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Microorganisms and migration of radionuclides in environment

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Abstracts

Amongst all the fission fragments, actinides and activation products coming from the nuclear energy processes, a dozen radionuclides are a hazard for Man and environment, due to their half-life and toxicity. For the last 3.5–4 billion years (Ga), microorganisms have been present on Earth in all sorts of surroundings, even the most hostile ones, characterized by extreme pH, temperature and/or radioactivity. To grow and multiply, they developed enzymatically induced oxido-reduction reactions by coupling reduction of metals/metalloids with oxidation of organic matter, which, in turn, may change soluble forms of radionuclides or stable elements into non-soluble forms. When this happens, microorganisms contribute to fix the radionuclides and prevent dissemination. *To cite this article: M. Simonoff et al., C. R. Chimie 10 (2007).*

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Résumé

Parmi les produits de fission, actinides et produits d'activation issus de l'énergie nucléaire, une douzaine de radionucléides constituent un danger pour l'homme et l'environnement du fait de leur période et toxicité. Les microorganismes, présents sur Terre depuis 3,5 à 4 milliards d'années (Ga), vivent dans tousles milieux, y compris les plus hostiles (pH, température, radioactivité). Pour assurer croissance et multiplication, ils sont à l'origine de réactions d'oxydo-réduction enzymatiques, dans lesquelles la réduction d'un métal (métalloïde) couplée à l'oxydation de matière organique peut transformer des formes solubles d'éléments stables ou radioactifs en composés insolubles. Dans ces conditions, les microorganismes tendent ainsi à limiter la dispersion de la radioactivité dans l'environnement. *Pour citer cet article : M. Simonoff et al., C. R. Chimie 10 (2007).* © 2007 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Radionuclides; Microorganisms; Bioremediation; Environment; Technetium; Selenium; Actinides

Mots-clés : Radionucléides ; Microorganismes ; Bioremédiation ; Environnement ; Technétium ; Sélénium ; Actinides

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1. Introduction

The aim of this review is to show how the migration of long-lived radionuclides (which are potentially harmful for Man and its environment) is strongly

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correlated with the action of microorganisms. The latter induce oxido-reduction reactions on numerous elements, and this is and will be an evermore frequent case in the future. This is directly correlated with life on Earth since its beginning. This review being intended for physico-chemists, the first part will be devoted to the origin and the diversity of microorganisms on Earth, with a particular interest on how life, which emerged on Earth several billion years ago, was implicated in the evolution of the original Earth crust. From this, we will infer on a geological timescale (the order of magnitude is the half-life of the radionuclides synthesized in nuclear processes) how and to what extent the microorganisms interact with nuclear wastes.

The first traces of life on Earth date from 3.5 to 4 billion years ago, when free oxygen did not yet exist in the atmosphere. As these microorganisms diversified, adapting to the conditions then encountered, they contributed to the formation of the environment of the primitive Earth. Using present techniques of micro- and molecular-biology, the number of microorganisms per gram of earth or water is estimated to be in the range from 10^6 to 10^9 . They are classified in three categories. Archaea, Bacteria and Eucarya, which developed from a common cell LUCA (Last Unicellular Common Ancestor). The microorganisms developed various strategies in order to obtain the elements (water, mineral salts, carbon, etc.) required to assure their growth and multiplication, and they are able to survive in media hostile to more complex forms of life (extremes of pH, salinity, radioactivity, temperatures $> 100 \,^{\circ}$ C). The activity of microorganisms concerns nearly every field of nature and human endeavor: e.g., agronomy (assimilation of nitrogen and phosphor by plants), geochemistry, formation of ore deposits, oxidation-reduction reactions and chemical transformations of all kinds, biotechnologies, etc.

The ability of the microorganisms to chemically transform numerous elements during oxidation—reduction reactions is independent of the nature (stable or radioactive) of the element involved. On a geological timescale and in spite of the heat and the radioactivity, a number of interactions between radionuclides and microorganisms could occur on nuclear waste storage sites after corrosion of concrete and containers, corrosion which, in addition, could be initiated and facilitated in certain cases by the microorganisms or their byproducts.

The present review, after dealing briefly with the source and diversity of our planets' population of microorganisms, will present their effects on metals and metalloids, analyzing particularly the consequences of their activity on key radionuclides in the environment (actinides, fission products from the electronuclear cycle).

2. Origin and diversity of microorganisms

The Precambrian (or cryptozoic) period extends from the birth of the Earth, -4.55 Ga till -0.540 Ga. It is subdivided into the Hadean (-4.55 to -4 Ga), the Archaen (-4 to -2.5 Ga) and the Proterozoic (-2.5 to -0.54 Ga) time lapses.

The evolution of the lithosphere [1-4] and the other external envelopes of the Earth occur simultaneously: the hydrosphere [5] appears and its physicochemical characteristics (temperature, salinity) evolve [6]; the atmosphere, reducing or neutral at first, slowly becomes enriched in free oxygen [7,8]. Three major hypotheses have been presented to account for the origin of life on Earth [9–12]:

- life already present in the interstellar medium arrived on Earth on asteroids and/or comets [13–16];
- life was created on the Earths' surface under conditions similar to the simulation experiments of Miller and his successors. In 1953, Miller [12] synthesized amino acids comparable to those of living systems. Although conditions similar to those which then existed were simulated, the appearance of life (and its autoreplication) in the mineral world remains to be demonstrated;
- life was created in the ocean depths in hot vents [17–20]. Recent work, however, does not support that hypothesis [21].

In 1969, Woese [22] studied the small ribosomal subunit RNA sequence of archaeabacteria, eubacteria, and eukaryotes. It is excluded that one of these lines gave rise to the others. The three groups descended from a common ancestor later called LCA or LUCA [23,24] and they co-existed precociously. The passage from a simple eukaryote to a hierarchized, then pluricellular, eukaryotic organism took place over time through the effects of bacterial endosymbiosis and oxygen. The association between prokaryotes and eukaryotes appears as one of the keys to the evolution of our bacterial universe [25,26]. The three biological groups according to Woese et al. [27] are shown in Fig. 1.

The primitive atmosphere of the Earth arose from the degassing of the magmas, then, from reductive, it probably evolved through the action of photosynthetic cyanobacteria [28-30] towards one progressively enriched in free oxygen to a level which will permit a fantastic adaptation [31] of the eukaryotic cell at the



Fig. 1. The three biological groups according to Woese et al. [27]. Reprinted from Wikipedia.

limit of the Precambrian and the Paleozoic (Cambrian). The next paragraphs will examine the first steps of life on Earth and its evolution. A valuable review on this topic is to be found in Ref. [32].

When the Earth is being formed, it receives a mass of organic matter from asteroids, meteorites and comets, the last of which is also the source of some terrestrial water. Volcanic activity is intense and tectonic plates begin to move as the first continents are formed. During the first four billion years, life forms consist mainly of bacteria. The image of the Precambrian Earth from space showed red periods due to iron minerals and sandstones, and white periods during global glaciations. Sudden changes of color occurred due to bacterial 'blooms', then continents and ocean margins become progressively green during the proterozoic chlorophyllian algae and the planet then becomes slowly blue. Life was present during the period 4.55-3.9 Ga, as testified to by the study of the carbon isotopes in the most ancient rocks presently known (Isua series, Greenland) dated 3.9 Ga [33-41]. From the beginning, the bacteria secrete varieties of mucilage and combine to form bacterial mats [42] which trap particulates or favor the precipitation of iron salts and carbonates, gradually laying down successive biomineral layers to form strucstromatoliths (Warrawoona tures called series. Australia, 3.5 Ga). In the different series corresponding to more recent periods, spherical bacteria, cyanobacteria, ferruginous bacteria, eukaryotic algae, etc., have been observed [43]. The principal accumulations of iron are found in the banded sedimentary layers. These thick, regular layers of iron oxide appear from 3.9 Ga (Isua series); 95% are formed between 2.6 and 2 Ga as Fe₂O₃ and Fe₃O₄ (the magnetite). The mats of photosynthetic cyanobacteria [29,42] produced the oxygen which was fixed by the iron dissolved in water. A major crisis in the biosphere probably occurred around 2 Ga.

As the supply of Fe^{III} disappeared, the cyanobacteria adapted and the banded ferriferous stromatoliths gave way to carbonated ones. Recent studies [44] show that the Archaea and Bacteria close to LUCA are capable of reducing Fe^{III} to Fe^{II}. LUCA was probably the first micro-organism capable of assuring respiration by the reduction of Fe^{III} to Fe^{II}.

The oxygen level begins to grow from 1.8 Ga on. Certain bacteria, including the cyanobacteria, consume carbon dioxide by their photosynthetic activity, lowering the partial pressure of carbon dioxide in the aquatic media and enhancing the precipitation of calcium carbonate.

The progressive increase of the oxygen level in the atmosphere revolutionizes the biosphere. During the major part of the Precambrian, the biosphere is a bacterial world functioning without sexuality. Cellular division by mitosis can take place only if the level of free oxygen is at least 1% of its present-day value. That date was reached at about 2 Ga, which is the date of the appearance of eukaryotes. An oxygen level of at least 7% of its present value is necessary for oxygen to diffuse through membranes, creating the first condition enabling the formation of a multicellular organism. Bacteria have proliferated on Earth since at least 3.9 Ga and the Precambrian biosphere has not disappeared.

At present, the Precambrian and the most recent organisms coexist [28–30]:

- the prokaryotes (Bacteria and Archaea) are of the size of a micron and are composed of a single compartment surrounded by a membrane, inside which are the cytoplasm and the chromosome made of DNA;
- the eukaryotes (mean dimension 10 µm) are defined by the presence of a nucleus containing the genetic material of the DNA, surrounded by a cytoplasm which can have the specialized organites (mitochondria, plasmids, peroxisomes, etc.), surrounded itself by a membrane defining the limits of the cell.

Among the Bacteria are found the photosynthetic organisms such as the cyanobacteria, as well as those living at high temperatures (thermophilic) and below 0 °C (psychrophilic). Some Archaea live in extreme environments: halophiles require high salt concentrations, thermoalcaliphiles grow only at temperatures of at least 70 °C and pH between 10 and 11, whereas the thermoacidophilic ones grow at 80 °C and pH below 2 (hot sulfurous source); the methanogens in absence of oxygen in swamps or marshes generate methane by reduction of carbon dioxide.

The recent discovery of microorganisms in deep marine sediments [18,45], as well as those living at the heart of the Earth's crust in deep basalt, considerably increases the extent of the biosphere. The message of the latest discoveries is very clear: we know but a small percentage of the species presently living in the biosphere (8×10^5 species are known and it is estimated that 10^6 species are present in the biosphere) [46].

3. Microbial colonization of radioactive environments

As seen above, microbial life in the biosphere is ubiquitous and fairly well known, but the level of microbial colonization of radioactive environments has only recently been investigated. Landa [47] studied mill tailings comprising uneconomic residues from mining and milling operations. Other environments that have received radioactive wastes have been explored for microbial content. Characterization of microorganisms attached to the walls of a pool storing nuclear materials from the Spanish nuclear plant was reported by Chicote et al. [48]. Several isolates were obtained and six different bacteria (affiliated to β -Proteobacteria, *Actinomycetales* and the *Bacillus/Staphylococcus* groups) as well as fungus (*Aspergillus* species) were identified.

The sediments contaminated in 1962 by a leak from a waste tank (USA DOE Hanford Site, Washington State) containing high concentrations of alkali, nitrate, aluminate, chromate, ¹³⁷Cs and ⁹⁹Tc have been studied by Fredrickson et al. [49]. Viable aerobic heterotrophic bacteria were found in most of the samples. Gram+ bacteria most closely related to *Arthrobacter* species were the most common among all isolated samples, but other phyla such as *Rhodococcus* and *Nocardia* were also found. Even the most radioactive samples (>10 μ Ci ¹³⁷Cs g⁻¹) yielded active bacteria and those from some isolates from samples with >20 μ Ci of ¹³⁷Cs g⁻¹ were closely related to *Deinococcus radiodurans* and were able to survive acute doses of ionizing radiation ~20 kGy. These organisms were highly resistant to desiccation.

At the Severnyi repository for low-level liquid radioactive wastes in eastern Siberia, Russia, water samples collected from a depth of 162–405 m below sea level [50] contained anaerobic denitrifiers, fermenters, sulfate-reducers and methanogens. A wide range of functional groups of microorganisms was detected and rates of several respiratory processes were higher in the zone of dispersion of radioactive waste than in the background areas. The authors noted that microbial gas production could result in a local increase in pressure in the repository and affect the consequent discharge of wastes, although key microbial activities (for example formation of insoluble sulfides from sulfate reduction) could also result in the immobilization of some radionuclides. Such processes have recently been shown to occur in microcosms prepared from sediments in two types of predwater lakes of the Moscow Oblast [51]. These results show that the radioactivity of several nuclear waste types is not necessarily inhibitory to all microbial life.

Nevertheless a recent study [52] using pure cultures of bacteria (*D. radiodurans, Pseudomonas putida* and *Schewanella putrefaciens*) in tests concerning the toxicity of actinides, metals and chelators showed that actinide toxicity is primarily chemical, not radiological, and that radiation resistance (for example in *Deinococcus*) does not ensure radionuclide tolerance.

In conclusion, the radioactivity does not eradicate the microbial populations in storage sites for nuclear wastes; at most it effects a selection, assuring the survival of several species among the multitude of microorganisms initially present.

4. Effects of microorganisms on radionuclides

The fate of the radionuclides in the environment will depend on pH, *Eh*, ligands and the presence of microorganisms. The environmental chemistry of actinides and some fission products (particularly Tc and Se) is very complex due to the multiple oxidation states [53]. Microbes will affect radionuclides directly by changing the oxidation state or indirectly by producing reducing compounds (H₂S, Fe^{II}, ligands). Microbial activity will deeply affect the solubility of radionuclides by the following mechanisms: biosorption, metabolism-dependent bioaccumulation, enzymatic biotransformations, biomineralization by ligands.

4.1. Biosorption

Sorption may be responsible for the accumulation of radionuclides on the surface or interface. Living cells and dead biomass can biosorb; dead biomass often sorbs more metal(loid)s than does the living one. Ligands such as carboxyl, amine, hydroxyl, phosphate and sulfhydril groups are involved. Biosorption is rapid and unaffected by temperature and can be described by the Langmuir, Freundlich and Brunauer–Emmett–Teller (BET) isotherms [54,55]. An extensive review of radionuclide biosorption is given by Lloyd and Macaskie [56].

4.2. Metabolism-dependent bioaccumulation

It is well known that it is possible for radionuclides and numerous stable elements to penetrate the cell by a classical transport system, as would an ion habitually involved in the physiology of the cell (Cs⁺ instead of K⁺, Sr²⁺ and Ra²⁺ instead of Ca²⁺). Once in the cell, the toxic metal (radioactive or not) is sequestrated by cystein-rich metallothioneins [57] or in the vacuole in the case of fungi [58]. This uptake by a metabolismdependent transport mechanism is poor for the heavier radionuclides, like actinides. Other explanations can be assumed: a recent review [59] explains intracellular accumulation of uranium by increased membrane permeability due to uranium toxicity and not to metabolism-dependent transport mechanisms.

4.3. Enzymatic biotransformations: bioreduction, biomethylation, biodegradation

Microorganisms can catalyze the direct transformation of toxic metals and metalloids to less soluble or more volatile forms via enzymatic mechanisms. Bioreduction can precipitate solid metal(loid)s by enzymatic reduction [60,61], or produce volatile derivatives (by biomethylation, for example, with Se, Te, Hg [62,63]); alkylated actinides are instable and few experiments have been carried out [64].

Under anaerobic conditions, several microorganisms can respire by the reduction of alternative electron acceptors. These oxido-reduction reactions are well recognized for nitrate, sulfate, and carbon dioxide in presence of microorganisms and in absence of oxygen and are called bioreduction or dissimilatory reductions. More recent studies [65] show that higher valence metal(loid)s, radioactive or not, also function as alternative electron acceptors during anaerobic respiration of specialist organisms (Fe^{III}, Mn^{IV}, U^{VI}, Se^{VI}, Tc^{VII}). Biomethylation can produce volatile methyl derivates, as for Se, Tc, Hg, I, and so decrease the concentration of soluble contaminants in water or soil under the action of microorganisms.

Biodegradation of associated organic compounds has been observed with citrate. Citrate can form with highly soluble radionuclide citrate complexes, then be in some cases degraded by microorganisms to give a precipitation of the radionuclide [66], as observed with *Pseudomonas aeruginosa* and *P. putida* [67].

4.4. Biomineralization by ligands produced by microbes

Ligands enzymatically generated, such as phosphate, carbonate and sulfide, are able to precipitate metals (stable or radioactive), and provide an appreciable way to eliminate radionuclides from solution. The microbial biomineralization by phosphate involves *Citrobacter* sp. (now *Serratia* sp.), since molecular methods identified the phoN phosphatase gene [68]. The role of a phosphatase in metal accumulation has been confirmed by the fact that the expression of the phoN gene clones in *Escherichia coli* conferred the ability to bio-precipitate uranyl phosphate [69].

Carbonates are ligands produced with *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*), *Pseudomonas fluorescens* [70–72] for precipitation of strontium carbonate, whereas the formation of uranyl carbonate species enhances the dissolution of uranyl [72].

Sulfides produced by sulfate-reducing bacteria, ubiquitously distributed in acid, neutral and alkaline conditions [73], have the potential to play a critical role in controlling the migration of radionuclides in a range of environments. The liberation of gas (H₂S) will accelerate a precipitate of high-valence element or its reduction.

5. Key radionuclides of environmental importance

The presence of radionuclides in the environment is the result, initially, of significant quantities of radioisotopes released as a consequence of nuclear weapons' testing in the 1950s and 1960s. Then accidental contamination, coming from nuclear activities, can arise, like Chernobyl catastrophe. Moreover, burden of anthropogenic radioactivity continues with the controlled discharge of process effluents produced by industrial activities bound to nuclear power. Wastes generated during uranium mining concern low- and high-level liquid and solid wastes produced from reactor operation and fuel reprocessing. Finally, some nuclear wastes have to be stored either in surface or in subsurface.

Thermal fission of 235 U generates more than 100 products in the range 72 Zn to 161 Tb with long or short half-lives. Interaction of the uranium nuclear fuel with α -particles and neutrons produced during fission, moreover, will generate the transuranic elements Np, Pu and Am in the reactors. Radionuclides present in some nuclear wastes arise from activation, corrosion of fuel cladding or assemblies (60 Co, 59 Ni, 93 Zr). The environment can be at risk from radionuclides with significant activity, long half-lives, large quantities and bioavailability. In the fission products and actinides produced, there are about a dozen elements with key radionuclides of environmental importance [74].

Table 1 gives some characteristics of key radionuclides of environmental importance: fission products have few oxidation states compared to actinides with multiple oxidation states for Am, Np, Pu; nonetheless

Th is essentially present as Th^{IV} , and uranium most frequently as U^{VI} and U^{IV} .

6. Fission products

Ref. [74].

The redox chemistry of the fission products is in general limited to some valencies. Both Cs and Sr exist in only one oxidation state and their environmental behavior is therefore not dependent on any redox chemistry. Their oxidation states account for their high bioavailability. Cs⁺ is analogous to K⁺ and enters cells via pathways for K^+ . Ca^{2+} , as other alkaline earth metals, Ba^{2+} , Ra^{2+} , forms complexes and insoluble precipitates. Technetium has, in particular, two environmentally stable oxidation states, VII, as the anion TcO_4^- (soluble), and IV with soluble and insoluble forms. For iodine, -I, 0, V are most frequently found as I⁻, I₂, IO₃⁻. Selenium has four environmentally important oxidation states. VI and IV as mobile and soluble forms in oxic media, and 0, elemental selenium. -II is very often bound to proteins or methylated.

6.1. Technetium (group VII)

Technetium is an artificial radioelement with an atomic number of 43. It belongs to the Mn group and has no stable isotope. The isotope ⁹⁹Tc is of great practical importance because it is one of the fission products with a relatively high yield in the thermal-neutron fission of ²³⁵U and ²³⁹Pu (about 6%) and it has a long half-life $(2.1 \times 10^5 \text{ a})$ [75]. Technetium-99 has been

released to the environment via nuclear weapons testing and nuclear facilities such as nuclear fuel reprocessing plants, nuclear power plants and waste disposal facilities. But it is also produced by the use of ^{99m}Tc $(T_{\frac{1}{2}} = 6.01 \text{ h})$ in nuclear medicine and decays directly to ⁹⁹Tc. Medical uses of ^{99m}Tc will continue to increase in the future. For those reasons, ⁹⁹Tc in the environment requires special consideration.

Technetium is known to exist in all valence states from +7 to -1. The dominant species in natural aqueous solutions in equilibrium with the atmosphere is the pertechnetate (TcO_4^-) , which has a high geochemical mobility [76] and availability for plants [74,75]. However, through a combination of factors [51,77-79] such as redox conditions and microbial activity [80-82], $Tc^{VII}O_4^-$ can be reduced and immobilized in insoluble forms in soils, in or around microorganisms. The redox chemistry of Tc is crucial in governing its mobility [75]. Under environmental conditions, $Tc^{VII}O_4^-$ can be transformed to lower oxidation states as Tc^{IV} species, amongst which the insoluble forms TcO2 under anaerobic conditions, as predicted by the Eh-pH diagram of Tc [83], while at valence 4 numerous soluble forms exist, such as TcO^{2+} , $TcO(OH)^+$, $TcO(OH)_2^0$ aq, $Tc(OH)_3^+$, $Tc(OH)(CO_3)_2^-$, and $Tc(OH)_2(CO_3)_2^{2-}$. Phosphorimager is of great help for the determination of Tc species [84]. The reduction of Tc can be achieved by abiotic mechanisms using zerovalent iron, or Fe^{II}-containing minerals under anoxic conditions; magnetite is particularly a efficient reductant for Tc^{VII} [85]. X-ray photoelectron spectroscopy XFS has confirmed that the Tc was precipitated on the surface of the magnetite as TcO₂. The retention of Tc as Tc₂S₇ on stibuite Sb₂S₃ has been studied by proton X-ray emission microbeam [86].

An example of TEM images of *S. putrefaciens* CN32 after enzymatic reduction of Tc is shown in Fig. 2 [87].

Microbial metabolism may affect Tc speciation by indirect (chemically mediated by either sulfide or Fe^{II}) and direct (enzymatic) mechanisms [61,80,87,88]. Microbial reduction of technetium has been studied very intensively. Sulfate-reducing bacteria, Desulfovibrio desulfuricans [89,90], metal-reducing bacteria, Shewanella putrefaciens [87] and Geobacter sulfurreducens [61] reduce Tc^{VII} at neutral pH. Under acidic conditions. Thiobacillus ferrooxidans and Thiobacillus thiooxidans reduce Tc^{VII} to low valency forms [91]. It was shown for the first time by Khijniak et al. [60] that haloalcaliphilic bacteria isolated from soda-lake environments are capable of reducing $Tc^{VII}O_4^-$ to Tc^V , Tc^{IV} and Tc^{III} at pH 10. Dissimilatory Metal-Reducing Bacteria (DMRB) include Shewanella putrefaciens [87], Shewanella alga,

Table 1 Some characteristics of key radionuclides of environmental importance: fission products have few oxidation states compared to actinides with multiple oxidation states for Am, Np, Pu

Element	Isotope	Half-life (a)	Oxidation states
Cobalt	⁶⁰ Co	5.27	II,III
Selenium	⁷⁹ Se	4.8×10^{5}	-II,0,IV,VI
Strontium	⁹⁰ Sr	29.1	II
Technetium	⁹⁹ Tc	2.15×10^{5}	IV,VII
Iodine	¹²⁹ I	$1.57 imes 10^7$	-I,0,V
Cesium	¹³⁷ Cs	30.17	Ι
Thorium	²²⁹ Th	7.34×10^3	IV
	²³⁰ Th	$9 imes 10^4$	
Uranium	²³⁸ U	4.47×10^{9}	II,IV,V,VI
Neptunium	²³⁷ Np	$2.14 imes 10^6$	III,IV,V,VI,VII
Plutonium	²³⁸ Pu	87.7	III,IV,V,VI,VII
	²³⁹ Pu	$2.41 imes 10^4$	
	²⁴⁰ Pu	6.55×10^{5}	
	²⁴¹ Pu	14.4	
Americium	²⁴¹ Am	432.7	II,III,IV,V,VI,VII
Th is essential	lly present as	Th ^{IV} , uranium as	U ^{VI} and U ^{IV} . From



Fig. 2. TEM images of *S. putrefaciens* CN32 after enzymatic reduction of Tc with different electron donors and solution compositions (bicarbonate-H₂). The cells are unstained, and the images result because the presence of reduced Tc. Reprinted by permission from the American Society for Microbiology [87], copyright 2000.

Geobacter metallireducens [87,92,93] and the sulfatereducing bacterium *D. desulfuricans* [88,89]. These microorganisms are capable of gaining energy for maintenance and growth by coupling the oxidation of organic C, from lactate, acetate or H₂, to the reduction of Tc^{VII}.

$$\begin{aligned} \mathrm{Tc}^{\mathrm{VII}}\mathrm{O}_{4}^{-}(\mathrm{aq}) \,+\, 3\mathrm{e}^{-} \,+\, 4\mathrm{H}^{+} &\rightarrow \mathrm{Tc}^{\mathrm{IV}}\mathrm{O}_{2}, \cdot n\mathrm{H}_{2}\mathrm{O}_{(\mathrm{s})} \\ &+\, (2-n)\mathrm{H}_{2}\mathrm{O} \end{aligned}$$

A direct enzymatic reduction of Tc^{VII} has been demonstrated by the Fe^{III}-reducing bacteria *G. metallireducens* and also with the widespread *E. coli* [81], and the sulfate-reducing bacteria *D. desulfuricans* [88]. Tc reduction was catalyzed by the hydrogenase component of the Formate Hydrogen Lyase complex (FHL). Precipitation of TcO_2 was at the periphery of the cell; these results were consistent with direct, hydrogen-dependent reduction of Tc^{VII} by a periplasmic hydrogenase. The identification of hydrogenase 3 of FHL as the Tc^{VII} reductase of *E. coli* opened the way for a program to screen organisms with naturally-enhanced activities against Tc^{VII} .

The addition of sulfate-reducing bacteria to mixed cultures of anaerobically grown soil bacteria increased Tc removal. This was mediated by an indirect mechanism utilizing microbially-generated H₂S. This metal sulfide formation also explained Tc accumulation by mixed anaerobic bacteria isolated from marine sediment [82].

Several extremophiles resistant to temperature, salinity or radiation have shown ability to reduce Tc^{VII} . *D. radiodurans*, the most radiation-resistant organism, capable of surviving acute exposures up to 15,000 Gy without lethality [94] is described as having a strictly aerobic respiratory metabolism, but under strict anaerobic conditions reduces Fe^{III}, coupled to the oxidation of lactate to CO₂ and acetate and Tc^{VII} in combination with humic acid or synthetic electron shuttle agents [95]. *Thermoterrabacterium ferrireducens* and *Tepidibacter thalassicum* (Gram positive thermophilic bacteria) precipitate Tc as Tc^{IV} with some amounts of soluble Tc^V at 65 °C (hydrogen, glycerol and lactate served as electron donors) [96].

In conclusion, numerous microorganisms are capable of immobilizing technetium in the environment under the most diverse conditions, thus favoring the retention of this long-lived fission product, produced in large amounts in the uranium electronuclear cycle.

6.2. Selenium (group VI)

Among the key radionuclides that must be taken into consideration with regard to environmental impact, ⁷⁹Se, a long-lived isotope ($T_{\frac{1}{2}} = 0.65 \times 10^5$ a) produced in very small amounts in uranium fission, has a special importance because of its role as an essential trace element for humans and animals. As an integral part of glutathione peroxidase, selenium contributes to the elimination of free radicals produced by oxygen or other inducers. Selenium is implicated in seleno-amino acids (the new 21st amino acid), selenoproteins, gaseous methyl-seleno compounds and contributes to the maintenance of homeostasia [63]. Moreover, selenium is definitely an essential element for many eukaryotes, bacteria and archaea [97-100]; its major role is in selenium-substituted amino acids, although it is also found in the active sites of some metalloenzymes.

In aerated environments, selenium can exist in several redox forms, including the elemental form Se⁰, which is solid, and the oxidized forms: selenate (Se^{V- IO_4)²⁻ and selenite (Se^{IV}O₃)²⁻. Since microorganisms are involved in the geochemical cycle of selenium, soil bacteria may transform the soluble forms Se⁶⁺ and Se⁴⁺ to insoluble Se⁰ or to volatile compounds (Se²⁻ selenide, dimethyl selenide...).}

Because of their proximity as group VIA elements, sulfur and selenium share many chemical and biochemical properties. In this regard, sulfate has been identified as an antagonist for selenate transport or toxicity in various microorganisms [101]. In the biogeochemistry of selenium [102,103], a selenium cycle has been proposed with reduction of selenate to selenite, selenium zero or sometimes selenide. The Se cycle [104–106] differs from the S cycle because $S^0_{(s)}$ is neither a product nor an intermediate in sulfate reduction, but arises from partial oxidation of sulfide [107].

Thin-section micrographs of *Rhodobacter sphaer*oides IL-106 grown under anaerobic photosynthetic conditions in the presence of 1 mM SeO_3^{2-} are shown in Fig. 3 [108].

Selenate and selenite seem to be the most abundant forms of bioavailable selenium in the environment and both can serve as electron acceptors. Like nitrate (denitrification), sulfate (sulfate reduction), and bicarbonate (methanogenesis), it is possible that selenate, selenite, and other oxidized metal or metalloids (Tc^{VII}, heavy metals, U^{VI}, Fe³⁺...) can oxidize deposited organic matter by dissimilatory processes and be implicated as electron acceptors in bacterial mineralization of organic matter in anoxic sediments. In bacterial anaerobic respiration, the organic matter concerned,

PHR

Se



Fig. 3. Inin-section micrographs of *Rhodobacter sphaeroides* IL-106 grown under anaerobic photosynthetic conditions in the presence of 1 mM SeO_3^{2-} . Arrows indicate the presence of electron-dense particles of selenium (Se). White particles correspond to polyhydroxybutyrate (PHB) granules. Reprinted by permission from American Society for Microbiology [108], copyright 2001.

usually acetate, succinate, lactate, is oxidized to CO_2 and is considered as an electron donor, as with, for example, the selenite or selenate electron acceptors:

$$4CH_{3}COO^{-} + 3SeO_{4}^{2-} \rightarrow 3Se^{0} + 8CO_{2} + 4H_{2}O + 4H^{+}$$
(1)

$$CH_{3}COO^{-} + H^{+} + 4SeO_{4}^{2-} \rightarrow 2CO_{2}$$

$$selenate acceptor$$

$$+ 4SeO_{3}^{2-} + 2H_{2}O$$

$$selenite$$
(2)

Several observations of $Se_{(s)}^0$ precipitation from selenate (as in Eq. (1)) in various bacterial cultures have been reported [109–113]. Selenate reduction to $Se_{(s)}^0$ by anaerobic bacteria growth on acetate electron donors with formation of carbon dioxide as the respiratory end product is the major sink for removal of selenium oxyanions in anoxic sediments and takes place with *Proteobacter* [114] and some species of *Pseudomonas* [111].

Bacterial $Se^0_{(s)}$ precipitation from selenite by several microbes, including algae [115] and bacteria [116], has been frequently reported. Microorganisms can reduce selenite to selenium, as is the case for the common aerobic soil bacterium *Bacillus subtilis* [117]. The accumulation of selenium by *R. sphaeroides* has also been reported [118]. *Ralstonia metallidurans* CH34 [119] reduces large amounts of selenite to elemental red selenium.

Macy et al. [120,121] isolated two organisms from a site with a high level of selenium; a *Thauera selenatis* reducing selenite to elemental Se⁰ and a *Pseudomonas* species which transforms selenate to selenite as in Eq. (2). Cells grown anaerobically in minimal medium on acetate plus selenate oxidized ¹⁴C-acetate to ¹⁴CO₂ with concomitant reduction of selenate to selenite and small amounts of Se⁰.

Multiple detoxification processes may occur during selenite reduction by microorganisms, since elemental selenium has been described as being deposited in the cytoplasm of *Wolinella succinogenes*, *D. desulfuricans* and *Pseudomonas alcaligenes* [122,123], in the periplasmic space with *E. coli* [124], and outside the cell for *Clostridium pasteurianum* and *E. cloacae* [125–128]. According to Tomei et al. [123], the particles containing elemental selenium found outside are related to cell lysis, while Losi and Frankenberger [126] suggested that the reduction reaction occurs close to the membrane of *E. cloacae*, possibly as a result of membrane-associated reductase(s), and that the precipitate is rapidly expelled by a membrane efflux pump. On the other hand, elemental selenium deposited inside or

outside cells has been described as being in spherical or spherical to oval-shaped structures [123,126], fibrillar and granular structures [129], amorphous aggregates [127] or selenium nanoparticles [128]. In *E. coli*, elemental selenium deposition has been observed both in the periplasmic space and in the cytoplasm. Kessi et al. [130] described the ability of *Rhodospirillum rubrum* to reduce selenite to Se⁰ and showed that elemental selenium was observed in the cytoplasm and could be expelled across the plasma membrane and the cell wall. *R. metallidurans* [119] can resist high concentrations of selenite (6 mM) and reduce selenite to elemental selenium, which accumulated essentially in the cytoplasm.

Several microorganisms, among which *T. ferroxidans*, can transform Se⁰ into volatile selenide. With selenate and selenite metabolized by *R. sphaeroides*, Corynebacterium [131], volatile products (as dimethyl selenide or dimethyl diselenide) account for only a small proportion of the accumulated selenium, most of which was present in organic form (selenomethionine) and as the red, elemental Se⁰ retained in or on the cell.

Several types of bacteria convert selenium compounds to volatile methylated forms and, in soils amended with organic or inorganic selenium, one observes the formation of volatile dimethyl selenide or dimethyldiselenide or of methyl selenite. This volatilization depends on microbiological activity, temperature, hygrometry, weather and the seasonal concentration of soluble selenium [132].

The immobilization of selenite and selenate by reduction to the much less bioavailable red elemental form of selenium may provide a useful approach for remediating selenium contamination [133]. The selenate or selenite bacterial respiration constitutes the major sink for selenium removal in anoxic sediments by sequestrering $Se^{0}_{(s)}$. The dissimilatory reduction of selenate to selenite could be responsible [107] for the relatively high levels of selenite in oxygenated ocean waters.

6.3. Cesium

¹³⁷Cs accounts for 65% of all isotopes activity in low-level wastes from the reprocessing plant at Sellafield, United Kingdom. ¹³⁷Cs persisted in a mobile form in the environment after the Chernobyl accident in 1986. It is an alkali metal, easily accumulated by microorganisms; ¹³⁷Cs reached a variety of trophic levels. Experiments on Cs uptake in *E. coli*, cyanobacteria, eukaryotic algae and fungi are detailed by Lloyd and Macaskie [56]. But currently ¹³⁷Cs is decontaminated using zeolithes (aluminosilicates), since microbial biosorbent offers low Cs uptake and no improvement compared to zeolithes.

6.4. Strontium

Biomineralization of ⁹⁰Sr has been demonstrated after exclusion from *P. fluorescens* [71] or *Citrobacter* and precipitation in the growth medium as crystalline strontium carbonate or insoluble phosphate, mediated via phosphatase activity. Several microorganisms can show Sr uptake (*Penicillium chrysogenum, Saccharomyces cerevisiae, Rhizopus*) [55,134]. Strontium is an alkaline earth metal like radium, from which ²²⁶Ra can be biosorbed by *Arthrobacter* [135] much more than by activated carbon.

6.5. Zirconium (group IV)

⁹³Zr ($T_{1/2} = 1.5 \times 10^6$ a) is present in some nuclear wastes and arises from fission, activation and corrosion of fuel cladding, but is considered to be biologically inert. In unreprocessed fuel, ⁹³Zr activity is about 20% of that of ⁹⁹Tc. Biosorption has been observed by microal-gae and cyanobacteria [136,137].

6.6. Cobalt and nickel (groups IX and X)

⁶⁰Co ($T_{\nu_2} = 5.27$ a) is generated by neutron activation. Iron-reducing bacteria are able to reduce from Co^{III} to Co^{III}. This valence is better absorbed by soils and offers possibility for in situ immobilization in contaminated areas [138].

⁵⁹Ni ($T_{1/2} = 7.6 \times 10^4$ a) arises from activation of fuel assemblies; microbial interactions have been almost unexplored, but stable nickel has been considerably studied and biosorption is poor with *Pseudomonas* algae, *Citrobacter* [70,139].

6.7. Iodine

The halogen ¹²⁹I ($T_{1/2} = 1.57 \times 10^7$ a) is a key radionuclide from nuclear wastes, with an important environmental impact because of its half-life, bioavailability and mobility. Little is known about its biogeochemistry. The species in the environment are iodide, iodine and iodate. Several microorganisms convert iodide I⁻ to volatile methyl iodide [140]. Enzymatic reduction of iodate (IO₃⁻) to iodide is possible with sulfate and Fe^{III}-reducing bacteria, and also by indirect mechanisms with the reducing compounds produced by bacteria (Fe^{II}, sulfide, iron monosulfide FeS) [141,142].

7. Actinides

Microbial interactions with actinides have been discussed in reviews by Banaszak et al. [143] and by Lloyd and Macaskie [56,74,144,145]. If ²³⁸U is the priority pollutant in medium and low-level radioactive wastes, other actinides can also be present (²³⁰Th, ²³⁷Np, ²⁴¹Pu, ²⁴¹Am). Actinide chemistry is complicated by the multiple oxidation states of most of the radionuclides, which govern their behavior in the environment. Fig. 4 shows the expected oxidation states for key actinides as a function of redox potential at pH 7.0. The standard redox potential of ferrihydrite/Fe²⁺ (approximately 0 V) [75] is more electronegative than the potentials for Pu^V/Pu^{IV}, Np^V/Np^{IV} and U^{VI}/U^{IV}. Thus Fe^{III}reducing bacteria have the metabolic potential to reduce these radionuclides enzymatically or via Fe^{II} produced from the reduction of Fe^{III} oxides.

Tetravalent actinides have high ligand complexing abilities [56] and are also immobilized in sediments containing active biomass [146]. Fe^{III}-reducing bacteria are able to transform actinides to cations more amenable to remediation, or to insoluble uraninite (UO₂) for the uranyl cation UO_2^{2+} . This is illustrated by the transformation of the highly soluble Np^V (NpO₂⁺), which is reduced to soluble Np^{IV} by *Shewanella putrefaciens*, with the Np^{IV} removed as an insoluble phosphate biomineral by a phosphate-liberating *Citrobacter* [147]. Fe^{III}-reducing bacteria can reduce Pu^{IV} to Pu^{III}, leading



Fig. 4. Expected oxidation states for key actinides as a function of redox potential at pH 7.0. Adapted by permission from Springer Verlag UK [143], copyright 1999.

to the solubilization of sediment-bound Pu^{IV} , but yield a trivalent actinide that is amenable to bioremediation with several microbially produced ligands [56]. In Fig. 4, one sees that Th^{IV} and Am^{III} are stable across most *Eh* values encountered in radionuclide-contaminated waters, but are removed easily through biosorption or biomineralization by several ligands, including biogenic phosphate [56].

7.1. Uranium

Uranium-bearing wastes from nuclear weapons production [148], mining [149], electronuclear production, and nuclear waste treatment have generally been disposed of in near-surface environments. Dispersion of toxic aqueous uranium species through groundwater is of great environmental concern. Consequently, uranium is a widespread groundwater contaminant at many sites where uranium was processed [150]. U^{VI}, the oxidized, soluble form of uranium, is mobile in groundwater. To prevent the spread of uranium contamination in the subsurface, possible methods could involve the reduction of mobile U^{VI} to U^{IV} , which is insoluble and precipitates [151–153]. The proposed reductive immobilization of uranium would be analogous to the formation of some subsurface uranium ores in which U^{VI} dissolved in aerobic groundwater enters an anaerobic zone, enriched in organic matter; U^{VI} is then chemically or biologically reduced to U^{IV}, which precipitates, forming the ore body [1,154]. Thus, uranium-contaminated subsurface environments receiving special enrichment in organic matter, chemical products of bacteria or microorganisms, could be places for precipitation of U^{IV} [155,156]. Although biooxidation of several metals including Fe^{II}, Mn^{II}, As^{III} is well documented [62], microbial oxidation of U^{IV} is not well explained and oxidation of Pu^{IV} or Np^{V} is not identified. Ehrlich [62] gives a detailed discussion concerning biosorption of various biomasses for different metals. There are also some examples of microbially-generated mineral deposits that have been known to sorb U^{VI}; for example, ferric (and manganese) oxides formed by a variety of microorganisms can sorb U^{VI} [62], like sulfide minerals, but with concomitant partial reduction of U^{VI} to U^{IV} in this case [157].

Geobacteraceae is frequently present among the natural populations near sites contaminated by uranium. Geobacteraceae have simple nutritional requirements and are able to fix atmospheric nitrogen. Even if abiological mechanisms for U^{VI} reduction can take place involving sulfides, Fe^{II} or hydroquinones [158,159], they seem limited compared to the naturally occurring, microbially-catalyzed U^{VI} reduction to U^{IV} in the subsurface [1,160,161].

Both Fe^{III}-reducing and sulfate-reducing microorganisms have been shown to precipitate uranium from contaminated waters [160,162]. The following equations show the reactions of various electron donors coupled to Fe^{III} and U^{VI} reduction under geochemical conditions containing 130 mM HCO₃⁻ [163].

 $\begin{array}{l} AH_2QDS \,+\, U^{VI}O_2(CO_3)_2^{2-} \rightarrow AQDS \,+\, U^{IV}O_2 \\ \\ +\, 2HCO_3^- \end{array}$

Several other microorganisms can use U^{VI} as an electron acceptor (e.g., *Shewanella putrefaciens*) and, in anaerobic conditions, acetate or hydrogen is oxidized with the concomitant reduction of U^{VI} to U^{IV} [151]. The black precipitate of U^{IV} as UO₂ (uraninite) is

The black precipitate of U^{IV} as UO_2 (uraninite) is very insoluble. Precipitate suspension after U^{VI} reduction indicates an extracellular amorphous mass of electron-dense material. Cells appear in some cases to be coated with enzymetically-reduced uraninite or produce, as for Spear et al. [164], amorphous uraninite particles. These amorphous and finely grained materials with nanometer-sized particles (Fig. 5) aggregated to form micrometer-sized particles easily disassociated from the cells [158]. This electron-dense precipitate is entirely extracellular with *Shewanella* [165] and with many other organisms [152–154,158–161]. No intracellular uranium was detected [75]. A uranium precipitate is absent from heat-killed suspensions. See an instance of this result in Fig. 6.

To date *Desulfovibrio vulgaris* is the only organism in which the enzyme system has been characterized: a tetraheme cytochrome C3 functions as a U^{VI} reductase in combination with hydrogenase, its physiological electron donor [161]. It has been postulated that electron transfer enzymes including hydrogenase are implicated in the U^{VI} reduction of some bacteria. As reduced U^{IV} is often observed in the space between the cytoplasmic membrane and cell wall, it is suggested that the terminal enzyme that donates electrons to U^{VI} is located at the outer surface of cytoplasmic membrane, or in a soluble form [166].



Fig. 5. Characterization of bioreduced uraninite (UO_2) nanoparticles. Transmission Electron Microscopy (TEM) image of flocculated UO_2 nanoparticles associated with *Desulfosporosinus* spp. bacteria (arrow). Inset: high-resolution TEM image of isolated particles. Reprinted by permission from Macmillan Publishers Ltd [178], copyright 2002.

Microorganisms that reduce U^{VI} in pure culture include a hyperthermophilic Archae [167], a thermophilic bacterium [168,169], mesophilic Fe^{III} and sulfate-reducing bacteria [151,160,161,164,170–172] and fermentive bacteria [66].

 U^{VI} is reduced by microbes in solutions containing organic or inorganic ligands or other cations [173, 174], or that contain other electron acceptors such as Fe^{III} oxides, sulfate, selenate [95,175,176]. A few studies have evaluated the impact of inorganic ions found



Fig. 6. Transmission Electron Microscopy (TEM) showing the U(IV) precipitates formed by *G. sulfurreducans* via enzymatic reduction of U(VI). TEM of thin sections of cells containing electron-dense U(IV) precipitated outside the cell and within the periplasm. Reprinted by permission from Taylor and Francis Group [75], copyright 2002.

in waste waters on uranium removal by bioreduction. Generally, high concentrations of bicarbonate, sulfate and nitrate ions appear to negatively impact the enzymatic removal of uranium [174,177]. Moreover, organic ligands influence the bioreduction of uranium. Organic compounds, through carboxyl, hydroxyl and other functional groups, interact spontaneously from coordinate-covalent bonds with metal cations, resulting in organic-metal complexes. Metal availability to assimilatory metal reduction bacteria is often affected by the presence of organic-metal complexes. Ganesh et al. [173] have evaluated four aliphatic ligands (acetate, malonate, oxalate, and citrate) and an aromatic one. The trends in uranium reduction varied with the nature and the amount of UVI-organic complex. Sulfatereducing bacteria rapidly reduced uranium from a monodentate aliphatic acetate complex. The reduction from multi-dentate aliphatic complexes (malonate, oxalate and citrate) was slower. Uranium from an aromatic complex was rapidly available for reduction. Selection of bacteria for rapid uranium reduction thus depends on the organic composition of the waste streams [179].

7.2. Thorium

Thorium is widely present in the environment at valency IV, so bioreduction is not possible, but, in presence of microorganisms, biosorption may occur. Biosorption of Th^{IV} on fungal masses has indeed been reported by several authors. The cation Th⁴⁺ is available only at low pH (~ 2). If the pH increases, the sorbed species will not be Th^{4+} , but species with one to four hydroxyl groups. At pH 4, hydrolyzed Th^{IV} forms colloidal Th(OH)4, increasing its uptake by some microorganisms. Andres et al. [180] studied thorium biosorption by Mycobacterium smegmatis at pH 1: the sorbed amount was 4% of the biomass dry weight and comparable to values for UO_2^{2+} with that strain. Some thorium removal as an insoluble biogenic phosphate has been reported [181], affording an interesting method for the precipitation of thorium phosphate in presence of NH₄⁺.

7.3. Neptunium

Neptunium-237 is an alpha-emitting radionuclide produced in ton quantities in nuclear reactors. It will eventually predominate in wastes after 10^4-10^7 years. It arises via decay of ²⁴¹Pu ($T_{1/2} = 14.9$ a) and ²⁴¹Am ($T_{1/2} = 433$ a) to ²³⁷Np ($T_{1/2} = 2.1 \times 10^6$ a). Neptunium-V forms complexes with ligands poorly and the α -emitting NpO₂⁺ cation is highly mobile, has high biological toxicity and is very difficult to remove from solution by physicochemical or biotechnical methods [182]. Np uptake is negligible with marine algae and microorganisms. So the solubility of Np does not have a seasonal cycle linked to microbial activity in a West-Cumbrian salt marsh [183], unlike Pu and Am. Nevertheless, biological reduction and removal of Np^V can be effected with *Shewanella putrefaciens* for reduction to Np^{IV} in conjunction with simultaneous precipitation as phosphate, produced by *Citrobacter* [56]. This preliminary study illustrates the potential for the biotechnological removal of Np^V, which is not easily achieved by chemical means.

7.4. Plutonium

Studies on the biogeochemistry of plutonium are far more challenging than those for the other actinides, due to its high radiotoxicity and complex redox chemistry. The most stable oxidation state of Pu is Pu^{IV}, but Pu^{III,V,VI} can also be found in the environment. Several studies attempted to understand the potential role of microorganisms in controlling Pu solubility. Peretrukhin et al. [182] utilized a stock solution of ²³⁹Pu of 2 mg/ml as the carbonate and added it to natural sediments. All the Pu mass was removed after 4 months, with only 34% of the removal being attributed to biosorption using heat-killed controls, implicating additional biochemical mechanisms. The reduction of Pu^{IV} to Pu^{III} was suggested by the authors and could be achieved by Fe^{III} -reducing bacteria. Pu^{IV} reduction may lead to solubilization of sediment-bound Pu^{IV}. and yield a trivalent actinide that reacts with a range of microbially produced ligands [56].

Pure cultures of *Rhizopus arrhizus* can cause bioreduction of Pu^{VI} and biosorption of Pu^{IV} at pH 4 and 9. The removal of Pu^{IV} as $Pu(OH)_4$ has been tested with *P. aeruginosa* immobilized on a plasma-treated polypropylene web [184]; from 1.7 nCi of Pu solution, up to 95% of the Pu was removed, but Pu removal (possibly as PuO₂) was particle-size dependent. A metal phosphate accumulating *Citrobacter* removed 50% of the ^{238/239}Pu from a 60 nM solution [185].

Although microorganisms have the potential to control the solubility of Pu in a range of environments, the precise mechanism of Pu-microbe interactions remains unclear [145].

7.5. Americium

Americium is stable in the trivalent oxidation state for a large range of environmental conditions. Studies of seasonal variations of actinides in water and sediments show that microbes play an important role in controlling Am^{III} solubility [79]. The summer solubility minima for Am^{III} correlate with maximal microbial biomass levels.

²⁴¹Am can be efficiently sorbed onto various algal biomasses at three orders of magnitude greater than for Pu. Biosorption of ²⁴¹Am by *R. arrhizus* was 40fold greater than that for Pu^{IV} at pH 2, comparable at pH 4 and less than Pu^{IV} at pH 7 [136]. Removal of 90% of ²⁴¹Am from a solution of 299 nCi/ml was observed with *Candida utilis* [145].

Enzymatically mediated biodeposition has been observed by Macaskie et al. [185] where phosphate biodeposition of ²⁴¹Am, with 100% removal from solution, was possible by *Citrobacter* sp.

Am is the most extensively studied of the trivalent actinides; some data for Cm^{III} show that it is partially removed by sterilized sediments and completely removed after 4 months from natural sediments [146].

8. Conclusion

The release of radionuclides from nuclear weapons' testing and from nuclear power plants in the environment is a subject of intense public concern. Low- and high-level chemical treatment methodologies for fuel reprocessing are well controlled operations, but nevertheless some nuclear wastes have to be stored in surface or subsurface deposits. From these deposits, in long timescale, radionuclides can migrate to the neighboring environment. The fate of an element, be it radioactive or not, depends greatly on microorganisms as it was revealed by the microbial diversity during Earth's history and their chemical effects on valencies of encountered metals(oids). The interaction of numerous microbial species with the key radionuclides coming from the fission of uranium constrains, most of the time, soluble products into non-soluble compounds. These products are easily isolated by man through bioremediation processes or are trapped into the geochemical environment. Their solubility and mobility properties are hopefully to be supervised by enzymatic transformation of radionuclides (biotransformation): uranyl UO_2^{2+} ion into U^{IV} as uraninite UO₂, pertechnetate TcO_4^- into Tc^{IV} as TcO_2 , selenite or selenate into elemental selenium Seo or gaseous forms of selenium. When reduced species are not intrinsically insoluble, appropriate ligands can change that. Therefore microorganisms, some acting under extreme environmental conditions, should help to confine or at least limit the spreading of radioactivity in the environment.

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