The Interaction of Plutonium with Bacteria in the Repository Environment

J.B. Gillow¹, A.J. Francis¹, D.A. Lucero², and H.W. Papenguth²

¹Brookhaven National Laboratory, Upton, NY, 11973

²Sandia National Laboratories, Albuquerque, NM, 87185

RECEIVED
MAY 16 2000
OSTI

Microorganisms in the nuclear waste repository environment may interact with plutonium through (i) sorption, (ii) intracellular accumulation, and (iii) transformation of chemical speciation. These interactions may retard or enhance the mobility of Pu by precipitation reactions, biocolloid formation, or production of more soluble species. Current and planned radioactive waste repository environments, such as deep subsurface halite and granite formations, are considered extreme relative to life processes in the near-surface terrestrial environment. There is a paucity of information on the biotransformation of radionuclides by microorganisms present in such extreme environments. In order to gain a better understanding of the interaction of plutonium with microorganisms present in the waste repository sites we investigated a pure culture (*Halomonas* sp.) and a mixed culture of bacteria (*Haloarcula sinaiensis*, *Marinobacter hydrocarbonoclasticus*, *Altermonas* sp., and a γ -proteobacterium) isolated from the Waste Isolation Pilot Plant (WIPP) site and an *Acetobacterium* sp. from alkaline groundwater at the Grimsel Test Site in Switzerland.

We examined the effects of various concentrations of ^{239}Pu -perchlorate and 1:1 ^{239}Pu -EDTA (10^{-8} to 10^{-6} M) on the growth of pure and mixed cultures of halophilic bacteria grown in medium containing 4 M NaCl and 1.7 M NaCl/1.4 M MgCl_2 , respectively, under anaerobic (denitrifying) conditions. In addition, to eliminate effects of growth media and pH change on Pu speciation, experiments were performed with washed resting cells of *Halomonas* sp. suspended

in high ionic strength solution and *Acetobacterium* sp in low ionic strength solution and contacted with 10^{-11} to 10^{-9} M ^{241}Pu -nitrate at a constant pH of 5.

Figure 1 shows ^{239}Pu association with the cells of *Halomonas* sp. and the mixed culture during growth in the presence of various concentrations and forms of Pu. During the growth of *Halomonas* sp., the pH of the medium changed from 7.0 to 8.5 as a result of bacterial action. Plutonium association with the bacterial cells was determined by separating the cells after filtration through a $0.4\text{ }\mu\text{m}$ membrane filter and analysis of Pu in solution before and after filtration. Samples containing Pu but without bacterial cells were used as controls. *Halomonas* sp. sorbed 0.013 to 2.1×10^{-8} mol Pu added as perchlorate per mg cells dry wt., and 0.057 to 0.99×10^{-8} mol Pu added as the EDTA complex per mg cells dry wt., depending upon the concentration of Pu added. On the other hand, with the mixed culture the pH of the medium did not change and remained constant at 6.5. The mixed culture sorbed 0.05 to 5×10^{-8} mol Pu added as the EDTA complex per mg cells dry wt. Toxicity of Pu to both the pure (*Halomonas* sp.), and mixed culture was observed at ~ 2 to 3×10^{-6} M as evidenced by a reduction in the optical density of the cell suspension, cell numbers and cell dry wt. It appears that at a lower cell density the bacteria may be able to sorb more Pu due to the absence of both clumping of cells and shielding of sorption sites at the cell-surface, hence the skewed data at higher Pu concentrations. An increase in Pu sorption by the growing cells may also increase the toxic effects.

Figure 2 shows association of ^{241}Pu with the resting cells of *Halomonas* sp. and *Acetobacterium* sp. that had been contacted with various concentrations of ^{241}Pu nitrate at pH 5 for 30 min. The halophile *Halomonas* sp. showed $\sim 36\%$ removal of the added Pu at both the

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

lowest and highest concentrations tested (0.96 to 8.3×10^{-13} mol mg cells dry wt.). In contrast, the non-halophilic *Acetobacterium* sp. showed only ~14% removal of the added Pu at the lowest concentration and ~7% at the highest concentration tested. When the same amount of Pu was added to the two cultures, there was greater Pu uptake by the halophile. Additional studies are under way to determine the functional groups involved in surface complexation and the chemical speciation of Pu.

These results show that the halophiles have a greater capacity for Pu removal than the non-halophilic bacterium. The oxidation state and chemical form of Pu, and possible intracellular transport processes, may regulate the amount of Pu taken up by microorganisms. Further studies are needed to determine the factors regulating the microbial mobilization and immobilization of Pu in the repository environment.

Figure 1. Uptake of various concentrations of ^{239}Pu by a growing culture of *Halomonas* sp. and a mixed culture of halophilic bacteria (*Haloarcula sinaiensis*, *Marinobacter hydrocarbonoclasticus*, *Altermonas* sp., and a γ -proteobacterium).

Figure 2. Uptake of ^{241}Pu -nitrate by resting cells of *Halomonas* sp. and the non-halophilic culture *Acetobacterium* sp.