Microbial Transformations of Plutonium and Implications for Its Mobility

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Abstract

The current state of knowledge of the effect of plutonium on microorganisms and microbial activity is reviewed, and also the microbial processes affecting its mobilization and immobilization. The dissolution of plutonium is predominantly due to their production of extracellular metabolic products, organic acids, such as citric acid, and sequestering agents, such as siderophores. Plutonium may be immobilized by the indirect actions of microorganisms resulting in changes in Eh and its reduction from a higher to lower oxidation state, with the precipitation of Pu, its bioaccumulation by biomass, and bioprecipitation reactions. In addition, the abundance of microorganisms in Pu-contaminated soils, wastes, natural analog sites, and backfill materials that will be used for isolating the waste and role of microbes as biocolloids in the transport of Pu is discussed.

Key Words

plutonium, microorganisms, nuclear waste, waste repository sites, toxicity, mobilization, immobilization, microbial metabolites, bioaccumulation, bioprecipitation, biocolloids

Introduction

There is considerable interest in understanding the behavior of plutonium in the environment because of its presence in surface soils at low concentrations (0.07-0.4pg/g) due to fallout from

nuclear testing, low-level radioactive wastes, contaminated soils and sediments from weapons production, and the disposal of Pu-containing nuclear wastes in deep geological formations. Plutonium occurs in soils and wastes as soluble- (organic- and inorganic-complexes) or insoluble-oxides and hydrolysis products of Pu(IV). The long half-life of Pu (>24,000 years) and the presence of microbes in groundwaters, sediments, soils, backfill materials, and wastes, and those introduced through mining operations, could potentially alter the mobility and stability of Pu.

Microorganisms may affect the solubility, bioavailability, and mobility of actinides by direct enzymatic or indirect nonenzymatic actions. These include (i) oxidation-reduction reactions, (ii) changes in pH and Eh, (iii) chelation, or the production of specific sequestering-agents, (iv) biosorption/bioaccumulation by biomass and biopolymers, (v) biocrystallization (precipitation and mineral formation), and (vi) biotransformation of actinides complexed with inorganic- and organic- (synthetic and naturally occurring) ligands. Solubilization of Pu could result from the formation of complexes with metabolites excreted by soil microorganisms. The microbes in the groundwater flowing through waste sites could adsorb radionuclides on to their cell surfaces or accumulate them within the cell, and eventually transport them away from the disposal sites. Although the microbial transformations of actinides, in particular of uranium, has been extensively studied, we have only limited information on the interactions of Pu with microorganisms.

Plutonium exists in several oxidation states (III, IV, V, VI, VII); the more stable ones are III, IV, and VI. The solution chemistry of plutonium is very complex. It undergoes disproportionation reactions and can exist simultaneously as Pu(IV), Pu(V), and Pu(VI) under oxidizing conditions. Although the role of microorganisms in the oxidation and reduction of U, Fe, and Mn has been extensively studied, there is little evidence to implicate that they are directly involved in the oxidation/reduction of Pu (Fig. 1). However, the microbes may indirectly affect the oxidation state and solubility of Pu by changing the Eh and pH of the medium, as well as by

producing sequestering agents. The chemical form of Pu (e.g., oxide, ionic, organic- or inorganic-complexes, co-precipitates), the availability of electron donors, electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, organic compounds), nutrients (nitrogen, phosphorus), and environmental factors (pH, Eh, temperature, moisture) all can affect microbial activity and the biotransformation of Pu. The extent of dissolution of Pu could rise considerably with an increase in microbial activity, particularly under anaerobic conditions due to the formation of stable complexes with metabolites and organic degradation products, or immobilization due to precipitation.

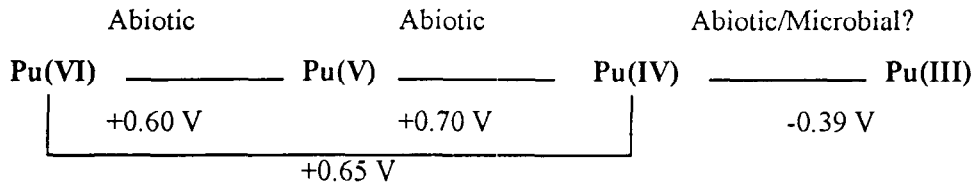

Figure 1. Plutonium oxidation states and redox potentials at pH 8 (Choppin, 1999).

Effect of Pu on Microorganisms

The effects of Pu on pure cultures of bacteria, actinomycetes, fungi, and mixed cultures of bacteria and soil microorganisms have been investigated and these studies have generally focussed on radiation effects versus metal toxicity, and the form and solubility of Pu.

Table 1 lists the effects of Pu on pure and mixed cultures of bacteria and fungi. The death of cells of *Salmonella typhimurium* exposed to ^{239}Pu citrate and the induction of mutations were an exponential function of radiation dose (Gafieva and Chudin, 1988). The growth of halophilic bacteria *Halomonas* sp. and mixed bacterial cultures consisting of *Haloarcula sinaiiensis*, *Alteromonas* sp, *Marinobacter* sp, and an unclassified species of *γ-proteobacterium* was retarded by ^{239}Pu-EDTA at concentrations > 1.0×10^{-5}M (Pansoy-Hjelvik, et al, 1997; Francis et al., 1998) (Figs. 2 and 3). Epifluorescence micrographs of *Halomonas* sp (WIPP-1A) showed a decrease in

cell numbers with increasing Pu concentration (> $1.0x10^{-6}$M) and also changes in cellular morphology with initial rods becoming shorter and more coccoid in appearance (Pansoy-Hjelvik, et al.1996). Wildung and Garland (1982) investigated the effects of Pu concentration, form, and specific activity on microbial types, and microbial activity (CO_2 evolution rate) in soils amended with carbon and nitrogen. The effects of Pu differed with type of organism and incubation time. For example, after 30 days of incubation, aerobic spore-forming bacteria and anaerobic bacteria decreased significantly by Pu levels in soil as low as 1 μg/g when Pu was added as hydrolyzable $^{239}Pu(NO_3)_4$ (solubility <0.1% in soil). Other classes of organisms, except the fungi, were affected at Pu levels of 10 μg/g; effects on fungi were seen only at levels of 180 μg/g. The effect of Pu on fungal colony-forming units was a function of its solubility in soil and specific activity; Pu-DTPA was effective at 1 μg/g. At concentration of 180 μg of Pu/g of soil the rate and extent of CO_2 evolution decreased in soils amended with carbon and nitrogen sources.

Radiation and Heavy Metal Effects (Pu Isotopes Effect). It was shown that the effects of ionizing radiation, rather than chemical effects, are the predominant cause of toxicity when bacteria are exposed to the higher activity isotopes of plutonium (Wildung and Garland 1982; Banazak et al 1999). For example, Wildung and Garland (1982) examined the effects of exposing soil fungi to ^{239}Pu and ^{238}Pu; two plutonium isotopes were used to differentiate between the chemical and radiolytic contributions to toxicity. In soils receiving ^{238}Pu-DTPA (0.6μg Pu/g soil) and ^{239}Pu-DTPA (180μg Pu/g soil), fungal colony forming units (CFU) were markedly reduced, relative to the controls. These treatments differentiated Pu concentration by a factor of 300 but they had equivalent radioactivity levels (10μCi/g) indicating that toxicity was due to radiation effects rather than to chemical effects. An analogous comparison of $^{239}Pu(NO_3)_4$-and $^{238}Pu(NO_3)_4$-treated soil at the 10μCi/g level showed the same effect, but toxicity at both mass levels was not as pronounced as in the DTPA-treated soils, reflecting the importance of solubility, as well as radiation level, on

toxicity (Wildung and Garland, 1982). Banaszak et al (1999) investigated the effect of Pu(IV)-NTA on *Chelatobacter heintzii*. When ^{239}Pu was the source of ionizing radiation, the loss of viability was caused by radiolytic effects, rather than chemical ones. At the same Pu concentration, deaths were much greater in ^{239}Pu exposures than in ^{242}Pu. No difference was noted between the exposures to 10^{-5} M ^{242}Pu and 10^{-6}M ^{239}Pu, which had the same activity but differed in concentration by an order of magnitude.

Dissolution of Plutonium by Microorganisms

Dissolution of the actinides can be brought about by direct and indirect actions of microorganisms. Direct action involves enzymatic oxidation to a higher valence or reduction to a lower valence state thereby affecting their solubility. Indirect dissolution involves the production of mineral acids, organic acid metabolites, oxidizing agents, and lowering the pH of the medium; thus, sulfuric acid is generated from the oxidation of sulfide minerals by autotrophic bacteria, organic acids from the metabolism of organic compounds by heterotrophic bacteria, and also metal-sequestering agents, such as siderophores, are elaborated. An increase in the activity of heterotrophic microbes due to the biodegradation of organic compounds can increase the solubility of the actinides. Several mechanisms have been proposed for such solubilization, including the production of organic acids, and of chelates. Often, a combined effect is important; for example, organisms may secrete organic acids which have a dual effect in increasing actinide dissolution by lowering pH, and by forming complexes. Microbially produced dicarboxylic acids, hydroxycarboxylic acid, polyhydroxy acids, and phenolic compounds, such as protocatechuic acid and salicylic acid, are effective chelating agents. A wide variety of heterotrophic microorganisms produce organic-acid metabolites, such as oxalic, isocitric, citric, succinic, hydrobenzoic, and coumaric acids which can form complexes with Pu via their carboxylic and phenolic groups.

Siderophores and extracellular microbial metabolites can effect the dissolution of actinides from their insoluble phases.

(i) Reductive Dissolution of Pu(IV) to Pu(III). Plutonium is present in the environment mostly as the oxides and hydroxides of Pu(IV) that have low solubility. The mobility and bioavailability of Pu(IV) is limited by its solubility. The solubility products of plutonium(IV) oxyhydroxide are estimated to be $10^{-56.8}$ and $10^{-57.8}$ The solubility of plutonium(III) hydroxide is much greater, $K_{sp}=10^{-22.6}$. Consequently, the reduction of Pu(IV) oxyhydroxides to Pu(III) is expected to increase the solubility of plutonium in the environment. The electrochemical potential for this reduction reaction is similar to that for iron and other metals known to be reduced by many microorganisms. The similarity in reduction potentials for hydrous PuO_2 and α-FeOOH suggest that those microbes able to reduce iron and other metals also might reduce Pu(IV) oxyhydroxides under similar conditions and, thus, reductively solubilize Pu(IV) to Pu(III). The persistence of the reduced soluble Pu(III) is affected by its stability, as well as by its interactions with organic and inorganic materials. Rusin et al (1986) showed that an iron-reducing bacterium *Bacillus* sp solubilized up to 90% hydrous PuO_2(s) in about 7 days under anaerobic conditions in the presence of nitrilotriacetic acid (NTA). In these studies, Pu(III) was present as a Pu-NTA complex. However, without NTA, only 40% of Pu was solubilized suggesting that a strong complexing agent is needed to keep the Pu in solution. Little dissolution of $PuO_2$was observed in sterile culture media or in the presence of non-iron-reducing bacteria, such as *Escherichia coli*.

(ii) Dissolution of Plutonium by Microbial Siderophores. Siderophores are low-molecular-weight iron chelators produced by microbes in response to a limited availability of soluble iron. As Pu(IV) and Fe(III) have chemical and biochemical similarities, and because of the ubiquity of siderophore-producing microbes, iron-sequestering agents produced by such microorganisms could be important in the complexation of Pu and other actinides, thus increasing

their solubility and bioavailability. Desferal, a polyhydroxamate chelate produced by microorganisms enhanced the dissolution of PuO_2 (Barnhart et al, 1980). The production of extracellular chelating agents resembling siderophores was observed for *Pseudomonas aeruginosa* grown in the presence of uranium and thorium (Premuzic et al, 1985). Brainard et al. (1992) determined rate-constants for the solubilization of hydrous $PuO_2(s)$ by the siderophores, enterobactin and desferrioxamine B, and by selected carboxylate amino polycarboxylate, and catecholate ligands. The rate constants showed that siderophores are extremely effective in solubilizing actinide oxides: on a per-molecule basis, enterobactin was $\sim 10^3$ times more effective than the other chelators tested in increasing the rate of solubilization of hydrous plutonium oxide. Notably, ferric siderophore complexes solubilize actinide oxides more effectively than the siderophores in the absence of iron.

(iii) Dissolution of Plutonium by Microbial Extracellular Metabolites. 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. These compounds also may be involved in transporting plutonium into the cells (Wildung and Garland, 1980; Wildung et al, 1987; Beckert and Au, 1976). 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. In the presence of known microbial metabolites and synthetic ligands (DTPA, EDTA, EDDHA), Pu(VI) was reduced to Pu(IV) before complexation, suggesting that the latter valence state would be the dominant one associated with organic complexes in soils. Studies with selected organisms indicated that Pu was both actively transported into, and sorbed on the cell, and these phenomena, as well as complexation of Pu by extracellular metabolites, were a function of the form

of Pu supplied (hydrolyzed and complexed forms), the organisms' type and growth characteristics, and the ability of the organism to alter extracellular pH (Wildung et al., 1987).

Immobilization of Plutonium

Plutonium may be immobilized by microorganisms through the following reactions: bioreduction, bioaccumulation, biosorption, precipitation, and mineral formation. In nature, bacteria interact with metal ions to immobilize and concentrate them, eventually generating minerals. Microbial biofilms bind significant quantities of metallic ions, and also serve as templates for their precipitation. These processes have received considerable attention because of their potential for bioremediating radionuclide-contaminated sites and waste-streams. Although the biochemistry of the interactions of toxic metals and uranium with bacterial cell walls, extracellular biopolymers, and microfossil formations has been extensively studied, we have limited information on microbial immobilization of Pu (McLean et al, 1996; Mackaskie et al, 1996; Francis 1998). Of particular concern is the long-term stability of the immobilized actinides which may undergo subsequent remobilization.

(i) Biosorption/Bioaccumulation of Plutonium. Biosorption is essentially a chemical action whereby the biomass acts as a surface upon which metals bind by ligand interactions or by ion exchange. The binding of metal ions to the surface of microorganisms can be described by Freundlich-, Scatchard-, or Langmiur-plots using data for metal concentrations bound and in solution. Both living and dead microorganisms possess abundant functional groups on their cell surfaces' that bind metal ions. Microbial surfaces consist of petidoglycon/techoic acid layers with an overall negative charge due to hydrophilic anionic functional groups, such as phosphate, carboxylate, and hydroxyl moieties, giving bacteria considerable ability to bind actinides (McLean et al., 1996). Polymers secreted by many metabolizing microbes also immobilize metals.

Desorption and recovery of the biosorbed radionuclides is easy. In contrast, bioaccumulation is a metabolically active process, usually occurring by an energy-dependent transport system in viable or growing cells where in the metals are taken up into cells and sequestered intracellularly by complexation with specific metal-binding components or by precipitation. All classes of microorganisms accumulate metals intracellularly. Localizing the metal within the cell permits its accumulation from bulk solution, although the metals cannot be easily desorbed and recovered. Radionuclide-binding to cell surfaces and polymers is a promising technology for remediating contaminated waters (Volesky and Holan,1995; McLean et al, 1996; Macaskie et al, 1996; Macaskie and Basnakova, 1998).

Kauri et al. (1991) investigated the ability of five strains of bacteria isolated from soil in Japan that had been contaminated with Pu by fallout for more than 40 years to bind low concentrations of Pu^{4+} during their growth. Although Pu was associated with all the bacterial strains studied, the association varied with the type of bacterial isolate indicating differences in the mechanisms of binding.

The interactions were examined between of ^{241}Pu nitrate and *Halomonas* sp. (from the WIPP site) and *Acetobacterium* sp., isolated from alkaline groundwater at the Grimsel Test Site, Switzerland, at pH 5.0. Although it was not verified, Pu was most likely in the pentavalent oxidation state. Both cultures were grown to late log-phase, washed in the appropriate electrolyte, and diluted to an OD of 0.4. *Halomonas* sp. biosorbed 9% (0.17×10^{-9} M) of the total ^{241}Pu in solution at the highest concentration tested (1.8×10^{-9} M) (Gillow et al. 2000). *Acetobacterium* sp. biosorbed 7% (0.11×10^{-9} M) of the total ^{241}Pu in solution at the highest concentration tested (1.5×10^{-9} M) (Gillow et al., 2000). On a dry-weight basis, *Acetobacterium* sp. sorbed 145 ng $^{241}Pu\ g^{-1}$ dry cells, and *Halomonas* sp. sorbed 351 ng $^{241}Pu\ g^{-1}$ dry cells. For both cultures, the amount of ^{241}Pu sorbed appeared to be a function of the ^{241}Pu added; however, there was no decrease in uptake

with increasing ^{241}Pu concentration tested in this study, so that, the cells probably could sorb more ^{241}Pu (surface sorption sites were not saturated). The extent of biosorption depends upon the form and chemical speciation of the Pu species present, with only a fraction of it being bioavailable in these studies.

Giesy et al. (1977) investigated the effect of naturally-occurring organics on Pu uptake by the fresh-water bacterium *Aeromonas hydrophila* and by the alga *Scenedesmus obliquus*. The organic matter was concentrated from the waters of Skinface Pond, near Aiken, South Carolina, and separated by membrane ultrafiltration into four nominal diameter-size fractions (F I > 0.0183; 0.0183 > F II > 0.0032; 0.0032 > F III > 0.009; F IV <0.009 μm). Each fraction was introduced into cultures of *Scenedesmus obliquus* and *Aeromonas hydrophila* at the concentrations found in nature. ^{237}Pu uptake was determined in log phase cultures after 6 h incubations. The initial Pu concentration in each flask was 1.1×10^{-4} μCi/mL $^{237}Pu(NO_3)_4$. Fractions I and II reduced ^{237}Pu uptake by *S. obliquus*, F IV increased uptake, and F III had no effect. ^{237}Pu uptake by *A. hydrophila* was no different in the presence of F I, F II, or F III than in tryptic-broth medium alone, whereas F IV increased its ^{237}Pu uptake.

The sorption of ^{239}Pu from aqueous nitrate medium was studied using the fungus biomass *Rhizopus arrhizus*. The biosorption of ^{239}Pu was maximal at pH 6-7, and this fungal biomass appears to be a promising sorbent treating radioactive effluents from the nuclear industry (Dhami et al. 1998). Li et al. (1995) showed that under optimal conditions a strain of *Rhizopus* species removed 99% of the ^{239}Pu from the wastewater. Alpha-energy spectral analyses in combination with SEM observations showed that the absorption occurred mainly on the cell walls. In most studies, it was not possible to determine whether the plutonium was incorporated into the cell, or was simply adsorbed onto the external cell surface.

(ii) Pu Uptake by Mycelium and Transport to Fungal Spores Studies were carried out on

the influences of different chemical forms and concentrations of ^{238}Pu in culture mediumat pH 2.5 and 5.5 on the uptake and transport of Pu by the mycelium to the spores of the common fungus *Aspergillus niger*. (Fig. 4). When Pu was added to the culture medium as dioxide microspheres, or as Pu-nitrate, or Pu-citrate, was transported to the spores; there was an almost linear relation between its transport and concentration. Raising the pH of the culture medium from 2.5 to 5.5 generally increased the transport of all three chemical forms. At Pu concentrations of 224 pCi/g, at both pH 2.5 and 5.5, the transport of Pu to the spores was approximately 3-fold greater from the nitrate- or citrate- form as from the dioxide microspheres. The derived transport factors indicated that Pu was concentrated in the mycelium and then further transported to the aerial spores of this fungus. A new, simple technique for collecting spores was developed to prevent cross-contamination of the spores with mycelial fragments, and by direct contact with the Pu-containing agar medium. The specific activities of the spores grown at pH 5.5 generally were at least twice those of the spores grown at pH 2.5. The uptake of Pu dioxide was approximately 33% of that from the nitrate- and citrate-forms at both pH levels. These findings suggest that this common soil fungus may solubilize soil-deposited Pu and render it more bioavailable for higher plants and animals (Au and Beckert, 1975; Beckert and Au, 1976). If a similar process occurs in Pu-contaminated soils, it could be an important link in transferring of soil-deposited Pu to humans and would also explain the apparent time-dependent increases in the uptake rate of Pu by plants grown in contaminated soils (Au, 1974).

Biotransformation of Plutonium-organic Complexes

Plutonium forms very strong complexes with a variety of organic ligands. Naturally occurring organic complexing agents, such as humic and fulvic acids, and microbially produced complexing agents, such as citrate, and siderophores, as well as synthetic chelating agents can

affect the mobility of Pu in the environment. Chelating agents are present in wastes because they are widely used for decontaminating nuclear reactors and equipment, in cleanup operations, and in separating radionuclides. The types of organic complexing agents used are carboxylic acids, such as citric-, hydroxy-acetic-, oxalic-, and tartaric- acids, and amino-carboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), and N-hydroxyethylene-diaminetriacetic acid (HEDTA). Many of these metal chelates either are poorly biodegraded aerobically, or undergo little anaerobic biodegradation; their biodegradation should precipitate the released ions as water-insoluble hydroxides, oxides, or salts, thereby retarding their migration. Although the biodegradation of synthetic chelating agents complexed with toxic metals has been investigated, little is known of the biotransformation of Pu complexed with natural organic compounds and chelating agents.

Effect of Microorganisms in Radioactive Wastes and Implications for Mobility of Plutonium

Microbial effects on low-level radioactive wastes disposed of in shallow land burial grounds and their potential effects in deep geological formations are of considerable interest from the standpoint of environmental remediation of contaminated sites and for assessing the performance of waste repositories. Microorganisms have been detected in backfill materials, natural analog sites, Pu-contaminated soils, low-level radioactive wastes, and waste-repository sites slated for high-level wastes (Tables 2-5).

Low-level radioactive wastes, and transuranic (TRU) wastes contain low levels of Pu in addition to other radionuclides and organic compounds (Francis 1990a,b). $^{238, 239, 240}Pu$ (gross alpha activity 1.7×10^5 pCi/L) were detected in leachate samples collected from the low-level radioactive-waste disposal sites: West Valley, NY, and Maxey Falts, KY (Husain et al. 1979., Weiss et al. 1979; Weiss and Colombo, 1980; Cleveland and Rees, 1981). Several aerobic and

anaerobic bacteria were isolated from the leachate samples; among them, were *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp. The radioactivity and the organic chemicals present in the leachate were not toxic to the bacteria which metabolized them producing-tritiated and carbon-14 methane (Francis et al., 1980a, b., 1990b).

Viable metabolically active microbes were detected at the Los Alamos National Laboratory (LANL) TRU waste burial site containing ^{239}Pu contaminated soil and flammable waste (Barnhart et al., 1980). Radiation-resistant bacteria are constantly being enriched in such environments containing nanocurie levels of alpha- and beta-activity (Barnhart et al., 1980). The potential effects of microorganisms on the long-term storage of radioactive waste could be significant. These effects include radionuclide mobilization by microbially produced chelates which can mediate dissolution of essentially insoluble plutonium dioxide, and possibly by gas pressurization of radioactive-waste storage vessels or enclosures due to microbially produced gases, such as carbon dioxide, methane, N_2O, and N_2. An analysis of the experimental results of actual and simulated waste-degradation studies showed that microorganisms produce far more gas than that produced by physical and chemical means including corrosion (Molecke, 1979).

Plutonium is the major radioactive component of concern in the TRU waste disposed of in the WIPP repository. Seventy-percent of this waste is cellulosic material. Biodegradation of cellulose under the hypersaline conditions in the repository can produce CO_2 and methane gas, as well as affect the solubility of Pu. Microbially produced gases could have significant ramifications for the long-term stability of the repository (up to 10,000 years). On the other hand, solublized Pu may affect the microbial metabolism. The rates of microbial respiration during the decomposition of several transuranic-contaminated waste materials were measured under environmental conditions representative of a geologic waste-repository in bedded salt. The major effect of activity on organic-matrix wastes was the generation of CO_2 gas. The experimental variables

studied included incubation temperature (25° to 70°C), atmosphere (aerobic and anaerobic), moisture content, brine content, and Pu level (0 to 40 μCi of alpha activity per g of waste). The maximum rate of evolution of CO_2 was 5.7 μg/day per g of waste. The addition of 300 mg (20 μCi) of defense-grade PuO_2 per g of waste reduced the rate of CO_2 generation by approximately 70%. The results indicate that microbes in existing drums of defense-related transuranic wastes have the potential to generate significant quantities of gas, both aerobically and anaerobically. CO_2 was the only gas detected in these studies (Caldwell, et al., 1988).

Kudo et al. (1997) investigated the interactions between sulfate-reducing anaerobic bacteria and Pu, in the presence and absence of bentonite, a backfill material for isolating of nuclear waste. The bentonite was obtained from Japan, and the anaerobic sulfate-reducing bacteria were isolated from a pulp- and paper-wastewater treatment plant. Distribution coefficients (Kd, in mL/g) were used as indices of radionuclide behavior. Pu Kd values for living bacteria ranged from 1804 to 112,952, depending on the pH, while the Kd values for dead bacteria were 1180 to 5931. Furthermore, higher Kd values of 39,677-106,915 for living bacteria were obtained for pHs between 6.83 and 8.25, while pH had no effect on the dead bacteria. These Kd values were obtained by using tracers for both ^{236}Pu and ^{239}Pu, to check the experimental procedures and for mass-balance calculations. Although the oxidation state of Pu was not specified, it is presumed to be the tetravalent state. In contrast, Pu Kd values of mixtures of living bacteria with bentonite, and sterilized (dead) bacteria with bentonite were 1194 to 83,648 and 624 to 17,236 respectively. The presence of live anaerobic bacteria with bentonite increased the Kd values of ^{239}Pu by 50-fold, in comparison to the mixture of dead bacteria with bentonite. The Pu Kd values for bentonite alone, both nonsterilized and sterilized, were approximately 10,000. The results suggest that anaerobic bacteria within the engineered barrier system (in this case, bentonite) will play a significant role in modulating the behavior of Pu in geologic repositories.

Biocolloids

Groundwater is expected to be the principal medium for the movement of radionuclides from the repositories. Colloidal transport of radionuclides is recognized as a potential mechanism of migration, and includes mineral fragments, humic substances, intrinsic colloids, and microorganisms. Significant numbers of bacteria exist in all subterranean environments including extreme environments, such as deep-sea hydrothermal vents, hot springs, and deep aquifers. They are present in the groundwaters of all the repository environments investigated, they fall within the colloidal size range of 1 nm to 1 μm, and can be transported with bulk-liquid flow. 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 biocollids. Microorganisms are important sources of colloids, as are their metabolic by-products and exocellular polymers. Microbes bioaccumulate and biosorb actinides intracellularly or extracellularly. Free-living bacteria are mobile suspended particles that may have radionuclide-sorbing capacities higher than that of the surrounding mineral phases. We have only limited information on the influence of microorganisms on the mobilization or immobilization of Pu in near-field and far-field environments.

Transport from the waste site can be facilitated by free-living suspended bacteria through bioaccumulation and biosorption. The chemical form of the Pu, i.e., its ionic-, organic-, and inorganic-complexes and its solubility affect its bioavailability to bacteria. Francis et. al. (1998) evaluated the interactions of dissolved ^{239}Pu (as Pu-nitrate, -perchlorate and -EDTA complex), under anaerobic conditions with a pure culture and a mixed culture of halophilic bacteria isolated from the WIPP repository environs to evaluate the potential transport as biocolloids from the waste site. The sizes of the bacterial cells ranged from 0.54 x 0.48 μm to 7.7 x 0.67 μm. The association

of Pu with suspended bacterial cells (10^6 to 10^9 cells ml^{-1}) at various growth phases in a medium containing NaCl or $MgCl_2$ brine was determined by sequential microfiltration. The amount of Pu associated with the suspended cell fraction (calculated as mol $cell^{-1}$) was very low (Pu 10^{-18} - 10^{-21}), and it varied with the particular bacterial culture (Fig. 5). These results support other studies which assessed the influence of bacteria on the migration of radionuclides from a deep spent-fuel repository, based on total number of bacteria present; they also concluded that negligible amounts of the released radionuclides bound to unattached bacteria (Pedersen, 1996). These studies also show an insignificant association of actinide with suspended bacteria suggesting that the other mechanisms may play a major role in regulating the mobility of actinides.

Summary

Microorganisms have been detected in Pu containing low-level radioactive wastes, transuranic wastes, Pu-contaminated soils, and in waste-repository sites under consideration for the disposal of nuclear waste. In general, Pu > $1.0x10^{-5}$M seems to affect most of the microorganisms studied. Its toxicity is due to radiation effects rather than metal toxicity, and is modulated by the chemical form and solubility of Pu. Dissolution of Pu by microorganisms is brought about by their production of organic acids, such as citric acid, extracellular metabolites, and siderophores. Immobilization of Pu by microbes may be due to indirect action by changing the Eh of the environment, and facilitating abiotic precipitation of Pu by reduction from higher to lower oxidation state, biosorption by bacteria, and bioprecipitation reactions. The chemical form and the type of the association of Pu with the bacteria has not been elucidated. Free-living bacteria suspended in the groundwater fall within the colloidal size range and may have Pu-sorbing capacity, giving them the potential to transport Pu in the subsurface. To date, we have only limited information on the interactions of microorganisms with Pu. Additional studies are needed to fully

evaluate the role of microbes in the mobilization and immobilization Pu in the environment and at the waste sites.

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Legends

Table 1. Concentrations of Pu affecting the growth or activity of microorganisms.

Table 2. Abundance of microorganisms in Pu-contaminated soils and radioactive wastes.

Table 3. Bacteria detected in radioactive waste repository environments.

Table 4. Number of bacteria at the natural analog sites.

Table 5. Bacterial population in backfill materials proposed for use at the radioactive-waste repositories.

Fig. 1. Plutonium oxidation states and redox potentials at pH 8 (Choppin, 1999).

Fig. 2. Effect of ^{239}Pu (1.0×10^{-5} M) on the growth of a pure bacterial culture, *Halomonas* sp. in brine. Pu(V) was added as a 1:1Pu-EDTA complex to the growth medium. Uninoculated medium containing Pu served as the control (redrawn from Francis et al., 1998; Pansoy-Hjelvik et al.,1997).

Fig. 3. Effect of ^{239}Pu on the growth of a mixed bacterial culture (*Haloarcula sinaiiensis*, *Marinobacter hydrocarbonoclasticus*, *Altermonas* sp., and a γ-proteobacterium) in brine. Pu(V) was added as a 1:1Pu-EDTA complex to the growth medium. Uninoculated medium containing Pu served as the control (redrawn from Francis et al., 1998; Pansoy-Hjelvik et al.,1997).

Fig. 4. Uptake of various chemical forms of plutonium added to agar medium by *Aspergillus niger* spores (reproduced from Beckert and Au, 1976, with permission).

Fig. 5. Association of ^{239}Pu with the bacterial cell fraction: Open bar is total ^{239}Pu in the unfiltered fluid column; shaded bar is ^{239}Pu associated with the cell fraction (0.4 μm filter fraction); open circle is the nunber of suspended cells (a) pure culture, *Halomonas* sp., and (b) mixed culture of bacteria (*Haloarcula sinaiiensis*, *Marinobacter hydrocarbonoclasticus*, *Altermonas* sp., and a γ-proteobacterium), (reproduced from Francis et al., 1998, with permission).

Table 1. Concentrations of Pu affecting the growth or activity of microorganisms

Microorganism	Pu form	Concn. (mM)	Quantity (ppm)	Activity (μCi/g or ml)	Ref
Salmonella typhimurium	^{239}Pu-citrate	NA	NA	NA	1
Chelatobacter heintzii	^{239}Pu-NTA	>0.001	>1.0	0.05	2
	^{242}Pu-NTA	>0.01	>10	0.50	2
HALOPHILIC BACTERIA (in liquid culture medium)					
Halomonas sp (WIPP-1A)	^{239}Pu-EDTA	>0.01	>2.5	>0.15	3
Mixed culture*	^{239}Pu-EDTA	>0.001	>0.25	>0.015	3
SOIL BACTERIA					
Aerobes	^{239}Pu$(NO_3)_4$	>0.04	>10.0	0.50	4
Spore formers	^{239}Pu$(NO_3)_4$	>0.004	>1.0	0.05	4
Anaerobes	^{239}Pu$(NO_3)_4$	>0.004	>1.0	0.05	4
Spore formers	^{239}Pu$(NO_3)_4$	>0.04	>10	0.50	4
SOIL FUNGI					
Fungi	^{239}Pu$(NO_3)_4$	>0.72	>180	10.0	4
Fungi	^{239}Pu-DTPA	>0.04	>10	0.50	4
Fungi**	^{238}Pu$(NO_3)_4$		0.6		4
Fungi**	^{238}Pu-DTPA		0.6		4
SOIL ACTINOMYCETES	^{239}Pu$(NO_3)_4$	>0.004	>1.0	0.05	4

NA - not available

* Mixed culture consisted of *Haloarcula sinaiiensis*, *Alteromonas* sp, *Marinobacter* sp, and *γ- proteobacterium.*

** The specific activity of ^{238}Pu is 300 times greater than that of ^{239}Pu. ^{238}Pu$(NO_3)_4$ and ^{238}PuDTPA caused a significant reduction in the growth of fungi measured as CFU(colony forming units) compared to ^{239}Pu$(NO_3)_4$ and ^{239}PuDTPA. The effect of the soluble Pu-DTPA complex was much more pronounced than that of Pu$(NO_3)_4$..

Reference: 1. Gafieva and Chudin 1988; 2. Banaszak et al., 1999; 3. Francis, et al., 1998; 4. Wildung and Garland, 1982.

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Table 2. Abundance of microorganisms in Pu-contaminated soils and radioactive wastes

Sample Source	Plutonium concentration	Microorganisms detected	Reference
SOIL			
Nevada Test Site Area 13 (Soil pH 8.4 - 9.2)	NA	Bacteria 2.9 ± 0.3 to 8.0± 0.7 x 10^6/g Fungi 3.1= 0.7 to 24.6 + 2x10^3/g	Au & Leavitt, 1982
Los Alamos (LANL) TRU waste shallow burial siteTA-54, Area C	NA	Bacteria aerobes: 2.3 x 10^6/g anaerobes: 3.3 x 10^6/g Actinomyctetes detected with depth, not counted Fungi 4.9 x10^4/g	Barnhart et al. 1979
SEDIMENT/WATER			
Rocky Flats Plant Pond B-1 Sediment) (Total Pu 81.5 mCi)	1000 dpm/g	Bacteria aerobes: 3 - 300 x10^6/ml anaerobes: 0.2 - 2.0 x 10^6/ml	Johnson et al, 1974
Water	NA	aerobes: 1.1 - 340 x10^3/ml Anaerobes: 2 - 7.8 x10^2/ml	
WASTE - SOLIDS			
^{239}Pu contaminated waste from a steel burial drum	NA	Bacteria aerobes: 33 - 9500/sample Fungi: 46 CFU/sample	Barnhart et al. 1979
Waste - leachates			
Maxey Flats, Ky			
Trench 19S: ^{238}Pu	170,000 pCi/L	Bacteria	Francis,
^{239,240}Pu	210,000 pCi/L	aerobes: 2.2x10^2 CFU/ml anaerobes: 3.2x10^2 CFU/ml	1990b
West Valley, NY			
Trench 8 ^{238}Pu	160,000 pCi/L	aerobes: 1. 4x10^3 CFU/ml	Gillow &
^{239,240}Pu	340 pCi/L	anaerobes: 7.6x10^2 CFU/ml	Francis, 1990

NA - not available

Table 3. Bacteria detected in radioactive-waste repository environments

Site	Bacteria detected	Reference
Repository Groundwater		
Waste Isolation Pilot Plant (WIPP), New Mexico, USA 5M NaCl brine (pH 7)	Far-field: $1.02 \pm 0.49 \times 10^5$ cells/ml Near-field: $1.24 \pm 0.13 \times 10^5$ cells/ml (denitrifiers, fermenters, sulfate reducers, methanogens)	Francis et al., 1998
Study Site Groundwater		
Nevada Test Site, USA	10^2 viable cells/ml (*Pseudomonas*, *Acinetobacter*)	Amy et al., 1992
Äspö Hard Rock Lab, Sweden granite fracture zone (pH 7.5)	$0.44 - 9.3 \times 10^5$ cells/ml (methanogens and homoacetogens)	Pedersen et al., 1996a
Grimsel Test Site, Switzerland granite shear zone (pH 10)	$3.97 \pm 0.37 \times 10^3$ cells/ml (fermenters and homoacetogens)	Gillow et al., 2000
Host Rock		
WIPP halite	$8.0 \pm 1.6 \times 10^4$ cells/g	Gillow (unpublished results)
Yucca Mountain tuff, Nevada, USA	$0.32 - 2.0 \times 10^5$ cells/g	Kieft et al., 1997

Table 4. Number of bacteria at natural analog sites

Analog site	Bacteria detected	Reference
Maqarin Spring, northern Jordan (pH 12.1)	1.3×10^5 cells/ml	Pedersen et al., 1997
Cigar Lake uranium ore deposit, Saskatchewan, Canada – sandstone w/ clay-hosted U ore	Groundwater $4.7 \times 10^2 - 4.4 \times 10^4$ cells/ml (denitrifiers, fermenters, sulfate reducers, methanogens) Ore $1.4 \pm 0.9 \times 10^5$ cfu/g viable cells (denitrifiers)	Francis et al., 1994
Pocos de Caldos, Brazil	Yes	West et al., 1992
Oklo, Gabon, Africa	Yes	Pedersen et al., 1996b

Table 5. Bacterial population in backfill materials proposed for use at the radioactive-waste repositories

Backfill	Bacteria detected	Reference
Wyoming bentonite	$5.32 \pm 0.34 \times 10^{11}$ cells/g <1.07 ± 3.54 x 10_2 cfu/g viable cells	Haveman et al., 1995
Avonlea bentonite	$6.29 \pm 0.75 \times 10^{11}$ cells/g 3.48 ± 0.56 x 104 cfu/g viable cells	Haveman et al., 1995
Canadian sand and bentonite buffer material	10^1 to 10^6 viable cells/g (*Acinetobacter*, *Pseudomonas*)	Stroes-Gascoyne et al., 1997

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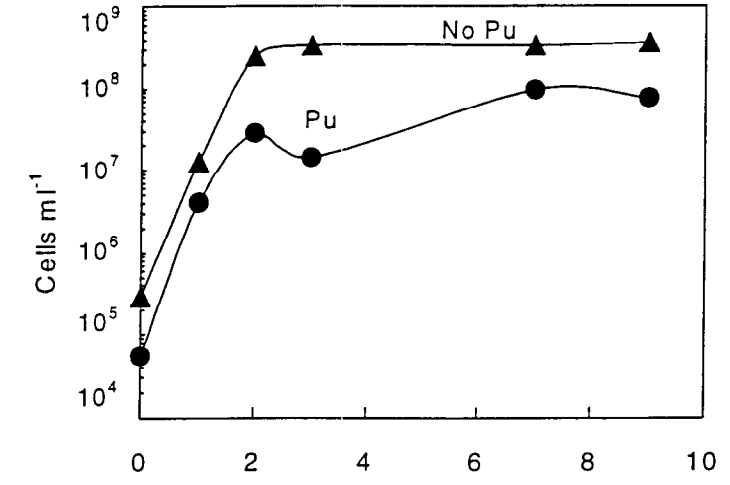
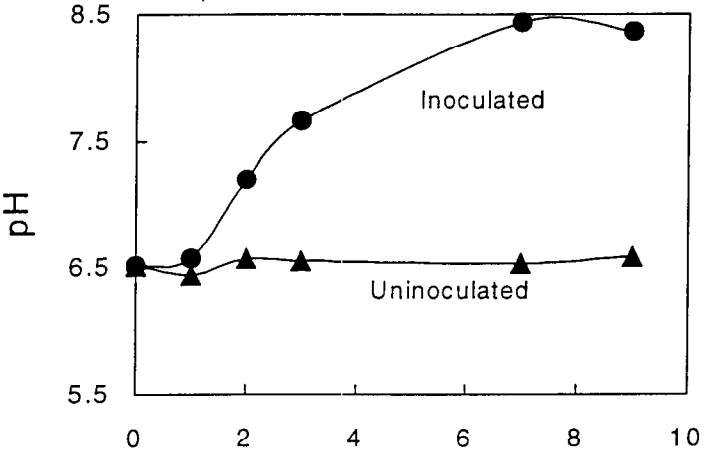
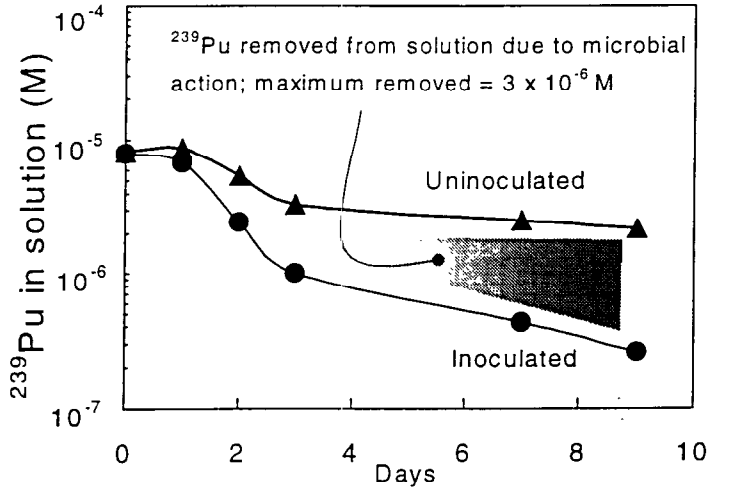
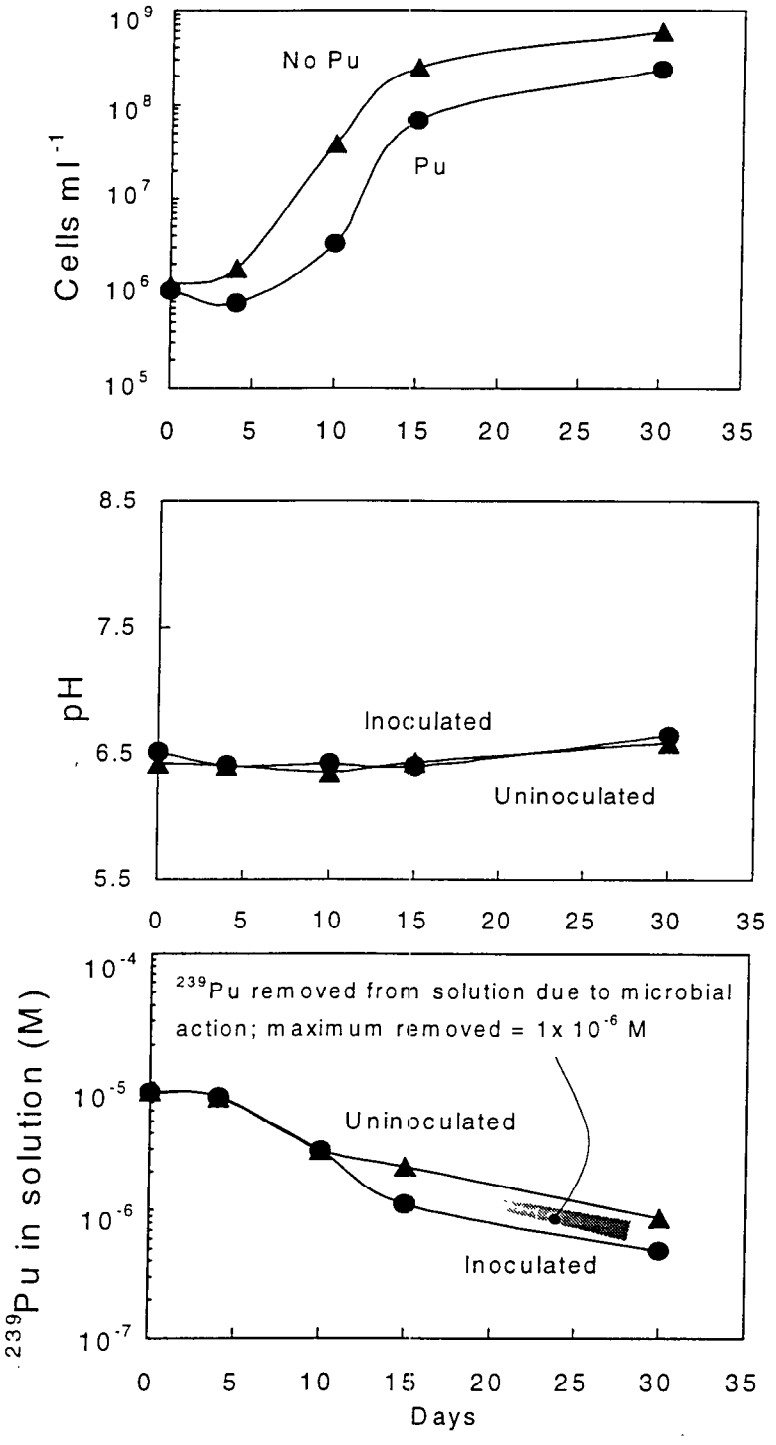
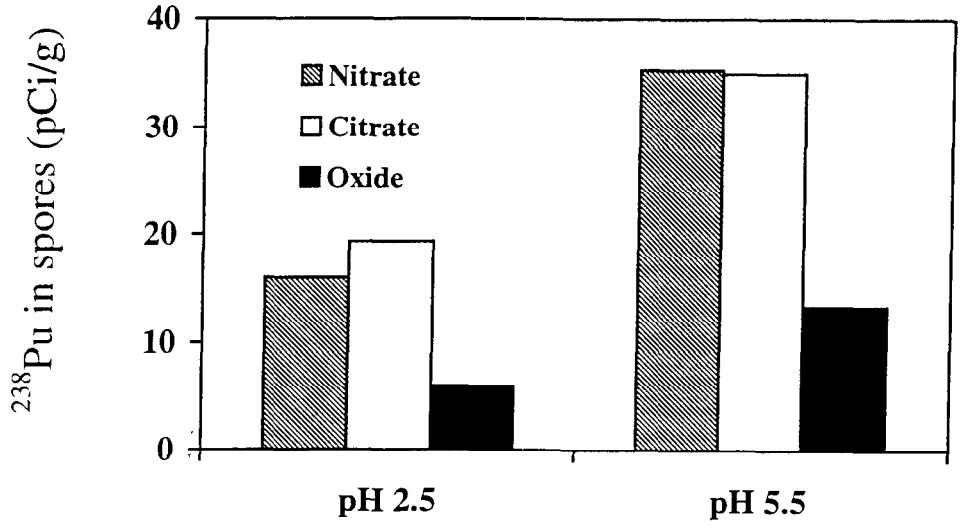

No Pu
Pu
Cells ml-1
10^9
10^8
10^7
10^6
10^5
10^4
0
2
4
6
8
10

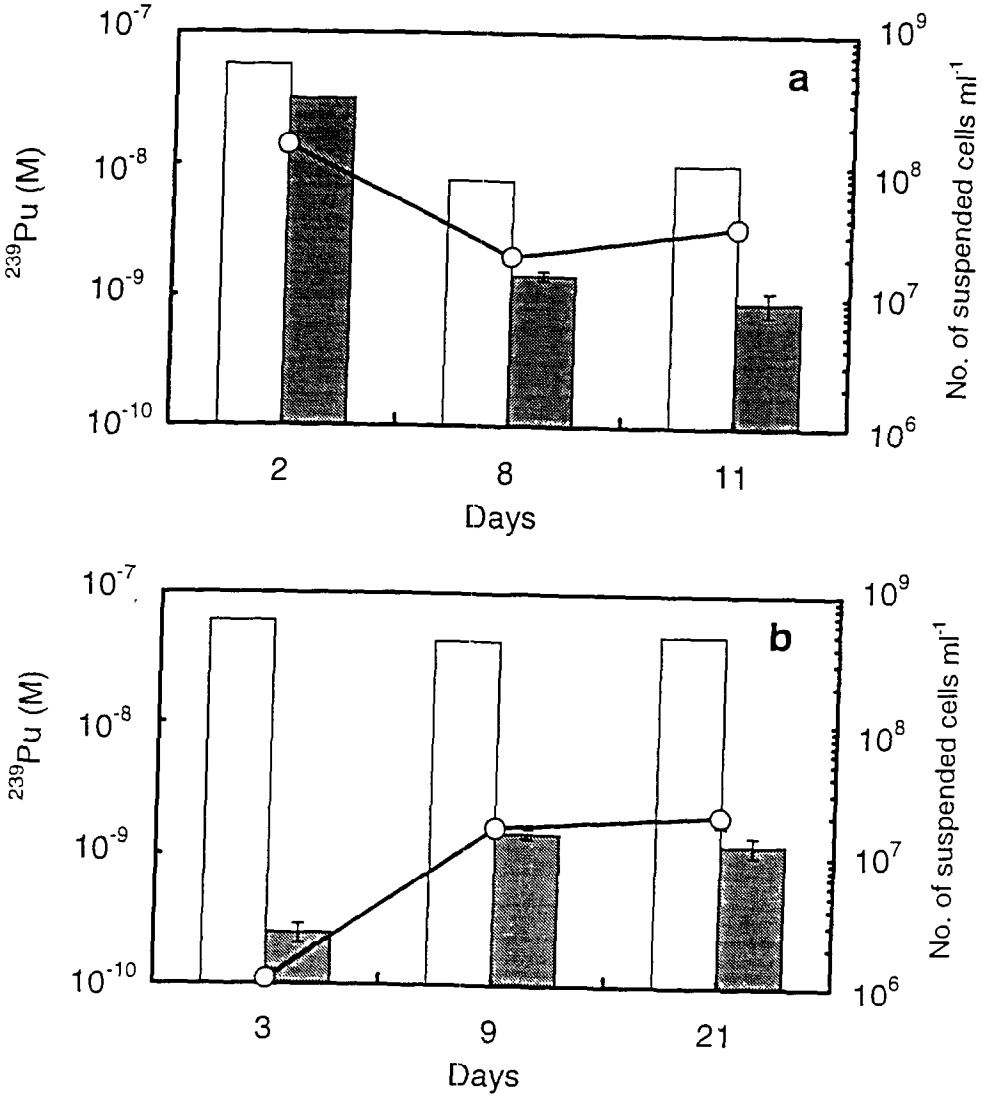

Inoculated
Uninoculated
pH
8.5
7.5
6.5
5.5
0
2
4
6
8
10


239Pu removed from solution due to microbial action; maximum removed = 3 x 10-6 M
Uninoculated
Inoculated
239Pu in solution (M)
10^-4
10^-5
10^-6
10^-7
0
2
4
6
8
10
Days


Cells ml^{-1}
10^9
10^8
10^7
10^6
10^5
No Pu
Pu
0 5 10 15 20 25 30 35
pH
8.5
7.5
6.5
5.5
Inoculated
Uninoculated
0 5 10 15 20 25 30 35
^{239}Pu in solution (M)
10^{-4}
10^{-5}
10^{-6}
10^{-7}
^{239}Pu removed from solution due to microbial action; maximum removed = 1x 10^{-6} M
Uninoculated
Inoculated
0 5 10 15 20 25 30 35
Days



Chemical forms of plutonium in agar medium


a
10^{-7}
10^{-8}
10^{-9}
10^{-10}
^{239}Pu (M)
10^{9}
10^{8}
10^{7}
10^{6}
No. of suspended cells ml^{-1}
2
8
11
Days
b
10^{-7}
10^{-8}
10^{-9}
10^{-10}
^{239}Pu (M)
10^{9}
10^{8}
10^{7}
10^{6}
No. of suspended cells ml^{-1}
3
9
21
Days