West *et al.* (1992) noted that immobilization of uranium was due to reprecipitation of uranium by the activities of sulfate- and iron-reducing bacteria in the reducing front of the uranium mine in Pocos de Caldas, Brazil. Percolation of uranium mine discharge water through soil lowered Se, U, Mo and SO_4^{2-} concentrations in mine waters by the activities of anaerobic bacteria *Clostridium* sp. and sulfate-reducing bacteria *Desulfovibrio* (Kauffman *et al.* 1986). Uranium mill effluents containing toxic amounts of Mn^{2+} and ^{226}Ra were treated under aerobic conditions with *Arthrobacter* sp., which oxidized Mn^{2+} to Mn(IV) hydroxide precipitate. During the process ^{226}Ra coprecipitated with Mn(IV) hydroxide (Mathur and Dwivedy 1988). However, under anaerobic conditions coprecipitated ^{226}Ra can be remobilized due to reductive dissolution of Mn(IV) to Mn^{2+} .

Treatment of uranium- and toxic-metal-contaminated sediment and sludge with the anaerobic bacterium *Clostridium* sp. removed a large fraction of soluble non-toxic metals such as Ca, K, Mg, Mn^{2+} , Na, and Fe^{2+} , enriched and stabilized Cd, Cr, Cu, Ni, Pb, U, and Zn, and reduced the overall volume

and mass (Francis *et al.* 1991b). A substantial fraction of uranium associated with the exchangeable, carbonate and iron-oxide fractions was released into solution due to indigenous anaerobic bacterial activity, but little uranium was detected in solution. The uranium released from exchangeable, carbonate and iron oxide fractions was subsequently immobilized due to reductive precipitation by the bacteria and many of the metals were redistributed with stable mineral phases, such as organic and silicate fractions (Francis *et al.* 1991a, b). In this treatment of wastes, the unique metabolic capabilities of the dual-action anaerobic bacteria were exploited to solubilize and/or precipitate radionuclides and toxic metals directly by enzymatic action and indirectly by the production of organic acid metabolites. The non-hazardous materials in the solid phase were solubilized and removed from the waste, thereby reducing its volume.

Anaerobic bacterial treatment of uranium-contaminated surface and ground waters and bicarbonate extracts of uranium-contaminated soils precipitated uranium as uraninite (Anderson *et al.* 2003; Phillips *et al.* 1995). Microbial reduction of uranium by *Geobacter* and *Schwenella* supplied with electron donor (carbon source acetate, lactate) in subsurface environments as part of the *in situ* bioremediation of contaminated aquifers and groundwater has been investigated extensively in recent years. Among the several remediation strategies being explored to mitigate the movement of uranium in surface water, groundwater, and subsurface environments, the reductive precipitation of U(VI) to U(IV) by indigenous microbial communities appears to be a promising treatment process. However, the reduced uranium is not very stable since it is readily re-oxidized upon exposure to air or oxidizing agents.

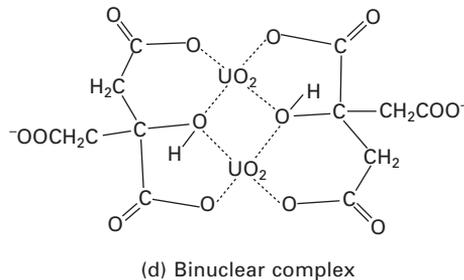
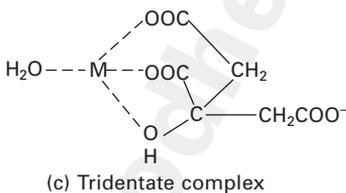
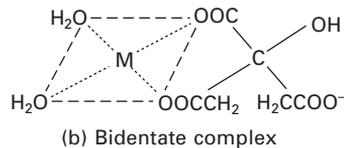
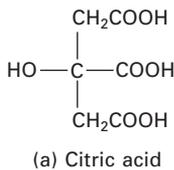
6.2.5 Biotransformation of uranium associated with organic ligands

Naturally occurring soluble organic complexing agents present at uranium-contaminated sites may affect not only the mobility of uranium but also the microbial transformation and reductive precipitation. Both U(IV) and U(VI) form complexes with anions, such as carbonate, nitrate, chloride, fulvic acid, humic acid, and EDTA, thus increasing the concentration of uranium in solution. Biotransformation of the complexed uranium can 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 soluble organic ligands.

Studies on the complexation of uranium with ketogluconic, oxalic, malic, citric, protocatechuic, salicylic, phthalic, and fulvic acids and catechol by potentiometric titration, EXAFS analysis, and electrospray ionization-mass spectrometry (ESI-MS) showed that ketogluconic acid formed a mononuclear complex with uranium involving the carboxylate group, while

1 malic acid, citric acid, and catechol formed binuclear complexes. Phthalic
2 acid formed a bidentate complex involving the two carboxylate groups,
3 while catechol bonded to uranium through the two hydroxyl groups. The
4 hydroxycarboxylic acids were bound in a tridentate fashion to uranium through
5 two carboxylates and the hydroxyl group (Dodge and Francis, unpublished
6 results).

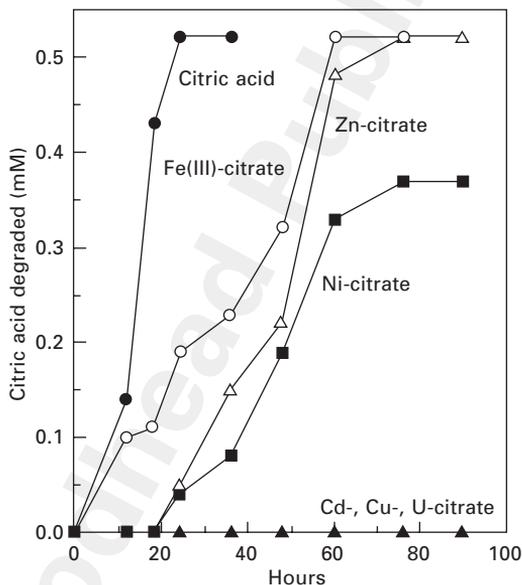
7 Citric acid is a naturally occurring, multidentate ligand which forms stable
8 complexes with various metal ions and has been investigated in most detail. It
9 is also used extensively as an extractant and decontamination agent. Citric acid
10 forms stable complexes with transition metals and actinides and can involve
11 formation of bidentate, tridentate, binuclear, or polynuclear complex species
12 (Fig. 6.3). Calcium, ferric iron and nickel formed bidentate, mononuclear
13 complexes with two carboxylic acid groups of the citric acid molecule.
14 Copper, ferrous iron, cadmium and lead formed tridentate, mononuclear
15 complexes with citric acid involving two carboxylic acid groups and the
16 hydroxyl group. Uranium has been shown to form a predominantly binuclear
17 complex with two uranyl ions and two citric acid molecules involving four
18 carboxylic groups and two hydroxyl groups. The solution pH, the ratio of
19 uranium to citrate, the temperature and the presence of other metals affect
20 the complex structure.



6.3 Citric acid (a) forms with metal ions a bidentate complex (b)
with two carboxyl groups; tridentate complex with two carboxyl and
the hydroxyl groups (c); and a binuclear complex (d) with uranium
involving with two carboxyl groups and the hydroxyl group (Francis
et al. 1992).

6.2.6 Biotransformation of uranyl citrate under aerobic conditions

The type of complex formed plays an important role in determining its biodegradability (Francis *et al.* 1992; Francis 1994). 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 *P. 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. 6.4). 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 (Joshi-Tope and Francis 1995).



6.4 Biodegradation of metal-citrate complexes is influenced by the type of complex formed between the metal and citric acid: a bidentate complex is readily degraded, whereas the tridentate, binuclear, and polynuclear species are recalcitrant (Francis *et al.* 1992; Joshi-Tope and Francis 1995).

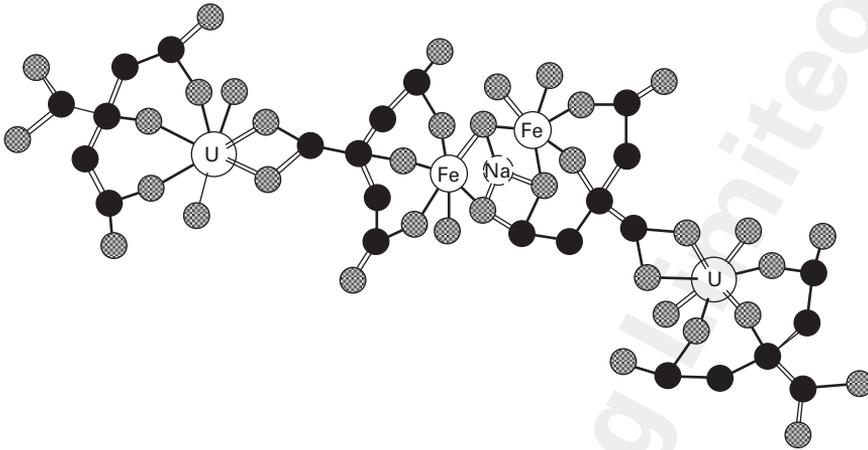
1 No relationship was observed between biodegradability and stability of the
2 complexes. The tridentate Fe(II)-citrate complex, although recalcitrant, was
3 readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)-
4 citrate form, denoting a structure–function relationship in the metabolism of
5 the complex. Biodegradation of the Fe(III)-citrate complex resulted in the
6 formation of carbon dioxide and ferrihydrite. Uranyl-citrate, however, was
7 not biodegraded and remained in solution.

9 6.2.7 Biotransformation of metal-citrate complexes under 10 denitrifying conditions 11

12 Biodegradation of various metal-citrate complexes by *Pseudomonas*
13 *fluorescens* incubated under anaerobic conditions in the presence of nitrate
14 as electron acceptor also was similar to that observed aerobically, but was
15 much slower. The bacterium completely utilized the nitrate and degraded
16 bidentate complexes of Fe(III)- and Zn-citrate, and only partially degraded
17 (~59%) the Ni-citrate complex. Ferric iron was in the colloidal form, and was
18 not reduced to Fe(II) under denitrifying conditions. Tridentate complexes of
19 Al-, Cd-, Cu-, and Fe(II)-citrate, and the binuclear U-citrate complex, were
20 not metabolized by the bacterium under denitrifying conditions (Joshi-Tope
21 and Francis, unpublished results).

23 6.2.8 Biotransformation of the ternary iron–uranium– 24 citrate complex 25

26 Citric acid forms mixed-metal complexes with various metal ions (Adin
27 *et al.* 1970; Binder 1971; Markovits *et al.* 1972; Manzurola *et al.* 1989).
28 The formation of the ternary 1:1:2 Fe-U-citrate complex involving the
29 hydroxyl group of citric acid was established by a combination of analytical
30 techniques including potentiometric titration, UV-vis spectrometry, gel-
31 filtration chromatography, time-of-flight secondary ion mass spectroscopy
32 (TOF-SIMS), and extended X-ray absorption fine structure (EXAFS) analysis
33 (Dodge and Francis 2003; Fig. 6.5). The coordination of the metal to citric acid
34 affects the biodegradation of the metal-citrate complexes. Biotransformation
35 of the ternary 1:1:2 Fe:U:citric acid complex by *P. fluorescens* showed that
36 it was recalcitrant. However, in the presence of excess citric acid, after
37 biodegradation of excess citrate, the 1:1:2 complex transformed to a 1:1:1
38 Fe-U-citrate species (Dodge and Francis 1997). These results suggest that
39 Fe-U-mixed-metal citric acid complexes resist biodegradation and may
40 persist in the environment.

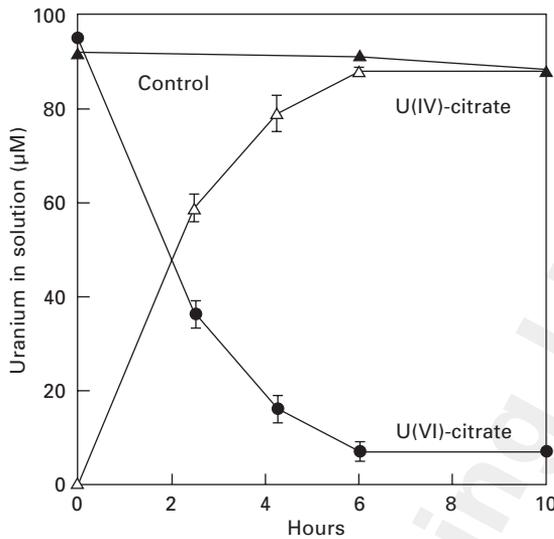


6.5 Proposed molecular structure for 2:2:4 Fe:U: citric acid complex, which is recalcitrant to biodegradation (Dodge and Francis 2003).

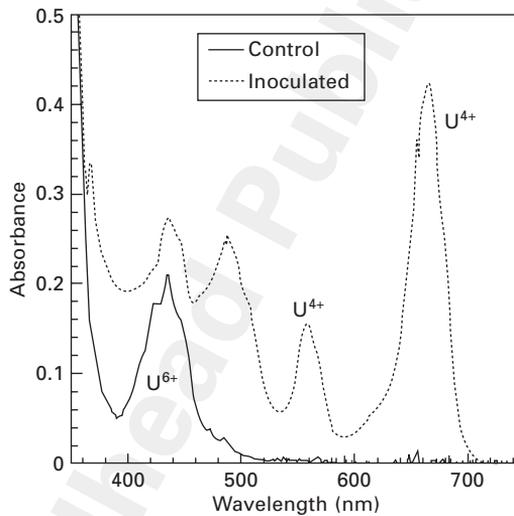
6.2.9 Biotransformation of uranyl citrate under anaerobic conditions

The presence of organic ligands affects the precipitation of reduced uranium under anaerobic conditions. For example, *Clostridium* sp. (ATCC 53464), which ferments glucose but not citrate, reduced U(VI)-citrate (Francis and Dodge 2008a) 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 (Ganesh *et al.* 1997). The reduced uranium was presumed to be complexed with an organic ligand, although its oxidation state and the nature of the complex were not determined. Other studies with the reduction of U(VI) by *Shewanella putrefaciens* in the presence of citrate, NTA, and EDTA showed that the reduced U(IV) remained in solution complexed with the organic ligand. However, the initial reduction rate of U(VI) in the citrate medium in which polynuclear U(VI)-citrate complexes were formed was much slower than those in the NTA and EDTA media (Suzuki *et al.* 2010). In a similar study, Gu *et al.* (2005) reported that in the presence of humic materials, the bioreduction of U(VI) did not result in its precipitation; rather, the uranium remained in the solution phase as the U(IV)-humic acid complex.

Studies with *Clostridium* sp. (ATCC 53464) and *C. sphenoides* (ATCC 53464) showed U(VI) complexed with citric acid 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 excess citrate (Fig. 6.6). The bacteria did not metabolize

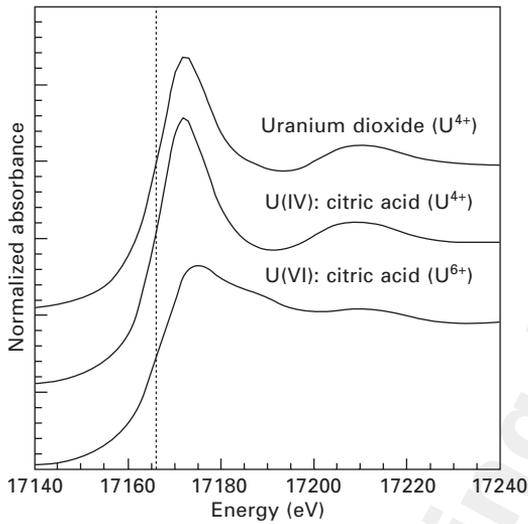


6.6 Bioreduction of U(VI)-citrate complex by *Clostridia*. *Clostridium* sp. or *C. sphenoides* reduced U(VI) complexed with citric acid to U(IV)-citrate, which remained in solution (Francis and Dodge 2008).

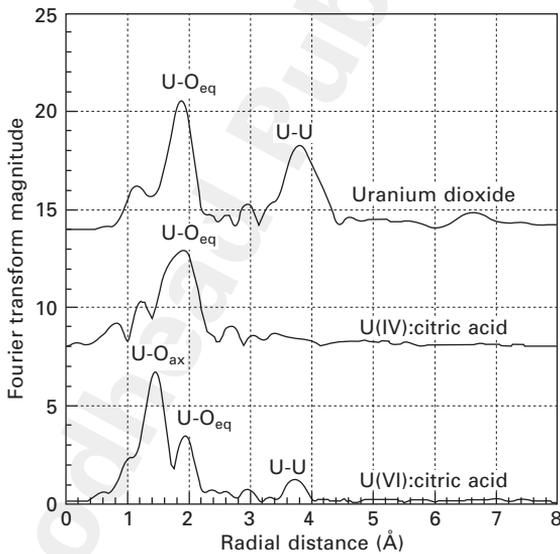


6.7 UV-vis spectra show reduction of U(VI) to U(IV) (Francis and Dodge 2008).

the citrate complexed to the uranium. UV-vis analysis showed reduction of U(VI) to U(IV) (Fig. 6.7). XANES analysis confirmed the reduced form of uranium in solution (Fig. 6.8), while EXAFS analysis (Fig. 6.9) showed that the U(IV) was bonded to citric acid as a mononuclear biligand complex



6.8 XANES spectra of uranium citrate before and after reduction by *Clostridium* sp. XANES spectra at L_{III} absorption edge show reduction of U(VI) to U(IV) by shift in absorption spectrum from 17,168 eV to 17,166 eV (Francis and Dodge 2008).

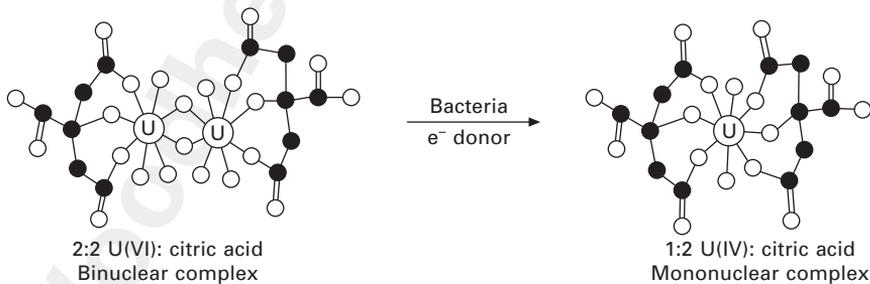


6.9 EXAFS analysis of U-citrate complexes before and after bacterial reduction. EXAFS analysis of the U(IV)-citrate complex shows no U-U interaction at 3–4 Å, indicating the presence of a mononuclear complex structure (Francis and Dodge 2008).

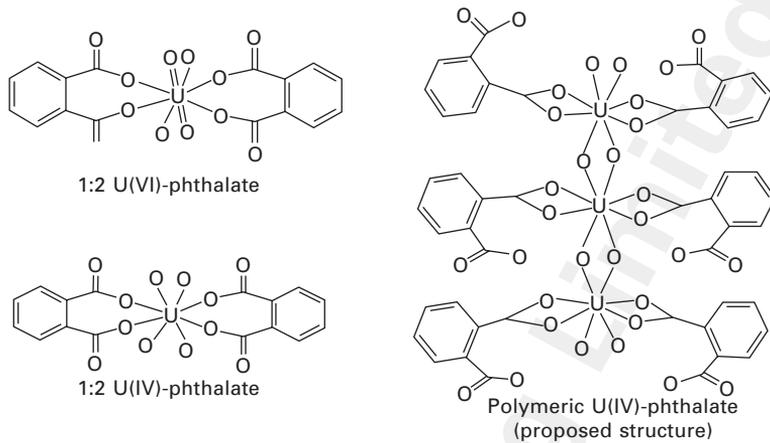
1 (Fig. 6.10). Under anaerobic conditions *Clostridium* sp. reduced the biligand
 2 binuclear U(VI)-citrate to the biligand mononuclear U(IV)-citrate complex.
 3 Furthermore, U(VI)-citrate was readily accessible as an electron acceptor
 4 for the microorganisms, despite their inability to metabolize the organic
 5 ligand complexed to the actinide. Metabolism of metal-organic complex
 6 is influenced by the nature of the complex. The bidentate complexes were
 7 readily metabolized by *C. sphenoides* under anaerobic conditions, whereas
 8 complexes that involve the hydroxyl group of citric acid, tridentate and the
 9 binuclear complexes were recalcitrant, consistent with the structure–function
 10 relationship as found with the metabolism of uranyl-citrate under aerobic
 11 conditions by *P. fluorescens* (Francis *et al.* 1992).

12 Phthalic acid, a synthetic as well as a naturally occurring compound
 13 present in radioactive wastes, forms mono- and bi-ligand complexes involving
 14 both carboxylate groups with uranyl ion, with the latter as the predominant
 15 species (Vazquez *et al.* 2009). Bioreduction of U(VI) by *Clostridium* sp. in
 16 the absence of phthalic acid resulted in the precipitation of U(IV); in contrast,
 17 the bacterium reduced U(VI)-phthalate complex to U(IV)-phthalate and it
 18 remained in the solution phase as a polymeric colloid. The formation of
 19 a colloid from the U(IV)-organic ligand complex has not been previously
 20 reported. No consumption or degradation of the organic ligand was detected
 21 (Fig. 6.11). These studies suggest that reduced uranium, when complexed
 22 with an organic ligand, can remain in solution; this finding is contrary to
 23 the conventional belief that reduced uranium will precipitate from solution.
 24 The persistence of reduced uranium complexed with chelating agents in
 25 subsurface environments is a major concern because of the potential for
 26 increasing the transport of the radionuclide.

27 The reduced U(IV) precipitates can be subjected to dissolution or
 28 mobilization due to oxidation by various oxidants such as dissolved oxygen,
 29 ferric iron, and nitrate, or through complexation by organic ligands such as
 30 siderophores, citrate and humic substances. Bacterially reduced uranium
 31 associated with organic ligand in solution phase is readily oxidized upon
 32



42 **6.10** Reduction of biligand U(VI)-citrate to monoligand U(IV)-citrate
 43 complex (Francis and Dodge 2008).



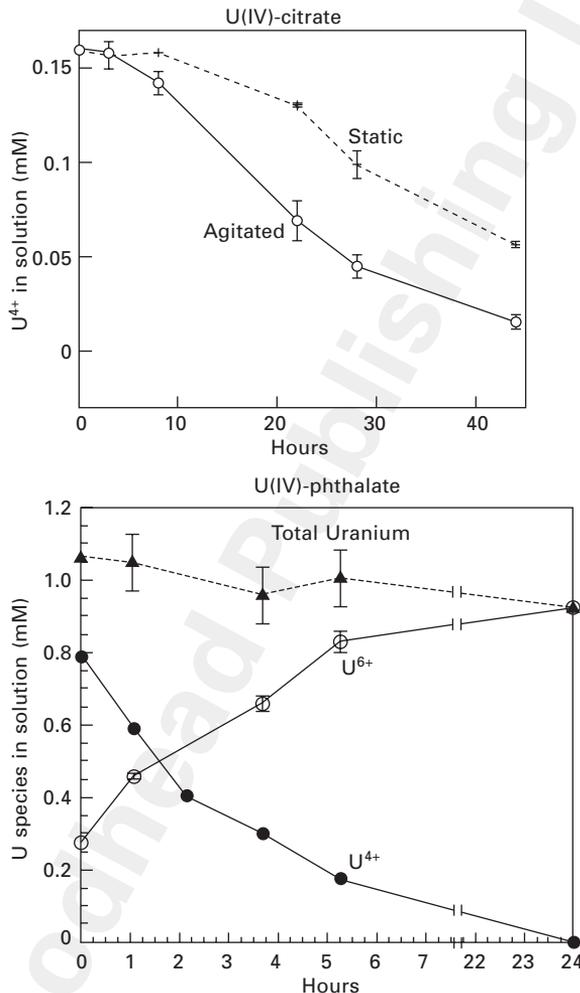
6.11 Reduction of U-phthalate by *Clostridium* sp. resulting in the colloidal polymeric uranium(IV)-phthalate complex (Vazquez *et al.* 2009).

exposure to aerobic conditions (Fig. 6.12, Vazquez and Francis, unpublished results), similar to the re-oxidation of bacterially reduced uranium in groundwater and subsurface environments. The rate and extent of re-oxidation of U(IV)-organic complexes may be influenced by the type of complex and its stability constant. Mobilization of U(IV) by complexing organic ligands is a concern in the stabilization of uranium through biological reductive precipitation or immobilization (Luo and Gu 2009, 2011). To prevent uranium from oxidative remobilization, amendments with organic substrates are often used to scavenge oxidants. Lee *et al.* (2010) reported that the reduced U(IV) or uraninite also form microaggregates and can be stabilized with microbial exudates or organic polymers. However, biotransformation of these organic compounds also generates metabolites such as organic acids with complexing capabilities to react with and mobilize U(IV).

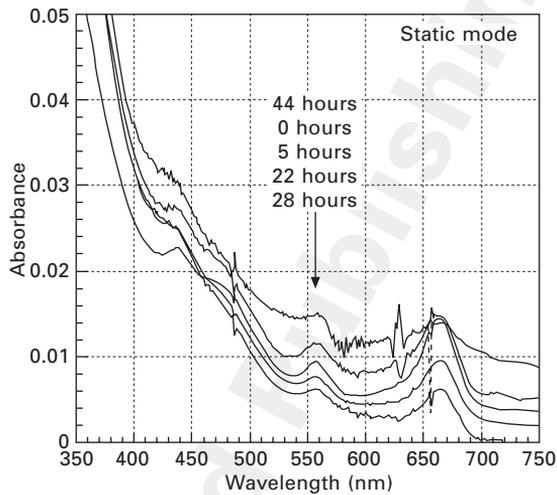
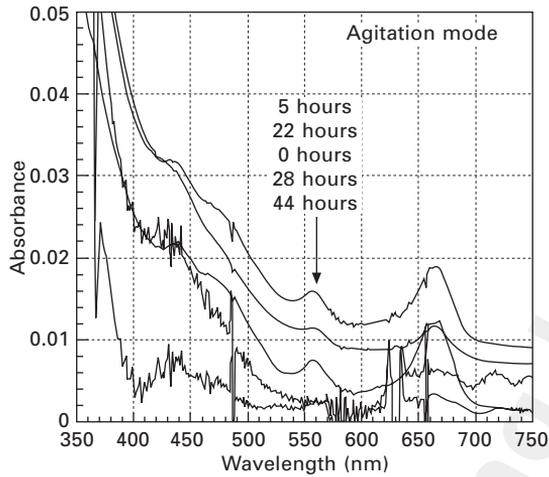
6.3 Biotransformation of plutonium

Plutonium can exist in several oxidation states (III, IV, V, VI, VII) and the solution chemistry is very complex. Plutonium(IV) is the most predominant and stable species 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, mineralogy, microbial activity, and redox conditions, affect the chemical speciation of Pu. Chemical characterization of

1 Pu at contaminated sites shows that its environmental form varies according
 2 to site, and depends on the waste stream. For example, at Rocky Flats,
 3 Colorado, the predominant form appears to be $\text{PuO}_2(\text{s})$ (Clark *et al.* 2006);
 4 at the Nevada Test Site (NTS), Pu was found to be associated with mineral
 5 colloids (Kersting *et al.* 1999). At the Rocky Flats site, studies show that
 6 Pu is associated with organic degradation product in a colloidal form (Xu *et*
 7 *al.* 2008). Plutonium speciation is affected by environmental bacteria (Neu
 8 *et al.* 2005). An increase in microbial respiration and activity will affect the



6.12 Re-oxidation of uranium(IV) complexed with organic ligands citric acid or phthalic acid. UV-vis spectra show the effect of oxygen under static and agitation mode on re-oxidation of bacterially reduced uranium (Vazquez and Francis unpublished results).



6.12 Continued

redox and Pu oxidation state by direct or indirect mechanisms of action. Bacteria catalyze the reduction and precipitation of soluble Pu(VI) and Pu(V) to Pu(IV). Microbes can solubilize Pu due to production of organic acids such as citric acid and sequestering agents such as siderophores and transport inside the cells. Keith-Roach *et al.* (2000) reported that Pu (and Am) concentrations corresponded with maximum biomass production and proposed that the data is in support of a bioaccumulation process (e.g., biosorption or bioprecipitation). Microbes may contribute to the generation and/or destabilization of Pu colloids. Plutonium in surface waters at the Rocky Flats Environmental Technology Site has been shown to be associated with

1 a 10,000 Da organic macromolecule (Santschi *et al.* 2002). This Pu-organic
2 species is possibly of microbial origin and may be labile.

3 In general, $^{239}\text{Pu} > 1.0 \times 10^{-5} \text{ M}$ seems to affect most of the microorganisms
4 studied (Wildung and Garland 1980, 1982; Francis 2001; John *et al.* 2001;
5 Neu *et al.* 2002). Its toxicity is due to radiation effects rather than metal
6 toxicity, and is modulated by the chemical form and solubility of Pu (Neu *et*
7 *al.* 2002). Radiation-resistant bacteria are constantly being enriched in such
8 environments containing higher levels of alpha- and beta-activity (Barnhart
9 *et al.* 1980). Plutonium is the major radioactive component of concern in the
10 TRU waste disposed of in the WIPP repository; 70% of this waste is cellulosic
11 material. Biodegradation of cellulose under the hypersaline conditions in the
12 repository can produce CO_2 and other gases, as well as affect the solubility
13 of Pu and other actinides (Francis *et al.* 1998).

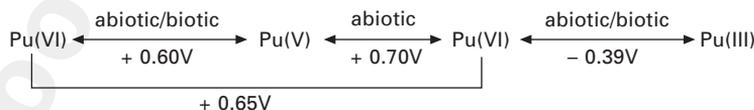
14 Immobilization of Pu by microbes may be due to indirect action by changing
15 the Eh of the environment, and facilitating abiotic precipitation of Pu by
16 reduction from higher to lower oxidation state, biosorption by bacteria, and
17 bioprecipitation reactions (Rusin *et al.* 1994; Gillow *et al.* 2001; Banaszak
18 *et al.* 1999; Panak and Nitsche 2001). The chemical form and the type of
19 association of Pu with the bacteria have not been fully elucidated.

21 6.3.1 Oxidation and reduction of plutonium

22 Microorganisms may directly or indirectly affect the oxidation and reduction
23 of Pu and affect its solubility. For example, a slight increase in microbial
24 activity (respiration) can alter the oxidation state of Pu(VI) to Pu(IV) because
25 of the very small differences in the reduction potential between Pu(VI),
26 Pu(V), and Pu(IV) (Choppin 1999; Fig. 6.13).

29 6.3.2 Dissolution of PuO_2

30 Solubility of Pu in marine and natural waters is limited by the formation of
31 $\text{Pu}(\text{OH})_4$ (amorphous) or PuO_2 (crystalline). Pu undergoes hydrolysis with
32 the formation of oligomers and polymers. Although much is not known of
33 the oxidative dissolution of PuO_2 by autotrophic (chemolithotrophic) bacteria
34 under aerobic conditions, dissolution by heterotrophic bacteria and fungi due
35 to production of metabolic products under aerobic and anaerobic conditions
36



39
40
41
42 **6.13** Microbial and abiotic reduction of Pu(VI) to Pu(III) (Choppin
43 1999).

has been reported. It is primarily due to production of organic acid metabolic products and iron-sequestering agents such as siderophores.

6.3.3 Oxidative dissolution of PuO₂ to Pu(VI)

Oxidation of crystalline PuO₂ or amorphous Pu(OH)₄ to Pu(VI) by autotrophic microorganisms has not been investigated. However, the oxidation of sulfide minerals by chemolithotrophic sulfur- and iron-oxidizing bacteria should result in the oxidation of PuO₂ to Pu(VI) or the dissolution of PuO₂ due to sulfuric acid production.

6.3.4 Dissolution of PuO₂ by heterotrophic bacteria and fungi

Dissolution of Pu by microorganisms is brought about by their production of organic acids, such as citric acid, extracellular metabolites, and siderophores (Au 1974; Bekert and Au 1976; Brainard *et al.* 1992; John *et al.* 2001; Wildung and Garland 1980). 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, thus increasing their solubilization and bioavailability. *Pseudomonas aeruginosa* isolated from a Pu-contaminated pond at Rocky Flats capable of bioaccumulation of uranium also elaborated several chelating agents for thorium and uranium when grown with these metals (Premuzic *et al.* 1985). 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 (Barnhart *et al.* 1980). Desferrioximine and enterobactin isolated from *Escherichia coli* solubilized hydrous plutonium(IV) oxyhydroxide (Brainard *et al.* 1992). Ruggiero *et al.* (2002) observed dissolution of Pu(IV) hydroxide by desferrioxamine siderophores and simple organic chelators.

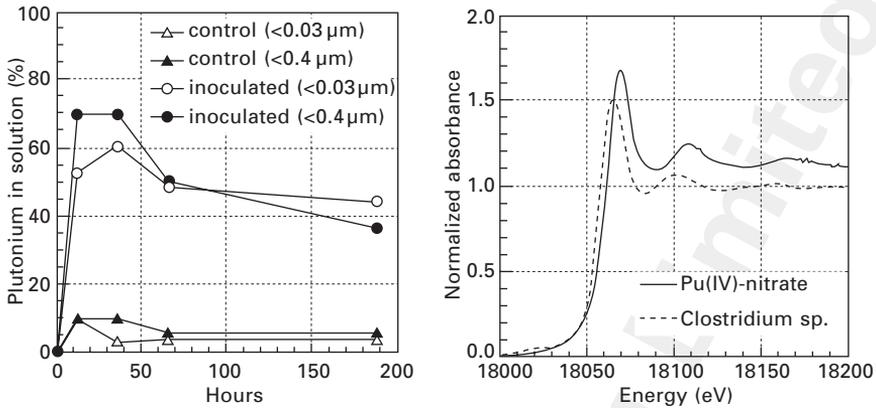
Microorganisms grown in the presence of plutonium produced complexing agents, such as citric acid and unidentified compounds capable of dissolving and mobilizing Pu in soils (Wildung and Garland 1980; Wildung *et al.* 1987). Several bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that increased the concentration of Pu in soil-column eluates relative to controls. Elution through soil effectively removed positively charged Pu complexes. The increased mobility of Pu in soil resulted from the formation of neutral and negatively charged Pu complexes (Wildung *et al.* 1987). Many of these compounds including citric acid also may be involved in transporting plutonium into the cells (Beckert and Au 1976).

6.3.5 Reductive precipitation of Pu

Reduction of Pu(VI) to Pu(V) to Pu(IV) is brought about by a wide variety of aerobic, facultative, and strict anaerobic bacteria. The direct enzymatic reductive precipitation of Pu(VI)_{aq} and Pu(V)_{aq} to Pu(IV)_s by the resting cells of facultative bacteria *Shewanella putrefaciens*, *S. oneidensis*, and *Geobacter metallireducens* under anaerobic conditions has been reported (Icopini *et al.* 2009). Direct interaction of Pu(+6) and Pu(+4) with cells of *D. aespoeensis* revealed bacterial reduction of PuO₂²⁺ to PuO₂⁺ and increased dissolution of cell-bound Pu. In contrast to the release of PuO₂⁺ from the cell surface into the surrounding solution, the biomass immobilized Pu as Pu(IV) polymers (Moll *et al.* 2006).

6.3.6 Reductive dissolution of Pu(IV) to Pu(III) by anaerobic bacteria

Bioreduction of Pu(IV) to Pu(III) by *Bacillus* sp. has been inferred (Rusin *et al.* 1994). Reduction of amorphous Pu(OH)₄ to Pu(III) by resting cell suspensions of *S. oneidensis* and *G. metallireducens* in the presence of a chelating agent EDTA was observed (Boukhalifa *et al.* 2007). Reductive dissolution of amorphous Pu(OH)₄ to Pu(III) by the growing culture of strict anaerobic, spore-forming bacterium *Clostridium* sp. has been reported (Francis *et al.* 2008b). *Clostridium* sp., ubiquitous in soils and wastes, is capable of reduction of Fe(III) to Fe(II), Mn(IV) to Mn(II), Tc(VII) to Tc(IV), and U(VI) to U(IV). The addition of Pu (1×10^{-5} M) had no effect upon growth of the bacterium. Commensurate with bacterial growth (as evidenced by glucose consumption, carbon dioxide and hydrogen production, a decrease in pH of the medium from 6.4 to 3.0 due to production of acetic and butyric acids from glucose fermentation, and a change in the Eh of the culture medium from +50 mV to -180 mV), Pu was rapidly solubilized as evidenced by an increase in Pu concentration in solution which passed through a 0.03 μ m filtration (Fig. 6.14). Selective solvent extraction of the culture by thenoyltrifluoroacetone (TTA) indicated the presence of a reduced Pu species in the soluble fraction. X-ray absorption near-edge spectroscopic (XANES) analysis of Pu in the culture sample at the Pu L_{III} absorption edge (18.054 keV) showed a shift of -3 eV compared to a Pu(IV) standard, indicating reduction of Pu(IV) to Pu(III). These results suggest that although Pu generally exists as insoluble Pu(IV) in the environment, under appropriate conditions anaerobic microbial activity could affect the long-term stability and solubility of Pu by its reductive dissolution.



6.14 Dissolution and reduction of ^{242}Pu (IV) to Pu(III) by *Clostridium* sp. Comparison of the sample absorption edge position at (18.059 keV) with that for Pu(IV)-nitrate (18.062 keV) showed a shift of -3 eV, confirming the presence of Pu(III) (Francis *et al.* 2008).

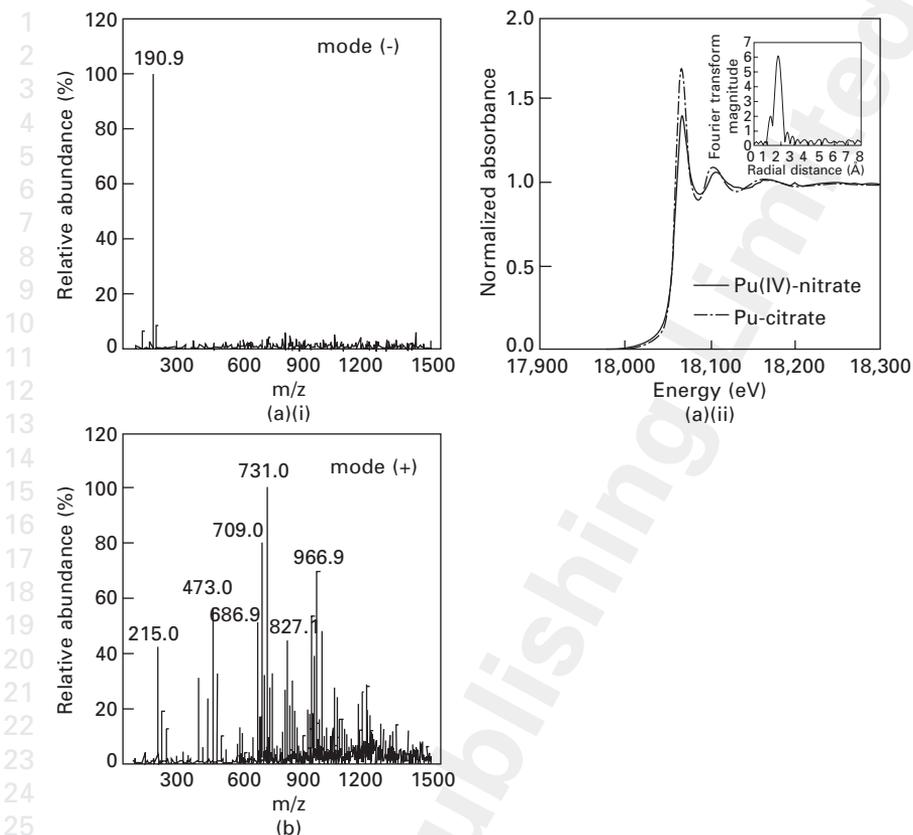
6.3.7 Biotransformation of plutonium complexed with organic compounds

Chelating agents are present in TRU and mixed wastes because they are widely used for decontaminating nuclear reactors and equipment, in cleanup operations, and in separating radionuclides. Plutonium forms very strong complexes with a variety of naturally occurring organic complexing agents, such as humic and fulvic acids, microbially produced complexing agents, such as formate, lactate, oxalate, tartrate, citrate, and siderophores, as well as synthetic chelating agents (NTA, EDTA) all of which can affect the mobility of Pu in the environment. Biodegradation should precipitate the released ions as water-insoluble hydroxides, oxides, or salts, thereby retarding their migration. Little is known of the biotransformation of Pu complexed with natural organic compounds and chelating agents.

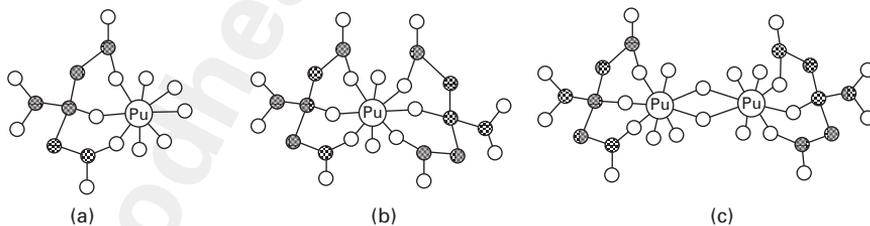
6.3.8 Biodegradation of Pu(IV)-citrate complexes

The Pu(IV)-citrate complex was characterized by ESI-MS, XANES and EXAFS to determine the oxidation state of the Pu and the nature of Pu complexation with citric acid (Francis *et al.* 2006). Pu was present in the tetravalent form predominantly as the mononuclear biligand complex, as well as the mononuclear monoligand and binuclear biligand complex also being present (Figs 6.15 and 6.16).

Citric acid (10^{-4} M) in the absence of ^{242}Pu was metabolized completely at a rate of $4.9 \mu\text{M}/\text{h}$. With 10^{-6} and 10^{-8} M ^{242}Pu present as the Pu-citrate complex, a slight decline of the rate and extent of citrate degradation was



6.15 Electrospray ionization–mass spectrometry (ESI–MS) of ^{242}Pu -citrate at pH 6.5 ((a), (b)) showed formation of mononuclear ($m/z = 473.0$), biligand ($m/z = 731.0$) and dinuclear ($m/x = 966.9$) complexes. XANES ((a)(ii)) and EXAFS ((a)(ii) insert) analysis showed Pu(IV) oxidation state and Pu-citrate predominantly as a mononuclear complex (Francis *et al.* 2006).



6.16 Proposed structures at pH 6 for (a) the monoligand 1:1 Pu: citric acid complex, (b) the biligand 1:2 Pu: citric acid complex $[\text{PuO}(\text{cit})_2]^{4-}$, and (c) the dimeric 2:2 Pu: citric acid complex. The open circles represent oxygen and the filled circles represent carbon atoms (Francis *et al.* 2006).

observed in comparison to the sample lacking Pu. During biodegradation a significant amount of Pu was released as a particle-reactive species that adsorbed to the cells ($>0.4 \mu\text{m}$), and possibly as a colloidal species ($>0.03 \mu\text{m}$).

6.3.9 Mobilization of Pu from contaminated soil due to microbial activity

Plutonium was solubilized due to indigenous microbial activity in a Pu-contaminated soil collected from Area 11 at the Nevada Test Site (NTS). In the citric acid-amended sample, Pu-concentration increased with time and subsequently decreased to below detection levels when the citric acid was completely consumed. In contrast, with glucose amendment, the concentration of Pu increased with the consumption of the carbon source and it remained in solution throughout the incubation. Pu speciation studies suggest that it exists in mixed oxidation states (III/IV) in a polymeric form as colloids. Although Pu(IV) is the most prevalent and is generally considered to be the more stable chemical form in the environment, these results suggest that microbial activity under aerobic and/or anaerobic conditions can affect the stability and mobility of Pu in contaminated environments and in radioactive wastes. Additional studies are needed to fully evaluate the role of microbes in the mobilization and immobilization of Pu in the contaminated environment and in the wastes.

6.4 Biosorption and bioaccumulation of uranium and plutonium

Biosorption and bioaccumulation of radionuclides has been observed in a wide range of microorganisms. It is still one of the most intensely investigated areas because of the potential use of biomass to remove radionuclides from waste streams. Bacterial cell walls, exopolymers, proteins, and lipids contain carboxylate, phosphate, amino, and hydroxyl functional groups which bind to metals and radionuclides. 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.

Biosorption processes essentially are chemical ones whereby the biomass acts as a surface upon which metals bind by ligand interactions or by ion exchange. Metal ions in relatively high quantities are retained by 'passive' sorption and/or complexation. Living and dead microorganisms possess

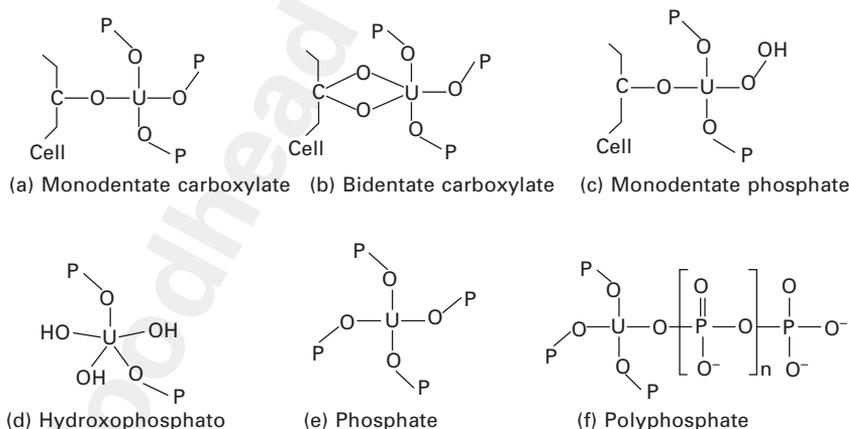
1 abundant functional groups, such as carboxyl, hydroxyl and phosphate, on
 2 their surface that bind metal ions (Fig. 6.17). Polymers secreted by many
 3 metabolizing microbes also immobilize metals. Desorption and recovery of
 4 the biosorbed radionuclides is easy. Radionuclide-binding to cell surfaces and
 5 polymers is a promising technology for remediating contaminated waters.

6 Biocrystallization, also called bioprecipitation or biomineralization, is the
 7 induction of metal precipitates and minerals by bacterial metabolism. Bacteria
 8 interact very strongly with metal ions and immobilize and concentrate them,
 9 eventually generating minerals. Microbial biofilms bind significant quantities
 10 of metallic ions naturally, and also serve as templates for the precipitation
 11 of insoluble mineral phases. The biochemistry of the interactions of metal
 12 ions with bacterial cell walls, extracellular biopolymers, and microfossil
 13 formations in immobilizing toxic metals is fairly well understood.

14 15 6.4.1 Uranium

16
17 Extracellular and intracellular association of U with bacteria was observed
 18 but the extent of its accumulation differs greatly with the species of bacteria.
 19 Extracellular association of uranium with bacterial cell surfaces is primarily
 20 due to physical and chemical interactions involving adsorption, ion exchange,
 21 and complexation and does not depend on metabolism. Intracellularly,
 22 uranium binds to anionic sites or precipitates as dense deposits. Intracellular
 23 accumulation involves transporting the metal across the cell membrane, which
 24 depends on the cell's metabolism. The mechanism of intracellular transport
 25 of uranium into the cell has not been understood.

26 Potentiometric titration, nuclear magnetic resonance spectroscopy (NMR),
 27 time resolved laser fluorescence spectroscopy (TRLFS), extended X-ray



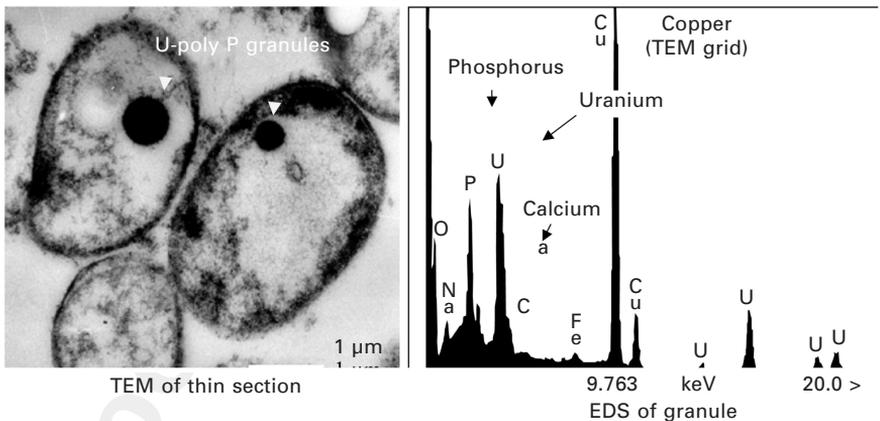
42 **6.17 Proposed predominant structures of uranium association with**
 43 **bacteria.**

absorption fine structure (EXAFS) and other spectroscopic techniques have been used to determine the functional groups involved in the complexation of uranium with bacteria.

Citrobacter sp. accumulated uranyl phosphate as polycrystalline HUO_2PO_4 at the cell surface. The phosphatase enzyme located on the cell surface of *Citrobacter* sp. cleaves glycerol-2-phosphate, liberating HPO_4^{2-} , causing precipitation of uranium. The cells have no saturation constraints and can accumulate several times their own weight of precipitated metal (Macaskie *et al.* 1992).

In *Halomonas* sp. U accumulated as electron-dense intracellular granules and was also bound to the cell surface (Fig. 6.18). 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 (Francis *et al.* 2004). 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 the 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



6.18 Extra- and intracellular accumulation of uranium by *Halomonas* sp. EDS shows U and O as major constituents of the intracellular granules (Francis *et al.* 2004).

1 bacterial species, and is determined in part by the rate at which it can be
2 degraded, for example in response to the presence of metals, and the amount
3 of inorganic phosphate secreted into the medium. In as much as all of the
4 uranium exposure studies reported were conducted with cells in the stationary
5 phase, the cells are responding to heavy metal stress by releasing phosphate
6 from the mineralization of cellular polyphosphate. In some studies reported
7 in the literature the cells were in fact incubated with uranium from several
8 hours to days. Under these conditions, the cells undergo lysis and release
9 inorganic phosphate (H_2PO_4^-) with the precipitation of uranium as uranyl
10 phosphate ($\text{UO}_2(\text{H}_2\text{PO}_4)_2$).

11 Uranium associated with the bacteria is not very stable, as it was removed
12 completely by Na_2HCO_3 from *Halomonas* sp., from an *Arthrobacter* sp. by
13 0.1 M NaHCO_3 , 0.1 M EDTA and Na_2CO_3 , and from *Bacillus* strains by EDTA.
14 Although bacteria possess a variety of functional groups, studies suggest that
15 cellular phosphate is the predominant functional group complexing with U.
16 Inorganic phosphate generated inside cells during starvation or under stress
17 bind U and other cations. EXAFS analysis showed that U is predominantly
18 complexed to phosphate with whole cells, whereas the lysed cells show
19 bidentate carboxylate bonding in addition to phosphate. SEM, TEM, and EDS
20 analyses of the bacterial cells exposed to uranium showed intracellular and
21 extracellular accumulation of uranium. EXAFS analysis showed the uranium
22 was associated to varying degree with phosphate: *Halobacterium halobium*
23 and *Bacillus subtilis* showed diphosphate bonding to uranium, *Halomonas*
24 *praevalens* showed monophosphate bonding to uranium, and uranium
25 was associated as uranyl phosphate or polyphosphate with *Pseudomonas*
26 *fluorescens*. Whole cells of *Halomonas* sp. had monophosphate bonding to
27 uranium, while the cell walls exhibited monophosphate as well as bidentate
28 carboxylate bonding to uranium.

29

30

31

6.4.2 Uranium association with biofilms

32 In natural and engineered settings, bacteria predominantly grow as biofilm
33 communities. Cells in a biofilm exhibit enhanced resistance and tolerance to
34 toxic metals compared with free-living ones. Granular biofilms consisting of
35 mixed bacterial species removed and immobilized uranium and chromium
36 from minimal media under anaerobic conditions. XPS analysis showed the
37 association of uranium with the granular biomass; XANES analysis revealed
38 the reduction of soluble Cr(VI) to Cr(III); and EXAFS analysis of the Cr-
39 laden granular biofilms demonstrated similarity to Cr(III) phosphate. These
40 studies demonstrate the potential application of granular biofilm-based
41 systems in treating metal-containing effluents and wastewater (Nancharaiah
42 *et al.* 2011).

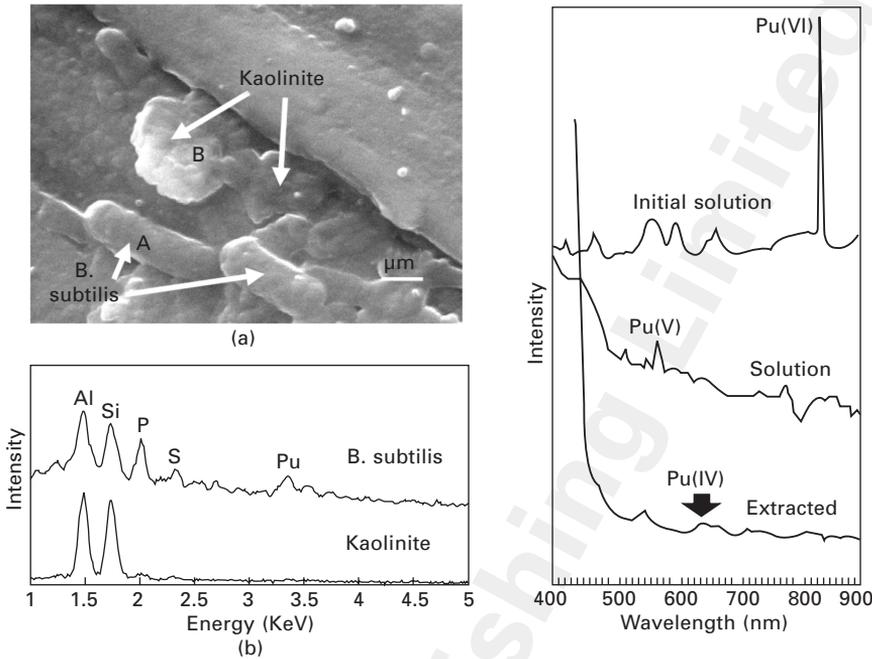
43 Several fungi solubilized UO_3 and U_3O_8 and subsequently accumulated

uranium in the mycelium. Accumulated uranium was biomineralized as well-crystallized uranyl phosphate minerals of the meta-autunite group with abundant uranium precipitates being found in the mycelium and encrusting the hyphae (Gadd and Fomina 2011).

6.4.3 Plutonium

Sorption studies of Pu(VI) with bacterial biomass have shown that the interaction with bacteria can cause changes in the oxidation state (Panak and Nitsche 2001; Renshaw *et al.* 2009) and most of the Pu(VI) was reduced to Pu(IV) and bound to the phosphate groups on the cell surface (Panak *et al.* 2002). The added Pu(VI) was sorbed to the bacterial cell surface through complexation with phosphate groups of the cells. Pu(VI) is reduced to Pu(V) with the dissolution of Pu(V) which disproportionated to Pu(IV) and Pu(VI) and subsequent complexation of Pu(IV) with the biomass (Panak and Nitsche 2001). Similar results were observed with *D. aespoeensis* and the cells immobilized Pu as Pu(IV) polymers (Moll *et al.* 2006).

Ohnuki *et al.* (2007b, 2010) investigated the interactions of U(VI) and Pu(VI) with *Bacillus subtilis*, kaolinite clay, and within a mixture of the two, directly analyzing their association with the bacterium in the mixture by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The accumulation of U by the mixture rose as the numbers of *B. subtilis* cells increased. Treating the kaolinite with potassium acetate (CH_3COOK) removed approximately 80% of the associated uranium while only 65% was removed in the presence of *B. subtilis*. TEM-EDS analysis confirmed that most of the U taken from solution was associated with *B. subtilis*. XANES analyses revealed that the oxidation state of uranium associated with *B. subtilis*, kaolinite, and with the mixture containing both was U(VI). The amount of Pu sorbed by *B. subtilis* increased with time, but did not reach equilibrium in 48 h; in kaolinite alone, equilibrium was attained within 8 h. After 48 h, the oxidation state of Pu in the solutions exposed to *B. subtilis* and to the mixture had changed to Pu(V), whereas the oxidation state of the Pu associated with both was Pu(IV). In contrast, there was no change in the oxidation state of Pu in the solution nor on kaolinite after exposure to Pu(VI). SEM-EDS analysis indicated that most of the Pu in the mixture was associated with the bacteria (Fig. 6.19). These results suggest that U(VI) and Pu(VI) preferentially are sorbed to bacterial cells in the presence of kaolinite clay, and that the mechanism of accumulation of U and Pu differs. U(VI) is sorbed directly to the bacterial cells, whereas Pu(VI) first is reduced to Pu(V) and then to Pu(IV), and the latter is associated with the cells. These results have important implications on the migration of radionuclides around the repository sites of geological disposal. Microbial cells compete with clay colloids for radionuclide accumulation, and because of their higher



6.19 Interaction of Pu with bacteria and kaolinite clay. SEM-EDS (a, b) and UV-vis analyses of the bacteria and clay mixture showed that added Pu(VI) was reduced to Pu(IV) and preferentially associated with bacteria (Ohnuki *et al.* 2007 b).

affinity and larger size, the microbes accumulate radionuclides and migrate much more slowly than do the clay colloids. Additionally, biofilm coatings formed on the fractured rock surfaces also accumulate radionuclides, thereby retarding radionuclide migration.

Lichens play an important role in the terrestrial food chain and cycling of elements. Uptake studies of $^{239}\text{Pu(VI)}$ and U(VI) by the lichen *Parmotrema tinctorum* showed that the accumulated Pu was evenly distributed on the upper and lower surfaces of *P. tinctorum*, in contrast to U(VI) , which accumulated in both cortical and medullary layers. UV/vis absorption spectroscopy showed that a fraction of Pu(VI) in the solution was reduced to Pu(V) by the organic substances released from *P. tinctorum*, and the accumulated Pu on the surface is reduced to Pu(IV) as Pu(IV) hydroxide, while the oxidation state of U(VI) remained unchanged. Since the solubility of Pu(IV) hydroxides is very low, it did not penetrate to the medullary layers, but is probably precipitated as Pu(IV) hydroxides on the cortical lichen surface (Ohnuki *et al.* 2004).

6.5 Biotransformation of other actinides and related elements

6.5.1 Neptunium

Neptunium-²³⁷ (²³⁷Np) is the most prevalent isotope of neptunium. It has a half-life of 2.14×10^6 years and decays by emitting an alpha particle. It is one of the minor actinides generated from the reprocessing of nuclear fuels. Neptunium has four oxidation states—3, 4, 5, and 6—and exists in solution under oxidizing conditions primarily as the Np(V)O₂⁺ species, which forms stable carbonate complexes. Under acidic conditions NpO₂⁺ disproportionates to Np(IV) and NpO₂²⁺. 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 NpO₂⁺, are similar to those of simple monovalent cations, i.e., low ligand-complexing abilities with a high environmental mobility. The neptunyl species NpO₂⁺, which is mobile and non-sorptive, is biologically reduced to insoluble Np⁴⁺ under anaerobic conditions (Lloyd *et al.* 2000b; Nagaoka 2005; Icopini 2007; Rittmann *et al.* 2002). Microbially enhanced chemisorption of Np has been reported (Macsakie and Basnakova 1998). Complexation of Np(IV) by fermentation intermediate products prevented its precipitation (Rittmann *et al.* 2002). Although Np⁴⁺ is easily oxidized in solution, it is stabilized in the presence of complexing ligands. For example, *Shewanella putrefaciens* reduced Np⁵⁺ to Np⁴⁺ which then was precipitated from solution as Np⁴⁺ phosphate (Lloyd *et al.* 2000b). Reduction of Np(V) to Np(IV) by cell suspension of *S. putrefaciens* MR-1 (Icopini 2007) and by the sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Nagaoka, 2005) have been reported. Neptunyl (NpO₂⁺), which is generally thought to be non-sorptive, showed significant sorption by *Pseudomonas fluorescens* cells (Songkasiri *et al.* 2002). XANES analysis of Np associated with the cells showed no reduction of Np(V). Significant sorption of Np(V) was observed with whole cells and cell components of *Shewanella alga* due to complexation with carboxyl and N-containing carboxyl ligands (Deo *et al.* 2010). This is in contrast to previous studies which showed negligible uptake of Np by *P. aeruginosa*, *Streptomyces viridochromogenes*, *Scenedesmus obliquus*, and *Micrococcus luteus* (Strandberg and Arnold 1988). Francis *et al.* (1998) observed large variation in Np(V) adsorption, 12–37% being associated with bacterial cells versus 30% adsorption recorded by Strandberg and Arnold (1988). Songkasiri *et al.* (2002) observed 15–20% Np adsorption to *Pseudomonas fluorescens* at pH 7. Anderson *et al.* (2007) reported that ²³⁷Np(V) adsorption at pH 7.5 was under 10% on rock surfaces and even lower on biofilm surfaces. Low sorption can also be attributed to the neptunyl ion, NpO₂⁺, which is a large ion with a single charge, so it is expected to be weakly complexed by anions. Np(V) was reduced to poorly soluble Np(IV)

1 in sediments by indigenous microorganisms under anaerobic conditions
2 and the sediment associated Np(IV) was somewhat resistant to oxidative
3 remobilization (Law *et al.* 2010). These studies suggest that the stability
4 of the reduced Np(IV) is influenced by the nature of the mineralogical
5 association in the sediment.

6.5.2 Americium

9 The most common isotopes of americium, ^{241}Am and ^{243}Am , have half-lives
10 of 432.2 and 7370 years, respectively. Americium is found in the areas used
11 for the atmospheric nuclear weapons tests between 1945 and 1980, as well as
12 at the sites of nuclear incidents, such as the Chernobyl disaster. Americium
13 can exist in multiple oxidation states, Am(III, IV, V, VI and VII). Am(III)
14 is the most stable and predominant form, and exists depending on pH and
15 water composition as soluble aquo ion, hydroxo complexes and carbonate
16 complexes such as AmCO_3^+ , $\text{Am}(\text{CO}_3)_2^-$, and $\text{Am}(\text{CO}_3)_3^-$. Microbial activity
17 may convert $\text{Am}(\text{OH})^{2+}$ species to Am(III)-carbonate complexes due to carbon
18 dioxide production; likewise, biodegradation of Am(III)-organic complexes
19 may precipitate Am as the hydroxide species.

20 Although Am has no known biological function, it has been proposed
21 to use bacteria for removal of Am and other heavy metals from rivers and
22 streams, for example by using *Citrobacter* bacteria to precipitate Am ions
23 with phosphate from aqueous solutions and then binding the metal-phosphate
24 complex at the cell walls (chemisorption) (Macaskie *et al.* 1994). Several
25 studies have also been reported on the biosorption and bioaccumulation of
26 americium by bacteria and fungi (Wurtz *et al.* 1986; Francis *et al.* 1998; Liu
27 *et al.* 2002). Uptake of Pu and Am by the freshwater bacterium *Aeromonas*
28 *hydrophila* (Giesy *et al.* 1977) and in nutrient media by *Escherichia coli*
29 and selected strains of marine facultative anaerobes accumulated significant
30 amounts of ^{241}Am (Wurtz *et al.* 1986). Bacterial uptake of water-soluble Am
31 appears to be primarily due to adsorption to the cell's surface; adsorption
32 is reversible and depends upon the nutrients present, the physiological state
33 of the cell, the pH, and the release of extracellular metabolic products.
34 Bacteria isolated from sediments and grown with ^{241}Am in minimal medium
35 produced exometabolites which formed soluble complexes with Am (Wurtz
36 *et al.* 1986). Thus, the potential exists for dissolution of Am in wastes by
37 microorganisms, thereby increasing its bioavailability and mobility.

6.5.3 Curium

41 Curium is usually present as Cm^{3+} in solution. Curium chemistry resembles
42 very closely that of americium. It forms strongly fluorescent complexes with
43 various organic compounds, but studies on the interaction between Cm and

microbes are limited. There is no evidence of its incorporation into bacteria. Time-resolved laser-induced fluorescence spectroscopy (TRLFS) is a very sensitive tool for directly determining the Cm speciation in biological samples because of the excellent luminescence properties of Cm³⁺. Biosorption of Cm(III) on the surface of the microorganisms *Chlorella vulgaris*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Halomonas* sp., *Halobacterium salinarum*, *Halobacterium halobium* and *Paracoccus denitrificans* (Ozaki *et al.* 2004, 2006) and sulfate-reducing bacteria *Desulfovibrio aespoensis* (Moll *et al.* 2004) has been reported. The organic compounds in the exudates of microorganisms affected the biosorption and coordination of Cm with the bacterial cells. The exuded organic carbon desorbed Cm(III) from the cell surfaces (Ohnuki *et al.* 2007a).

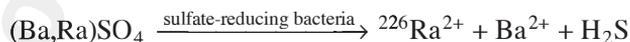
6.5.4 Radium

Radium is a naturally occurring radionuclide formed by the decay of uranium and thorium in the environment. It occurs at low levels in virtually all rock, soil, water, plants, and animals. Radium-226, the most common isotope, is an alpha emitter, with accompanying gamma radiation, and has a half-life of about 1600 years. Microorganisms can indirectly affect the radium mobility in the environment.

Remobilization of coprecipitated radium by sulfate-reducing bacteria

The effects of sulfur-oxidizing bacteria on the release of ²²⁶Ra²⁺ 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 ²²⁶Ra²⁺ in the effluent. At many uranium mining and milling sites, soluble radium is removed as a coprecipitate with BaSO₄ by the addition of BaCl₂ to sulfate-rich tailing effluents. The resulting (Ba,Ra)SO₄ precipitate is allowed to settle, yielding a radioactive sludge and a supernatant low in ²²⁶Ra²⁺ for discharge to the environment (McCready *et al.* 1980).

The disposal of radioactive sludges must ensure that ²²⁶Ra²⁺ 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 usable carbon source with the reduction of SO₄²⁻ to H₂S and concurrent release of Ba²⁺ and ²²⁶Ra²⁺ (Fedorak *et al.* 1986):



These results suggest that ultimate disposal of these wastes should ensure

1 that they are maintained under aerobic conditions to minimize the activity
2 of sulfate-reducing bacteria.

3

4 6.5.5 Thorium

5
6 The most common form of thorium is thorium-232, found naturally at very
7 low levels in soil, rocks, and water. Soil commonly contains an average of
8 around 12 ppm of thorium. Thorium occurs in several minerals including
9 thorite (ThSiO_4), thorianite ($\text{ThO}_2 + \text{UO}_2$) and monazite. Thorianite is a rare
10 mineral and may contain up to about 12% thorium oxide. Monazite, a rare
11 earth and thorium phosphate mineral, is the primary source of the world's
12 thorium. There is growing interest in using Th as nuclear fuel. Waste products
13 such as mill tailings produced from mining and refining activities may result
14 in the contamination of the water and the environment. While we have a
15 greater understanding of the contamination of the environment due to uranium
16 mining waste, very little is known of the impacts of Th mining and processing.

17 Thorium compounds are stable in the +4 oxidation state. Th(IV) readily
18 hydrolyzes in water and easily forms colloids and polynuclear species.
19 Thorium forms complexes with citric acid (Bobtelsky and Graus 1954) very
20 similar to U(IV)- and Pu(IV)-citrate. Little is known of the biotransformation
21 of Th-citrate or other Th-organic complexes. The role of microorganisms
22 in the dissolution of Th and rare earth elements from monazite ore has not
23 been investigated. Also the biotransformation of Th by autotrophic and
24 heterotrophic microorganisms and the presence and fate of Th-organic
25 complexes is not known. Biosorption of Th by microbial biomass has been
26 investigated (Tsezos and Volesky 1981). Complexation of Th by metabolic
27 products produced by *Pseudomonas aeruginosa*, grown in its presence of have
28 been reported (Premuzic *et al.* 1985). *In situ* biofilms affect the adsorption
29 and immobilization of Th. The amount of $^{234}\text{Th}^{4+}$ that adsorbs to biofilms
30 at the Äspö Hard Rock Laboratory (HRL) in Sweden is extremely variable,
31 reaching up to 75% of the available ^{234}Th (excluding colloidal-bound Th),
32 though it is usually 5- and 10-fold lower than the amount that adsorbs to rock
33 surfaces (Anderson *et al.* 2007). The bacterial adsorption figure from Äspö
34 is similar to that reported by Francis *et al.* (1998), where 74% of the Th was
35 associated with bacterial cells. Sorption of Th to bacteria has been noted to
36 decrease at higher pH levels by Sar *et al.* (2004), who demonstrated that the
37 maximum for Th sorption is at pH 4, beyond which sorption decreases.

38

39 6.6 Biotransformation of fission and activation 40 products

41

42 Fission and activation products produced from nuclear reactors, nuclear
43 weapons testing, and nuclear power plant accidents include ^{99}Tc , ^{79}Se ,

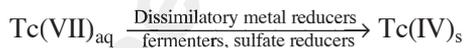
^{60}Co , ^{135}Cs , ^{129}I , tritium, and carbon-14. These radionuclides can undergo extensive microbiological transformations resulting in the mobilization or immobilization in the environment or in the nuclear wastes disposed of in shallow-land and deep geological formations.

6.6.1 Technetium

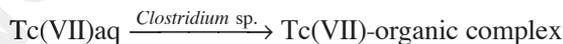
Technetium is produced in large quantities by the fission of ^{235}U during nuclear power generation and defense-related activities, including nuclear testing and reactor operations. There are 19 known isotopes with mass numbers from 92 to 107 with half-lives ranging from a few seconds to 2.1×10^5 years. Tc can exist in oxidation states 0, +3, +4, +5, +6, and +7; however, the predominant ones of environmental concern are 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 under environmental conditions. Tc(V) and Tc(VI) undergo disproportionation reactions to form Tc(IV) and Tc(VII) (Yoshihara 1996). Microorganisms affect the dissolution or precipitation of Tc by oxidation–reduction reactions and by complexation with organic by-products and macromolecules.

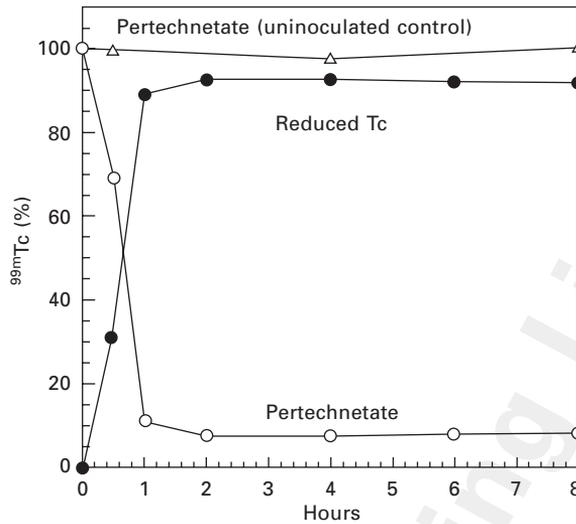
The dominant oxidized form of Tc(VII) is the pertechnetate (TcO_4^-) anion, which is highly soluble in water. It is reduced to technetium(IV) hydrous oxides, such as $\text{TcO}_2 \cdot n\text{H}_2\text{O}$, either chemically or biologically. A wide variety of microbes have been shown to enzymatically reduce soluble Tc(VII) to Tc(IV) solid under anaerobic conditions. Microbially generated ferrous iron and sulfide are known to indirectly reduce Tc (Lloyd *et al.* 2000a; Zachara *et al.* 2007).

Chemolithotrophic, haloalkaliphilic, aerobic, facultative, and anaerobic (fermentative and sulfate-reducing) bacteria reduced pertechnetate ion to an insoluble form (Lloyd and Macaskie 1996; Lyalikova and Khizhnyak 1996; Gearing *et al.* 1975; Pignolet *et al.* 1989; Henrot 1989; Tagami and Uchida 1996; Peretrukhin *et al.* 1996; Wildung *et al.* 2000; Francis *et al.* 2002; Khijniak 2003):



Clostridium sp reduced Tc(VII) to Tc(IV) under anaerobic conditions (Fig. 6.20). 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 (Francis *et al.* 2002).





6.20 Reduction of pertechnetate by *Clostridium sphenoides* (Francis *et al.* 2002).

Solubility of Tc(IV) in the presence of organic ligands

Reductive precipitation of Tc(IV) oxides ($\text{TcO}_2(\text{s})$) is an effective means of immobilizing Tc. However, under anoxic conditions Tc(IV) oxides are subject to dissolution by complexing agents such as ethylenediaminetetraacetate (EDTA), diethylenetriamine pentaacetic acid (DTPA), oxalate, citrate, humic acid and fulvic acids (Gu *et al.* 2011). A substantial amount of reduced Tc is associated with bacterial cells and with bacterial macromolecules, most probably as an organic complex (Francis *et al.* 2002). Technetium(IV) bound to the cells, associated with macromolecules and complexed with organic ligands, could affect the mobility. The nature and stability of the Tc-organic complexes is not fully known. Studies have shown that carboxyl groups are among the most important functional groups for complexing Tc(IV) and other actinide ions by forming binuclear compounds. Reduced Tc(IV) was re-oxidized rapidly under oxic conditions (Gu *et al.* 2011).

Speciation of microbially reduced Tc

The predominant reduced Tc species identified include TcO_2 , $\text{Tc}(\text{OH})_4$ and 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_2 , whereas with non-sulfate reducers it is present as Tc oxide and hydroxide species. Technetium absorption by soil has been attributed to soil microbial activity. About 98% of technetium is absorbed within two to

five weeks by soils and sterilization of the soil eliminated this absorption. Peretrukhin *et al.* (1996) 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. Tc may be present in 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.

6.6.2 Selenium

Selenium-79 is present in spent nuclear fuel and in reprocessing wastes. Its yield is low (about 0.04%) and it has a half-life of 327,000 years. ⁷⁹Se would be released from the spent fuel or vitrified waste as soluble selenate. Selenium occurs naturally as a trace element in most soils, rocks and waters, and it accompanies sulfur in volcanic effluents. It is found in a number of inorganic forms, including selenide, selenate, and selenite. Selenium is an essential micronutrient for many organisms, is known to protect cell membranes against oxidative damage, and can be easily bioconcentrated in the food chain. In the presence of nitrate, the reduced forms of selenium are oxidized and mobilized.

Microorganisms play a major role in the transformation of Se. The pathways and mechanisms involved in the cycling of Se in the environment have been extensively investigated (Dowdle and Oremland 1998; Dungan and Frankenburger 1999; Stolz *et al.* 2006; Alexander 1977; Ehrlich and Newman 2009). A wide variety of bacteria and fungi are involved in (1) the oxidation of elemental selenium to selenite and selenate, (2) the reduction of selenite and selenate to elemental selenium, and (3) the methylation of selenium to dimethylselenide (CH₃)₂Se and dimethyldiselenide (CH₃)₂Se₂. Isotopic enrichment of Se depending on the microbial species and selenium species has been reported. The dissimilatory reduction of Se(VI) via Se(IV) to Se(0) has been shown to be a significant and rapid environmental process.

In contrast to sulfate reduction in which the final product is H₂S, elemental selenium, rather than hydrogen selenide, H₂Se, accumulates. Numerous bacteria can reduce Se oxyanions, which are used as electron acceptors during the oxidation of organic matter in anoxic environments. Methylation of selenium is carried out by a variety of bacteria and fungi (Stolz *et al.* 2006; Peitzsch *et al.* 2010). Dimethyl selenide is released from soil into the atmosphere and the volatilization of the methylated compound is pronounced when the microflora is provided with a methyl donor (such as methionine or cysteine), readily available carbon and selenate, selenite and seleno-amino acids.

Although no studies have been conducted with ⁷⁹Se, it is expected that the transformation, fate, and transport of ⁷⁹Se in nuclear wastes and in

1 contaminated environments are similar to those of naturally occurring Se in
2 terrestrial and aquatic environments (Peitzsch *et al.* 2010). Investigations by
3 Vandergraaf *et al.* (1997) of $^{75}\text{Se}(\text{IV})$ adsorption to biofilm-coated granite
4 coupons in flow cells indicated that Se sorption is attenuated but not retarded
5 by biofilms; radiometric analysis indicated that 10% adsorbed to naked rock
6 surfaces while only 1% sorbed to biofilm surfaces, suggesting that biofilms
7 are an effective barrier preventing the sorption of Se to granite.

8 9 6.6.3 Strontium

10
11 Strontium-90 is a by-product of the fission of uranium and plutonium in
12 nuclear reactors, and in nuclear weapons. It is found in waste from nuclear
13 reactors and in contaminated reactor parts and fluids. Strontium-90 has a
14 half-life of 29.1 years. It behaves chemically much like calcium, and therefore
15 tends to concentrate in the bones and teeth.

16 Strontium-binding activity in *Micrococcus luteus* is localized on the
17 cell envelope and is sensitive to pretreatment. Bound Sr can be displaced
18 by chelating agents, divalent cations or H^+ . Other monovalent cations are
19 less effective at displacing Sr. Strontium binding in *M. luteus* is reversible,
20 though both ion exchange, mediated by acidic cell surface components, and
21 intracellular uptake may be involved (Faison *et al.* 1990). Mixed cultures of
22 bacteria isolated from low-level radioactive waste leachates preferentially
23 accumulated ^{85}Sr in mineral salts medium containing a mixture of radionuclides
24 (Francis 1990). The bacteria accumulated $^{85}\text{Sr} > ^{60}\text{Co} > ^{137}\text{Cs}$.

25 Immobilization of Sr as SrCO_3 by bacteria has been reported (Anderson
26 and Appanna 1994). *Pseudomonas fluorescens* when grown in medium
27 containing Sr-citrate metabolized citrate and precipitated Sr as crystalline
28 SrCO_3 due to production of CO_2 from citrate metabolism. This study shows
29 the potential microbial immobilization of Sr in contaminated environments.
30 The bacterium also metabolized yttrium-citrate with the precipitation of
31 yttrium as yttrium phosphate (Appana and Huang 1992).

32 In soils ^{90}Sr is likely to be present as an exchangeable form or bound to
33 soil organic matter, iron (hydr)oxides, or insoluble carbonate or phosphate.
34 Microorganisms can affect the association of the above-mentioned forms of
35 Sr in soils in the following way: (1) dissolution of carbonate and phosphate
36 phases, clays, and other minerals due to production of organic acids and
37 sequestering agents, (2) reductive dissolution of iron and the release of Sr
38 associated with the iron oxides, (3) biodegradation of the organic carbon
39 associated Sr fractions, and (4) immobilization due to precipitation reactions,
40 i.e., formation of strontium carbonate, microbial formation of strontium
41 calcite phase and by biomass/exopolymers.

6.6.4 Cesium

The radioactive isotopes ^{135}Cs and ^{137}Cs , with half-lives of 30.17 and 2.3 million years respectively, are produced from nuclear fission. Small amounts of ^{134}Cs and ^{137}Cs were released into the environment during all nuclear weapon tests and some nuclear accidents. ^{137}Cs is the principal source of radiation in the zone of alienation around the Chernobyl nuclear power plant. It was also found in the plumes emanating from the leakage at the Fukushima reactors in the Japan earthquake incident in 2011.

Cesium is a structural analog for potassium and physiologically behaves like the K^+ ion. Bioaccumulation of Cs by several microorganisms has been reported; consequently, there is considerable interest in using microorganisms to remove radioactive Cs from waste streams and contaminated sites. Mixed cultures of bacteria isolated from low-level radioactive waste leachates accumulated $^{85}\text{Sr} > ^{60}\text{Co} > ^{137}\text{Cs}$ in mineral salts medium containing a mixture of radionuclides (Francis 1990). Microbial cultures concentrated ^{137}Cs and ^{226}Ra less than uranium (Strandberg *et al.* 1981).

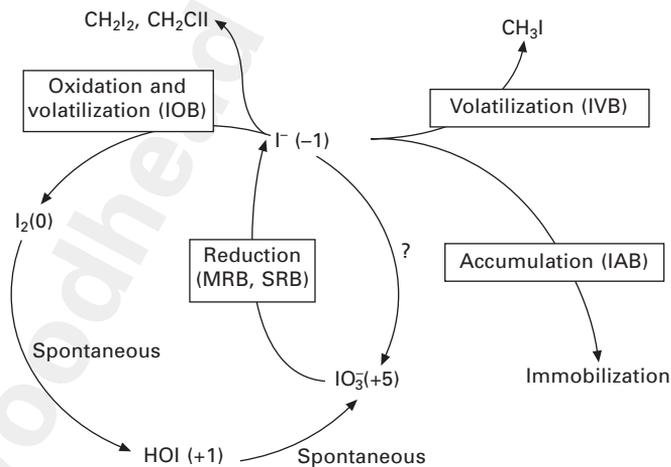
Cesium-accumulating bacteria isolated from soil displayed the rod-coccus growth cycle and contained mesodiaminopimelic acid, mycolic acids, and tuberculostearic acids (Tomioka *et al.* 1992). Cesium uptake was optimal at pH 8.5. Potassium and rubidium inhibited Cs accumulation, suggesting that Cs is taken up through the potassium transport system (Tomioka *et al.* 1994).

Ohnuki *et al.* (2003) examined the accumulation and distribution of Cs in yeast cells (*Saccharomyces cerevisiae*) in the presence of other elements by the micro-PIXE (particle induced X-ray emission) and by energy dispersive spectroscopy (EDS) coupled to a scanning electron microscope. Micro-PIXE analysis of cells grown in the presence of Cs showed that Cs was uniformly distributed in the cells and PIXE revealed the presence of Cs, P, K and Fe, whereas only P and S were detected by the EDS. Cells exposed to Cs showed an increase in Cs peak intensity, and a decrease in P, K and Fe with time. However, the nature of the association of Cs with the cells (extra- or intracellular) remains unclear, as does the long-term fate of biomass associated Cs.

6.6.5 Iodine

Iodine-129 (half-life 1.57×10^7 years) is one of the most persistent radionuclides released into the environment from nuclear power plant accidents, notably Chernobyl and most recently Fukushima, and spent nuclear fuel reprocessing 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

1 processes such as volatilization (CH_3I), oxidation of I^- to I_2 , reduction of IO_3^- to
 2 I^- , and bioaccumulation by bacterial cells both intracellularly and extracellularly
 3 (Amachi 2005; Fig. 6.21). Microbial volatilization of organic iodine was
 4 observed in soil slurries and seawater samples by aerobic bacteria through
 5 methylation of iodide (I^-) to form methyl iodide (CH_3I). The volatilization
 6 of iodide was also found in iodide-rich natural brine water. In addition to
 7 the organic iodine compounds, a significant amount of molecular iodine (I_2)
 8 was produced. Axenic cultures of bacteria produced diiodomethane (CH_2I_2)
 9 and chloriodomethane (CH_2ClI). Iodide-oxidizing bacteria, which oxidize
 10 I^- to I_2 , were isolated from seawater and natural brine water. Sulfate-reducing
 11 bacteria *Desulfovibrio desulfuricans* and metal-reducing bacteria *Shewanella*
 12 *putrefaciens* have been shown to reduce iodate to iodide. Iodate (IO_3^-) is
 13 electrochemically or biologically reduced to I^- prior to uptake by rice plants.
 14 Changes in iodine redox states could have important effects on the mobility
 15 of iodine in natural systems. Conditions that are known to influence microbial
 16 activity and survival of microorganisms affected iodine sorption. Incubation
 17 of soil samples with varying levels of biomass, oxygen concentration, and
 18 soil water content showed iodine immobilization by soil microorganisms.
 19 Pure cultures of soil bacteria and fungi incorporated radioiodine (Bors and
 20 Martens 1992). Radioiodine (^{125}I) adsorption by soil treated with nutrient
 21 showed elevated levels of microbial biomass with increased adsorption of
 22 radioiodine. Anaerobiosis during the incubation period lowered adsorption.
 23 Migration of radioiodine in water-saturated soil columns was influenced by
 24 the quantity of microorganisms present. Soils high in organic substances
 25 and soil biomass exhibited higher radioiodine sorption compared to clay
 26 minerals, and the iodine sorption process was predominantly irreversible
 27 (Bors and Martens 1992).



6.21 Biotransformation of iodine (Amachi *et al.* 2005).

In terrestrial environments iodine concentrations accumulated in soils at $\sim 5 \text{ mg kg}^{-1}$ worldwide, 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}) (Amachi *et al.* 2005). Such high iodine accumulation in soils and sediments has been attributed at least in part to microbial effects, although the mechanism of the 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 (Amachi *et al.* 2005). 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 $3.54 \pm 3.80 \text{ } \mu\text{mol iodide per gram of bacteria}$. 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 (Amachi *et al.* 2005). 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 mechanisms of microbial transformations of iodine and the chemical speciation of the bioaccumulated iodine in bacteria and the stability of such species (Amachi 2008).

6.6.6 Carbon-14

Carbon-14 is a radioactive nuclide with a half-life of 5730 years. Apart from its natural and continuous production in the upper atmosphere, $^{14}\text{CO}_2$ is also being released directly from nuclear tests and nuclear power plants and being readily assimilated by plants through photosynthesis with a potential concentration in the food chain. The direct ^{14}C releases from nuclear power plants are dominated by gaseous emissions, mainly in the form of $^{14}\text{CO}_2$, with subsequent incorporation in plants by photosynthesis.

In addition, the spent ion exchange resins used for the purification of reactor water constitute solid radioactive waste and contain varying concentrations of ^{14}C as well as other radionuclides. Bacterial populations in a reactor-spent resin (cation, anion, and mixed resin) and in influent and effluent cooling water samples from the reactor at BNL were analyzed (Francis and Quinby 1981). Bacteria present in the resin and water samples, when incubated with the resin, produced CO_2 , albeit at a slow rate, indicating that these organisms may play a significant role in the degradation of the resin, the radiolytic decomposition products of the resin, or other organic compounds present in the resin, and may release $^{14}\text{CO}_2$, $^{14}\text{CH}_4$ and other carbon-14 compounds.

1 6.6.7 Tritium

2 The microbial oxidation of gaseous tritium to tritiated water has been studied
3 because tritiated water is more readily bioavailable than molecular tritium,
4 and may result in significant contamination of food and water. Consequently,
5 maximum permissible concentrations of HTO are about 100 times lower than
6 than for gaseous tritium. These studies have been undertaken in light of the
7 increasingly large atmospheric tritium discharges from nuclear fuel reprocessing
8 plants, development of nuclear fusion technology, radioactive waste leachate
9 evaporation practices at the disposal sites, and the nuclear industry.

10 Soils exposed to molecular tritium under laboratory conditions showed
11 that molecular tritium was converted to tritiated water within the soil column
12 (Murphy *et al.* 1976), and soil conditions that promoted soil microbial
13 activity increased tritiated water production. Soil sterilization greatly
14 decreased the conversion of molecular tritium, whereas reinoculation with
15 soil microorganisms restored the conversion of molecular tritium to tritiated
16 water. Exposure of natural (unsterilized) clay loam or of a sterilized soil
17 inoculated with a soil water extract yielded over 95% conversion of tritium
18 to tritiated water. Bacteria isolated from the soil were able to catalyze this
19 reaction in solution. Molecular tritium oxidation rates in various soils by
20 soil microorganisms ranged from 12% to 66% per hour, and were generally
21 independent of the soil type or soil chemical properties (McFarlane *et al.*
22 1979). The authors suggested that these rates are sufficiently rapid to account
23 for significant oxidation of HT if it were present in the environment. Fallon
24 (1982) studied the incorporation of gaseous tritium (T_2 , HT, or both) by
25 soils. The optimal temperature and pH values ranged from 20°C to 50°C,
26 and from pH 4 to 7, respectively, and the tritium metabolism declined at the
27 wet and dry extremes in soil moisture content. Hydrogenase enzymes have
28 been suggested to play a role in the metabolism of tritium (Fallon 1982).

29 McFarlane *et al.* (1978) and Murphy *et al.* (1976) demonstrated that elemental
30 tritium (gas) in the environment is metabolized by soil microorganisms, and
31 they produced tritiated water as the end product. Rapid exchange of gaseous
32 tritium into soil water can occur and should be taken into consideration in
33 soil uptake studies. T_2 -HT uptake in soils can occur by oxidation of $2HT +$
34 $O_2 \rightarrow 2HTO$, or through an exchange reaction involving no net transfer of
35 electrons, $H_2O + HT \rightarrow HTO + H_2$ (Anand and Krasna 1965; Adams *et al.*
36 1981; Klibanov and Huber 1981). *In vivo* studies with *Methanobacterium*
37 *thermoautotrophium* (Daniels *et al.* 1980; Spencer *et al.* 1981) show that the
38 exchange reaction can be up to 40 times more rapid than the net oxidation
39 reaction. The exchange reaction has an equilibrium constant $K = (HTO)(H_2)/$
40 $(H_2O)(HT) = 6.25$ (Anand and Krasna 1965; Klibanov and Huber 1981).
41 Tritium from HTO can exchange with exchangeable hydrogen atoms of
42 organic compounds with functional groups such as OH, —COOH, NH₂, and
43

NH and can be subsequently metabolized by microorganisms in the course of methane production. As an additional tritium source, bacteria are able to use tritium from tritiated water by enzymatic reactions in the methane biosynthesis.

Microbial generation of carbon-14 and tritiated gases

Radioactive gaseous compounds such as CH_3T , HTO , ^3H , and other tritiated hydrocarbons as well as ^{85}Kr , ^{222}Rn , $^{14}\text{CH}_4$ and other ^{14}C hydrocarbons have been identified as evolving from burial trenches from low-level waste disposal sites (Lu and Matuszek 1978; Husain *et al.* 1979). Often tritiated methane is one of the most abundant compounds, and it has been estimated that 200–6000 mCi/year of CH_3T is released to the environment (Matuszek 1980). Microorganisms play a significant role in the generation of radioactive gases directly through their metabolic activity, and indirectly enhance the release of trapped gases such as radon which result from the radioactive decay of radium. Particular attention is given to methanogenic bacteria because of anoxic conditions that prevail in the waste sites and their possible contribution to the release of carbon-14 and tritiated methane.

Gaseous compounds such as CO_2 and H_2 , generated by biological decomposition and/or radiolytic degradation of the wastes, may be reduced to CH_4 by methane bacteria under anaerobic environments if the radioactivity is not sufficient to inhibit bacterial activity. Tritium and carbon-14 released into the environment as tritiated and ^{14}C methane from the waste may also be oxidized to $^{14}\text{CO}_2$ and HTO once they reach the aerobic layer in the soil by microorganisms.

Complex organic materials can be degraded by microorganisms to simple organic acids, alcohols, aldehydes, ketones, esters, and gases such as H_2 , H_2S , CO_2 and CH_4 . In anaerobic environments, the methane-producing bacteria are the terminal organisms in the microbial food chain, and the organic acids, alcohols, H_2 , and other simple organic compounds serve as energy sources for the growth of these bacteria. For example, cellulose in an anaerobic environment is hydrolyzed to the disaccharide cellobiose by cellulolytic bacteria. Cellobiose is degraded by a variety of organisms, producing fatty acids and alcohols, which in turn are metabolized by a different group of organisms, producing acetate, formate, CO_2 , and H_2 as products. These simple compounds serve as substrates for methane bacteria which produce methane.

Release of other radioactive gases by microbial activity

Radon-222 is one of the naturally occurring radioisotopes and is a member of the ^{238}U decay series. When ^{226}Ra decays by α -emission, it transmutes to

1 its daughter ^{222}Rn , an inert gas having a half-life of 3.82 days. Radon-222
 2 may be formed in aquatic systems through an enrichment of ^{226}Ra , which
 3 is dissolved naturally in waters. The occurrence of ^{222}Rn associated with
 4 biologically produced gases such as N_2 , N_2O , CH_4 , and CO_2 from sediments
 5 in a production reactor cooling pond at the Savannah River Plant, Aiken,
 6 South Carolina, was investigated by Fliermans *et al.* (1978). Radon-222 is
 7 transported into the atmosphere by both physical and biological mechanisms,
 8 and the rates of ^{222}Rn flux by biological gassing appear to be less important
 9 than that released by physical transport processes from the terrestrial systems.
 10 In contrast, the biological production and transport processes may be
 11 significant in the overall flux or release of radioactive gases from low-level
 12 waste disposal, particularly from sites located in humid regions.

14 **6.7 Microbiological studies of low- and** 15 **intermediate-level wastes, and high-level waste** 16 **repository sites**

18 Microorganisms have been detected in low-level radioactive wastes, TRU
 19 wastes, Pu-contaminated soils, backfill materials, natural analog sites, and
 20 waste-repository sites selected for high-level radioactive wastes (Anderson
 21 *et al.* 2011; Amy *et al.* 1992; Pedersen *et al.* 1996; West *et al.* 1985, 1986,
 22 1992; Kieft *et al.* 1997; Haveman *et al.* 1995; Stroes-Gascoyne *et al.* 1997;
 23 Francis 1990, 2001; Gillow *et al.* 2001).

26 6.7.1 Microbial population distribution in radioactive 27 wastes and related sites

29 Low-level and TRU wastes contain low levels of Pu in addition to other
 30 radionuclides and organic compounds (Francis 1990). $^{238,239,240}\text{Pu}$ (gross
 31 alpha activity 6.3×10^3 Bq/L) was detected in leachate samples collected
 32 from the low-level radioactive waste disposal sites at West Valley, New
 33 York, and Maxey Flats, Kentucky (Husain *et al.* 1979; Weiss *et al.* 1979;
 34 Weiss and Colombo 1980; Cleveland and Rees 1981). Several aerobic and
 35 anaerobic bacteria were isolated from the leachate samples; among them
 36 were *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp.
 37 The radioactivity and the organic chemicals present in the leachate were not
 38 toxic to the bacteria, which metabolized them, thereby producing tritiated and
 39 carbon-14 methane (Francis *et al.* 1980a, b, c). Viable, metabolically active
 40 microbes were detected at the Los Alamos National Laboratory (LANL) TRU
 41 waste burial site containing ^{239}Pu -contaminated soil and flammable waste
 42 (Barnhart *et al.* 1979, 1980) and in high-level nuclear waste-contaminated
 43 vadose sediments at the Hanford Site (Fredrickson *et al.* 2004).

Both low-level and intermediate-level radioactive wastes contain a large portion of organic materials (e.g., cellulose) and a significant amount of inorganic nutrients (e.g., NO_3^-). Microbial degradation of these materials has been a major concern for long-term repository performance assessment. An example is the WIPP, which is located within a salt bed in southern New Mexico, and was designed for disposal of defense-related transuranic wastes. Microbial degradation of organic carbon-rich materials in the WIPP repository has been studied for its impact on repository pressurization and water chemistry (Gillow and Francis 2011; Wang *et al.* 1997). Unlike low-level and intermediate-level wastes, high-level radioactive wastes generally contain no organic materials and are thus not conducive to microbial activity.

6.7.2 Microbial gas generation and implications for radioactive waste disposal in the subterranean environment

Microorganisms play a significant role in the biodegradation of organic compounds present in LLW, ILW and TRU wastes. In general, the levels of radioactivity in the wastes are not toxic to microorganisms. Of particular concern is the gas generation due to decomposition of the wastes which may result in the container pressurization and loss of integrity, pressurization of containment areas in the repository, and settlement of the waste contents followed by subsidence. Biodegradation of organic constituents of the waste under aerobic and anaerobic conditions results in the production of gases such as CO_2 , N_2O , N_2 , H_2 , H_2S , and CH_4 . If the wastes contain carbon-14 and tritiated compounds, the gases generated as a result of biodegradation will also be radioactive. The production of these gases is influenced by the types of microorganisms, the presence of electron donors (organics), electron acceptors (oxygen, nitrate, sulfate, carbon dioxide), and other environmental factors.

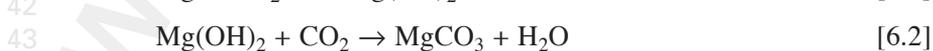
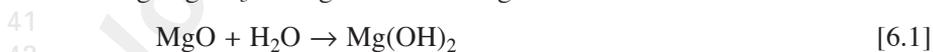
Long-term studies on gas generation from ILW have been conducted in Finland. Other countries such as Germany, Switzerland and South Korea are evaluating the potential impact of gas generation from LLW and ILW (Small *et al.* 2008; Kim *et al.* 2010). More detailed studies on the microbial gas generation and their impacts on the long-term performance of the WIPP site are summarized below.

6.7.3 Microbial gas generation studies at the WIPP repository

Microbially produced gases could have significant ramifications for the long-term stability of the repository (Wang and Francis 2005). The actinides in the

1 waste may affect the overall microbial metabolism. The rates of microbial
 2 respiration during the decomposition of several transuranic-contaminated
 3 waste materials were measured under environmental conditions representative
 4 of a geologic waste-repository in bedded salt. The major effect of activity
 5 on organic-matrix wastes was the generation of CO₂ gas. The experimental
 6 variables studied included incubation temperature (25° to 70°C), atmosphere
 7 (aerobic and anaerobic), moisture content, brine content, and Pu level
 8 (0 to 40 µCi of alpha activity per gram of waste). The maximum rate of
 9 evolution of CO₂ was 5.7 µg/day per gram of waste. The addition of 300
 10 mg (20 µCi) of defense-grade PuO₂ per gram of waste reduced the rate of
 11 CO₂ generation by approximately 70%. The results indicate that microbes
 12 in existing drums of defense-related transuranic wastes have the potential
 13 to generate significant quantities of gas, both aerobically and anaerobically.
 14 CO₂ was the only gas detected in these studies (Caldwell *et al.* 1988). The
 15 potential effects of microorganisms on the long-term storage of radioactive
 16 waste could be significant. An analysis of the experimental results of actual
 17 and simulated waste-degradation studies showed that microorganisms produce
 18 far more gas than that produced by physical and chemical means, including
 19 corrosion (Molecke 1979).

20 The Waste Isolation Pilot Plant (WIPP), located in a salt bed (2200 feet
 21 below ground) in southern New Mexico, is designed by the US Department of
 22 Energy for permanent disposal of defense-related transuranic wastes. In this
 23 high-salinity environment, microbes present in the repository are dominated
 24 by halophilic or halotolerant bacteria, with a population of $1.02 \pm 0.49 \times$
 25 10^5 cells/mL in the far field and $1.24 \pm 0.13 \times 10^5$ cells/mL in the near field
 26 (Francis *et al.* 1998). Microbes detected in the WIPP include denitrifiers,
 27 fermenters, sulfate reducers, and methanogens (Francis *et al.* 1998). The
 28 WIPP can be categorized as an organic carbon-rich repository. Based on
 29 the inventory estimates, wastes to be emplaced to the WIPP contain a large
 30 quantity of organic materials and various nutrients (nitrate and sulfate). The
 31 organic materials are dominated by cellulose, rubbers and plastics. There
 32 is a concern for the long-term performance assessment of the repository
 33 with a potential CO₂ generation from biodegradation of organic materials,
 34 especially for the scenario in which a large volume of brine is introduced
 35 into the repository by human intrusions (Wang and Francis 2005; Gillow and
 36 Francis 2011). The generation of CO₂ can potentially impact the mobility of
 37 actinides and the closure disposal room. In order to mitigate the detrimental
 38 effect of microbial CO₂ generation on the WIPP performance, a sufficient
 39 amount of MgO is added to the repository as a backfill to sequester CO₂ by
 40 forming MgCO₃ through the following reactions:



Microbial gas generation has a significant impact on the evolution of disposal room pressure. The pressure buildup is attributed to both microbial gas generation and anoxic metal corrosion (Wang *et al.* 1997).

6.7.4 Microbiological studies at the experimental underground laboratory and high-level waste repository sites

Research is underway on the interactions between microorganisms and radionuclides under conditions typical of a repository for high-level radioactive waste in deep hard rock environments at a depth of approximately 500 m at Äspö Hard Rock Laboratory (HRL) in Sweden, and at the Atomic Energy of Canada Limited (AECL) underground laboratory in Whiteshell, Canada. Similarly, microbiological studies have been carried out at the Waste Isolation Pilot Plant (WIPP) site where transuranic (TRU) waste is disposed of in a deep geological salt formation at Carlsbad, New Mexico, and at the Yucca Mountain site at Las Vegas, Nevada, proposed for HLW but abandoned recently for technical reasons. Several of the pertinent microbiological studies conducted at the various sites are highlighted.

Several studies have been conducted on microbial activity related to nuclear waste disposal in deep geologic repositories (Anderson *et al.* 2011; Amy *et al.* 1992; Pedersen *et al.* 1996; West *et al.* 1992; Kieft *et al.* 1997; Haveman *et al.* 1995; Stroes-Gascoyne *et al.* 1997). A survey of microbial population distributions in water and in subterranean soil samples from deep mines designated for disposal of high-level radioactive waste in Europe revealed the presence of a variety of organisms representing autotrophic and heterotrophic groups that include native organisms and organisms introduced from mining operations (West *et al.* 1985, 1986, 1992). Even extreme environments, such as the hypersaline groundwaters at the WIPP site and the extremely low-nutrient granodiurite pore-waters in Switzerland, harbor microorganisms capable of interacting with actinides in TRU waste (Gillow *et al.* 2001).

6.7.5 Experimental underground laboratory studies

The deep biosphere therefore harbors a great diversity of microbial species and metabolic processes. In deep oligotrophic subsurface granitic rock environments, fracture biofilms harbor approximately $2\text{--}5 \times 10^6$ cells cm^{-2} . The cells in these biofilms are spatially distinct and are surrounded by an extracellular polysaccharide matrix that constitutes up to 60% of the total organic carbon. Under *in situ* conditions, fresh subsurface fracture walls consisting of glass and rock surfaces are rapidly colonized by microbial biofilms that thinly coat the entire surface. Individual cells are generally

1 separated, but extracellular films cover the surface between cells. Subsurface
2 biofilm formation affects adsorption by acting as a barrier between the
3 water and underlying mineral sorption mechanisms, providing sorption
4 sites for radionuclides, that has been found to influence the adsorption and
5 immobilization of Am, Np, Pm, Th, and U (Stroes-Gascoyne *et al.* 2000;
6 Francis *et al.* 1998; Anderson *et al.* 2007, 2011).

7 *In situ* studies at the Äspö Hard Rock Laboratory (HRL) tunnel showed
8 that the concentrations of biogenic iron-oxides, lanthanides, and actinides
9 correlated positively with the iron oxidizing bacteria *Gallionella* biomass.
10 In both the AECL underground laboratory in Canada and the Äspö HRL
11 in Sweden, subsurface biofilms involved in the generation of biogenic iron
12 oxides have a high adsorption capacity for trace metals and radionuclides,
13 concentrating these elements from extremely dilute sources (Brown *et al.*
14 1994; Anderson *et al.* 2011). Fresh bacteriogenic iron oxides (three months
15 old) from Äspö concentrated REE up to 1×10^4 fold higher than groundwater
16 levels and the older biofilms (i.e., >2 years old) from the same environment
17 have 1×10^6 fold higher concentrations of REE than the groundwater. These
18 studies show that microorganisms can influence, and sometimes even control,
19 the migration behavior of radionuclides in deep geological environments
20 typical of proposed sites for radioactive waste repositories.

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6.7.6 Yucca Mountain site

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34 examined thus far.

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43 More complete information on the organisms contained within the YM
community was obtained by characterization of DNA extracted directly from

YM rock (Horn *et al.* 2003). Quantitative analysis of the total number of bacteria present in a Yucca Mountain rock sample aseptically collected from the Exploratory Studies Facility (ESF) was determined using phospholipid fatty acids (PLFA) analysis, which is a direct indicator of viable or potentially viable cells (Horn *et al.* 2004). It was estimated that one sample contained 6×10^4 cells per gram of dry rock (3 pmol (pM) of PLFA/g dry rock); and another deeper-dwelling distal sample had 4×10^4 cells per gram of dry rock (2 pM of PLFA/g dry rock), using a conversion factor of 2×10^4 cells/pM PLFA. No diglycerides were detected, an indicator of absence of dead bacteria. The PLFA analysis showed a preponderance of Gram-negative organisms.

Microorganisms identified in YM span a wide phylogenetic range, and include groups of organisms known to reside in dry environments. The great diversity of microorganisms detected at the YM site further confirms that microorganisms in a subsurface environment are highly adaptable and sufficiently diverse for carrying out any specific metabolic reaction that the environment permits. Temperature, radiation, relative humidity, water availability, aerobic and anaerobic conditions, availability of nutrients, will affect microbial activity.

The temperature of the subsurface environment will limit the type of bacteria. During the period of thermal perturbation resulting from waste package emplacement, the temperature in the repository drifts could exceed 120°C (the upper temperature limit for the presence of microorganisms) and, for a waste package surface, could be as high as 170°C . Therefore, microbes initially present in the drift will be severely limited in growth, if not totally eliminated, by heating for a few hundred years at the early stage of the repository. Microbes may migrate into the repository with infiltrating fracture flow, once temperatures decrease. Infiltrating organisms that survived the heating period may colonize if conditions are favorable for growth. Even after the peak temperature, the in-drift temperature will remain above 50°C for the duration of the 10,000-year regulatory period. Therefore, the microbial population is expected to be dominated by thermophiles and hyperthermophiles.

Water is essential for microbial growth. In order for microorganisms to grow, the relative humidity in the environment needs to be 75–100% (Brown 1976). In a full-scale test conducted at Atomic Energy Canada Ltd (AECL), a simulated waste container (maximum heat output 85°C) was buried for 2.5 years, surrounded by compacted buffer materials. Extensive microbial analysis of this test has shown that most viable organisms in the buffer material disappear around a moisture content of 15%, corresponding to RH 95–96% (Stroes-Gascoyne 1996). The activity and availability of water for microbial growth in the unsaturated zone at Yucca Mountain will be dependent upon thermal–hydrologic conditions. During the thermal pulse, relative humidity

1 on the waste package surface can be lower than 10%. In some realizations,
2 relative humidity recovers (back to 100%) after approximately 1000 years.
3 In many other realizations, relative humidity remains below 90%, and even
4 below 70% in some cases, throughout the regulatory period, thus limiting
5 microbial growth and activity. In addition, low water activity generally
6 corresponds to low liquid-water availability. Low saturation of liquid water
7 fractures on the drift wall will limit the transport growth substrates to
8 microbial cells and, therefore, the activity of the cells.

9 Experiments have been performed to define conducive and inhibitory
10 environmental conditions that pertain to growth of Yucca Mountain
11 microbial communities in modified Yucca Mountain groundwater (Horn
12 *et al.* 2004). Experimental data show that nitrogen and sulfate sources are
13 apparently sufficient to support microbial growth, even in unconcentrated
14 Yucca Mountain groundwater. Both phosphate and organic carbon have
15 been shown to have significant effects on microbial growth. Phosphate
16 increased cell densities by approximately 1.5 orders of magnitude. The
17 addition of a carbon source, glucose, resulted in increases of one order of
18 magnitude in simulated groundwater medium. Yucca Mountain groundwater,
19 however, has extremely low levels of organic carbon and phosphate. Only
20 trace concentrations of organic carbon (up to 1.1 mg/L) have been reported
21 in qualified measurements of Yucca Mountain groundwater. The materials
22 introduced into emplacement drifts will not contain any organic carbon,
23 except for a trace amount of reduced carbon in metal or metal alloys. These
24 carbons, like graphite, are expected to be refractory and, thus, will not be
25 biodegradable. Therefore, the extremely low organic-carbon supply in the
26 repository will limit heterotrophic microbial activity. Based on the repository
27 loading design, the expected maximum surface dose rate from one unbreached
28 canister designed to contain spent nuclear fuels is 1.7 Mrad/year. Therefore,
29 it is expected that radiation may inhibit microbial growth in the repository.
30 This assessment is consistent with an AECL test that used a simulated waste
31 container with a maximum heat output of 85°C. The test has shown that
32 the surface of nuclear fuel waste containers is sterilized 9 to 33 days after
33 emplacement (Stroes-Gascoyne, 1996).

34 35 6.7.7 Overall impact of microbial activity on yucca 36 mountain near-field chemistry 37

38 Microbial growth and activity at Yucca Mountain are limited by multiple
39 factors: (1) the in-drift temperatures during the thermal pulse will exceed
40 the temperature tolerance of all known microbes for a significant portion of
41 the repository time; (2) the relative humidity and the liquid-water saturation
42 in the repository are predicted to be low for a significant duration, thus
43 further limiting microbial activity; and (3) the extremely low organic carbon

supply in the repository will limit heterotrophic microbial activity. Due to these environmental constraints, the microbial activity in the repository is expected to be low, and its impacts on drift chemistry can be negligible. The overall effect of microbial activity on the near-field chemistry in the Yucca Mountain repository is expected to be negligible because of limited nutrient supply and harsh environmental conditions.

6.8 Conclusion

Microorganisms play a significant role in the transformations of radionuclides generated from the nuclear fuel cycle and thus regulate the mobility and stability of the radionuclides in nuclear wastes and in the environment. Key microbial processes involved in the mobilization or immobilization of selected radionuclides of interest are summarized in Table 6.1. Among the radionuclides, biotransformation of uranium has been extensively studied, whereas we have only limited understanding of the microbial transformations of other radionuclides. The biochemical mechanisms and the enzymes involved in the biotransformation of radionuclides and the impact on the solution chemistry are not fully understood. The organic degradation products, metabolites including the specific sequestering agents produced by fungi and bacteria, affect the solubility of both the oxidized and reduced species of radionuclides. Manipulation of the bioaccumulation and bioprecipitation reactions can result in the formation of more stable mineral phases and immobilization of the radionuclides. Fundamental understanding of the mechanisms of microbial transformations of several chemical forms of the

Table 6.1 Summary of key microbial processes and transformations of radionuclides

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

NA not applicable; ND not determined.

¹Dissolution due to oxidation from lower to higher valence state.

²Reductive precipitation due to enzymatic reduction from higher to lower valence state.

³Dissolution due to oxidation from lower to higher valence state, changes in pH, production of organic acids and sequestering agents.

⁴Association of radionuclides with suspended bacteria, which can be transported as biocolloids.

1 radionuclides under various environmental and microbial process conditions
2 is needed for assessing the microbial impact on the long-term behavior of
3 radionuclides released from nuclear power plants, fuel reprocessing plants,
4 during on-site storage, shallow land-burial, and disposal in deep geological
5 formations as well as in developing appropriate management and remediation
6 strategies for contaminated sites.

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