Microbially-Enhanced Chemisorption of Heavy Metals: A Method for the Bioremediation of Solutions Containing Long-Lived Isotopes of Neptunium and Plutonium

LYNNE E. MACASKIE* AND GABRIELA BASNAKOVA

School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Immobilized cells of a *Citrobacter* sp. removed neptunium and plutonium negligibly from solution using an established technique that used biologically-produced phosphate ligand (P_i) for metal phosphate bioprecipitation. Removal of these transuranic radionuclides was enhanced by prior exposure of the biomass to lanthanum in the presence of organophosphate substrate to form cell-bound $LaPO_4$. Polyacrylamide gel-immobilized cells removed little Np and Pu per se, but preloaded $LaPO_4$ promoted the removal of Np and Pu upon subsequent challenge in a flow-through column. Approximately 2 μg of Np was loaded per 1 mL, column, when the experiments were stopped after 10 mL, with maintenance of approximately 90% removal of the input metal. Transuranic element removal by this technique, generically described as microbially-enhanced chemisorption of heavy metals (MECHM), is via a hybrid of bioaccumulative and chemisorptive mechanisms.

Introduction

Application of biotechnology to uranium removal from wastewater is well-established (*1*−*4*), but little attention has been paid to the removal of long-lived, α-emitting transuranic elements (*5*). Simple chemical precipitation is the easiest and most economical method, but the low bulk metal concentrations and formation of poorly-settling colloidal precipitates may limit the effectiveness of this approach. Uptake of ^{241}Am was shown by *Escherichia coli* (*6*, *7*) and by a *Citrobacter* sp. (*3*, *8*, *9*), this removing many heavy metals by metal phosphate precipitation via the release of phosphate ligand (*8*, *9*) produced by the activity of a radioresistant (*3*) cell-bound phosphatase (*3*, *8*, *9*). In contrast to Am, the removal of Np and Pu is problematic. Previous studies using *Citrobacter* (*3*, *8*) and other organisms (*10*) gave little removal of ^{237}Np. Similarly, attempts to remove ^{239}Pu gave only 50% removal at steady state under conditions where the removal of ^{241}Am was complete (*8*).

The recalcitrance of Np is attributable to the solution chemistry of the pentavalent actinide species. Np(V), the most common species in neutral solution (NpO_2^+), does not form insoluble phosphates (although $HNpO_2PO_4$ is insoluble; *11*). Plutonium, normally Pu(IV) in solution (*12*, *13*) is thought to predominate instead as Pu(V) in natural waters (*14*), behaving similarly to Np(V). Np(V), although almost completely disproportionated into Np(IV) and Np(VI) in 6 M HNO_3, forms stable Np(V) at neutral pH, although Np(IV) is stabilized in solutions containing complexing ligands (*15*) that can occur (e.g., EDTA, citrate) in wastes (*16*). Most waste inventories pay scant attention to the metal speciation, and this will vary according to the conditions (redox potential, pH, radiolysis, UV light) and the composition of the background ionix matrix. Hence we set up a model matrix for use in a preliminary study.

A previous study using a 'surrogate' mixture of La(III), Th(IV), and U(VI) as a model (*17*) for AnIII, -(IV), and -(VI) species established that Th(IV) was removed poorly (*17*, *18*) under conditions where La(III) and U(VI) were removed efficiently using polyacrylamide gel (PAG)-immobilized cells in a flow-through reactor (*17*). The recalcitrance of Th was attributed to the amorphous nature of the thorium phosphate (*17*, *18*) and to the low availability of free Th^{4+} for phosphate precipitation in the presence of citrate, routinely incorporated to suppress actinide hydrolysis (*19*) and, in the case of Pu^{4+}, to suppress the formation of polymeric species (*20*, *21*). Th and Pu were removed more efficiently in the presence of La^{3+} with the formation of a co-crystal of lanthanum/thorium phosphate (*17*). This is in accordance with a generic model (microbially-enhanced chemisorption of heavy metals, MECHM) that postulates that predeposition of the phosphate crystal of a 'benign' metal promotes the subsequent deposition of a metal that does not precipitate easily (*3*, *8*). The study (*17*) was unable to distinguish between an intercalation mechanism and simple addition of $Th(HPO_4)_2$ onto an already-formed, 'nucleating' deposit of $LaPO_4$; this could be difficult to confirm by solid-state methods in the absence of published data for reference compounds. In the case of Pu and Np, the use of sufficient metal to produce enough solid for analysis presents difficulties in handling and final disposal of the active nuclides. Also, crystallographic studies are problematic; growth of sufficiently large reference crystals is very difficult (*11*).

Regardless of the actual mechanism of MECHM (intercalative or additive), the principle of this states that the removal of a recalcitrant metal is promoted in the presence of a previously deposited metal phosphate. This was tested here, using La as the 'priming' metal in columns containing immobilized cells of *Citrobacter* sp. that were subsequently challenged with the α-emitting isotopes ^{237}Np or ^{239}Pu 'spiked' with the high-active tracer isotopes ^{239}Np (γ-emitter) and ^{241}Pu (β-emitter), respectively. In addition to ^{237}Np and ^{239}Pu, the latter isotope can contribute much of the radioactive burden to fresh wastewater (*22*).

Materials and Methods

Cell Growth and Immobilization. The *Citrobacter* sp. strain N14 (used under license from Isis Innovation, Oxford, U.K.) was grown and immobilized in PAG as described previously (*17*, *18*). Each preparation (5 g fresh weight of biomass) was divided into 50 replicate columns (working volume 3 mL; Pierce) each containing 0.1 g wet weight of biomass in 1 g of shredded material, held within the column by porous frits (Pierce) below and above the gel (bed volume, approximately 1 mL).

Column Priming with a Deposit of $LaPO_4$ and Column Testing with La. The columns were challenged with priming solution (room temperature), comprising 50 mM MOPS/

* Corresponding author Tel: 0044-121-414-5889; Fax: 0044-121-414-6557; e-mail: L.E.Macaskie@bham.ac.uk.

S0013-936X(97)00852-3 CCC: $14.00
Published on Web 01/01/1998

TABLE 1. Radioisotopes Used in This Study[a]

isotope	half-life and emission	concn in challenge (mass)	concn in challenge (radioactivity)
^{239}Pu	2.44×10^4 yr (α)	39.30 nM	21.6 Bq/mL
^{241}Pu	14.5 yr (β)	0.18 nM	188 Bq/mL
[^{241}Pu	14.5 yr(β)	1.80 nM	1686 Bq/mL]
^{237}Np	2.10×10^6 yr (α)	1.0 μm	6.3 Bq/mL
^{239}Np	2.33 day (γ)	ND	360 counts min^{-1} mL^{-1}

[a] Initial tests in brackets used 0.1 mL of stock ^{239}Pu and 0.2 mL of ^{241}Pu in a final volume of 10 mL. Subsequent studies (for more convenient handling) used 0.1 mL of ^{239}Pu and 0.02 mL of ^{241}Pu; the final concentration (^{239}Pu + ^{241}Pu) was 39.5 nM in a final carrier of 24 mM NO_3^-. The volume of ^{237}Np taken (0.05 mL) was supplemented with 0.05 mL of ^{239}Np (concentrated stock solution was 1200 counts s^{-1} mL^{-1}; the mass concentration was not determined, ND). The total Np taken was 0.1 mL in a final concentration of NO_3^- in the challenge solution of 80 mM.

NaOH buffer/2 mM citrate buffer (pH 7), 5 mM glycerol 2-phosphate, and 1 mM lanthanum nitrate. La and citrate were mixed first. The columns were challenged (50 mL upflow; Watson Marlow Flow Inducer) at a flow rate (F) that gave an appropriate steady-state removal of La (ca. 80% or 23% removal of the input La as described), with La removal monitored by assay of inflow and outflow solutions using arsenazo III (*17*, *25*). The primed columns were stored at 4 °C, statically, in La test solution, which comprised 10 μM $La(NO_3)_3$ in a 'carrier' flow of 50 mM MOPS/NaOH/0.5 mM citrate buffer, pH 7, and 5 mM glycerol 2-phosphate (10 mL). Assay of residual La was done with arsenazo III as above.

Preparation of Transuranic Element Solutions. ^{237}Np (bulk, α-isotope), separated from its high-active β-daughter ^{233}Pa, and ^{239}Np (γ-tracer) were gifts from BNFL. All Np experiments were done within 3 days. ^{239}Pu (bulk, α-isotope) and ^{241}Pu (β-tracer) were from AEA Fuel Services, Harwell, U.K. The metals were supplied in carriers of 8 M (Np), 2 M (^{239}Pu), or 1 M HNO_3 (^{241}Pu) and when prepared in test challenge solutions (below) were neutralized just prior to use with NaOH (4 M) to give a final pH of approximately 7. Column challenge solution was prepared fresh by introduction of stock actinides (in HNO_3) into a polypropylene scintillation vial. To this was added, in order, 1 mL of 5 mM trisodium citrate/citric acid buffer, pH 6.9; 2.5 mL of 200 mM MOPS/NaOH buffer, pH 7; 0.1 mL of 500 mM glycerol 2-phosphate (sodium salt); and 6.4 mL of distilled water and the appropriate volume of NaOH. The final volume was made to 10 mL with water; the final actinide concentrations were approximately 40 nM (Pu) and 1 μM (Np) (Table 1).

Valence testing (for Np; Pu was assumed to be similar) of the carrier solution was done by paper chromatography with radioactive spots (^{239}Np γ-tracer) visualized using a phosphorImager (*3*, *23*). The R_f values of Th(IV) and U(VI) for reference were determined in parallel and visualized by spraying with arsenazo III (composition as in ref 17) since the specific γ-activity of these was very low at convenient mass loadings (*3*).

Challenge of the Immobilized Cells with Actinide Elements. The column was prepared as described previously (*8*, *24*). For use, the columns were taken from storage, and a cotton wool filter (0.5 g of nonabsorbent cotton wool in the barrel of a Pasteur pipet) was inserted at the distal end to trap any outflow particulate species. Columns that had been primed and tested against 10 μM La were drained and challenged with a flow (downward, using a 'pull' pump to maintain negative pressure in the column) of metal-unsupplemented test solution (F = 6 mL/h, 10 mL), until the top of the bed was just visible above the meniscus. Freshly prepared test actinide solution (above) was introduced, and the column outflow solution was collected in approximately 2-mL fractions, with the volume estimated by weight. The Np and Pu contents of the input and outflow solutions were determined by γ-counting (for ^{239}Np: Canberra high resolution germanium detector connected to a Nuclear Data multichannel analyzer) or by scintillation counting of ^{241}Pu in 10 mL of Hisafe II scintillation cocktail (Pharmacia) in a Tri-Carb 2700 liquid scintillation analyzer (Packard Instrument Company, Meriden CT).

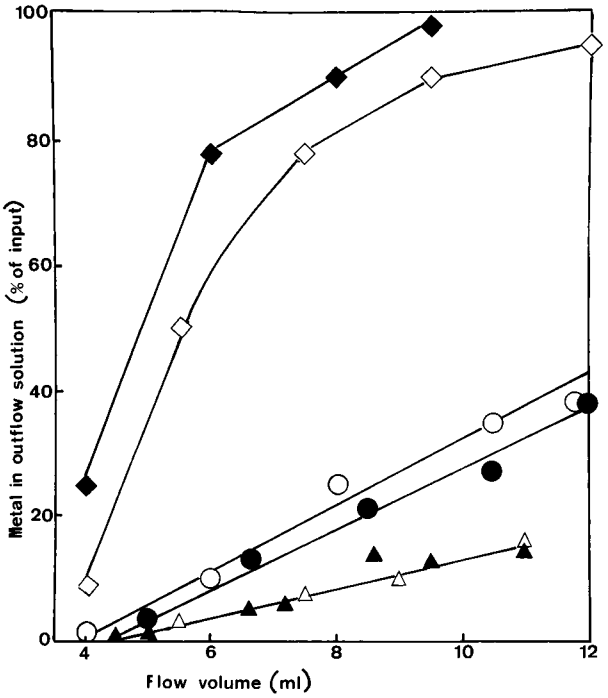

FIGURE 1. Columns of PAG-immobilized cells of *Citrobacter* sp. were challenged with solutions of Pu (filled symbols) or Np (open symbols) as shown in Table 1 in challenge solution as described in the text, either without prior exposure to La (◇, ♦) or following priming at an efficiency (set by the flow rate of the priming solution through the column) of 77% (for subsequent challenge with Np, ○) or 83% (for subsequent challenge with Pu, ●) removal of the input La. A second set of experiments reduced the level of priming to 21% (for subsequent challenge with Np, △) and 24% (for subsequent challenge with Pu, ▲) removal of the input La. Determination of the transuranic element concentration in the column inflow and exit solutions was by γ-counting or by β-counting for the probes ^{239}Np and ^{241}Pu as described in the text.

Results and Discussion

Neptunium migrated at R_f positions corresponding to U(VI) and Th(IV) and between these. The valence was suggested to be a mixture of species, but the exact speciation in the tests would depend on the time taken to reach the disproportionation equilibrium of Np(V) within the run time of each column (<2 h) and the microenvironmental conditions within the column. The formation constant of actinide(VI) phosphates is approximately 1−2 orders of magnitude higher than for the actinide(V) species, and that of the actinide(IV) species is more than 7 orders of magnitude greater than actinide(VI) (*15*). Deposition of actinide(IV) and -(VI) phosphates is retarded in the presence of citrate (*25*), but deposition of both is accelerated in the presence of preexisiting nucleation foci (*8*, *25*). Very similar results were obtained with Np and Pu throughout (Figure 1). Columns not primed with $LaPO_4$ gave rapid breakthrough of the actinides. Some removal occurred initially, probably attributable to sorption onto the biomass; similar results were seen previously using a phosphatase-deficient mutant. (*8*). Column activity was largely lost within approximately 10 mL

(Figure 1), and it was concluded that phosphatase activity alone did not permit efficient or sustained removal of either element. Columns previously primed with $LaPO_4$ (the identity of which was checked using X-ray diffraction analysis; *17*) at an efficiency of ca. 80% removal of the La during priming removed approximately 70% of both actinides at the 10-mL stage. Columns were not challenged beyond 12 mL due to the accumulating radiohazard, but the activity of the primed columns was substantially enhanced as compared to native columns.

For development of a realistic process, a greater efficiency would be required. A conceptual, hypothetical model was formulated previously, the application of which, we postulate, could increase the activity of the bioreactor (*8*, *24*). This model assumes that a column operating at a slow flow rate during priming would deplete the substrate at an early stage in the column and that deposition of the priming $LaPO_4$ would be restricted to proximal column areas. The enzyme at the distal end of the column would be redundant; no substrate would remain, and accordingly, the distal areas of the column would remain devoid of priming deposits. A fundamental assumption of the model is that nascent phosphates of neptunium and plutonium are not precipitated effectively from solution unless there is a preformed nucleating surface (the requirement for nucleation deposits was shown previously for Th(IV) and U(VI); *8*,*25*); hence columns primed under optimal conditions (high phosphatase activity, slow flow rate) would then remove Np and Pu inefficiently because deposition of $LaPO_4$ is confined to a proximal small area of the column, in heavy deposits of a relatively small surface area/volume ratio. This was observed in other tests using uranyl ion; deposition of yellow HUO_2PO_4 was confined to the bottom of the column at slow flow rates but visible throughout the column at high flow rates. Hence, a column of low phosphatase activity or a column run at a high flow rate would permit the spread of residual substrate throughout the length of the column and a more even distribution of priming deposit (nucleation sites) along the length of the column. Thus, a column made less effective during priming should remove Np and Pu more efficiently during challenge due to improved distribution and enhanced priming deposit surface area. This was tested using columns that removed La at an efficiency of only ca. 23% during priming by application of a rapid *F*. As predicted, the efficiency of these columns was greater that those primed at ca. 80% efficiency of La removal; at the 10-mL stage, the actinide removal efficiency was still nearly 90% (Figure 1). Approximately 2 μg of Np was loaded per 1 mL of column when the experiments were stopped after 11 mL.

It could be argued that higher flow rates could allow greater penetration to occluded areas of the bioreactor. Against this, other studies showed that use of a low phosphatase activity preparation had the same effect as a rapid flow rate during priming; however, the columns still gave a slow breakthough of both actinides with increasing flow volume. For bioprocess use, it would be necessary to regenerate the column after, e.g., 10 column volumes (or introduce another priming layer). A simple wash technique could suffice, for example, a 50-fold concentration of uranium was achieved by washing the U-loaded column with carbonate solution (*25*).

It is possible to intercalate NpO_2^{2+} (but not NpO_2^{+}) into a lattice of HUO_2PO_4 (*26*), but in this case the possibility of selective desorption of Np (or Pu) from the lattice with carbonate would be lost since all actinides form very strong carbonate complexes (*15*). Use of $LaPO_4$ as the priming layer could permit a continuous sorb−desorb cycle for actinide concentration, with conservation of the immobilizing matrix of $LaPO_4$, while at the same time retaining the potential for regeneration of the priming surface with a fresh charge of $LaPO_4$ via the biochemical activity.

Acknowledgments

G.B. was supported by a grant from the BBSRC (ROPA Award PAC6/2677). The authors wish to thank S. Napier and M. John of BNFL for the provision of laboratory facilities and Drs. G. Zabierek and H. Janewska of the Radiation Safety Office, University of Birmingham, for their invaluable assistance and advice.

Literature Cited

(1) Kauffman, J. W.; Laughlin, W. C.; Baldwin, R. A. Microbiological treatment of uranium mine wastes. *Environ. Sci. Technol.* **1986**, *20*, 243.

(2) Tsezos, M.; Bell, J. P.; McCready, R. G. L. The continuous recovery of uranium from Elliot Lake bioleach using immobilized biomass. *Biotechnol. Bioeng.* **1989**, *34*, 10.

(3) Macaskie, L. E.; Lloyd, J. R.; Thomas, R. A. P.; Tolley, M. R. The use of microorganisms for the remediation of solutions contaminated with actinide elements, other radionuclides, and organic contaminants generated by nuclear fuel cycle activities. *Nucl. Energy* **1996**, *35*, 257.

(4) Macaskie, L. E.; Yong, P.; Doyle, T. C.; Roig, M. G.; Diaz, M.; Manzano, T. Bioremediation of uranium-bearing wastewater: biochemical and chemical factors affecting bioprocess application. *Biotechnol. Bioeng.* **1997**, *53*, 100.

(5) Macaskie, L. E. The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: biodegradation and bioaccumulation as a means of treating radionuclide-contaminated streams. *CRC Crit. Rev. Biotechnol.* **1991**, *11*, 41.

(6) Geisy, J. B.; Paine, D. Uptake of americium-241 by algae and bacteria. *Prog. Water Technol.* **1977**, *9*, 845.

(7) Wurtz, E. A.; Sibley, T. H.; Schell, W. R. Interactions of *Escherichia coli* and marine bacteria with ^{241}Am in laboratory cultures. *Health Phys.* **1986**, *50*, 79.

(8) Macaskie, L. E.; Jeong, B. C.; Tolley, M. R. Enzymically-accelerated biomineralization of heavy metals: application to the removal of americium and plutonium from aqueous flows. *FEMS Microbiol Rev.* **1994**, *14*, 351.

(9) Macaskie, L. E.; Empson, R. M.; Cheetham, A. K.; Grey, C. P.; Skarnulis, A. J. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymically-mediated growth of polycrystalline HUO_2PO_4. *Science* **1992**, *257*, 782.

(10) Strandberg, G. W.; Arnold, W. D. Microbial accumulation of neptunium. *J. Ind. Microbiol.* **1988**, *3*, 329.

(11) Weigel, F.; Hoffman, G. The phosphates and arsenates of hexavalent actinides. Part II. Neptunium. *J. Less-Common Met.* **1976**, *44*, 125.

(12) Allard, B.; Rydberg, J. Behavior of plutonium in natural waters in Plutonium Chemistry. *Am. Chem. Soc. Symp. Ser.* **1983**, *No. 216*, 275.

(13) Katz, J. J.; Seaborg, G. T.; Morss, L. R. *Chemistry of the Actinide Elements*; Chapman and Hall: London, 1986.

(14) Nelson, D. M.; Lovett, M. B. Oxidation state of plutonium in the Irish Sea. *Nature* **1978**, *276*, 599.

(15) Lieser, K. H.; Muhlenweg, U. Np in the hydrosphere and in the geosphere. *Radiochim. Acta* **1988**, *43*, 27.

(16) Delegard, C. H. Solubility of $PuO_2{\cdot}xH_2O$ in alkaline Hanford high level waste solution. *Radiochim. Acta* **1987**, *41*, 11.

(17) Yong, P.; Macaskie, L. E. Bioaccumulation of lanthanum, thorium and uranium, and use of a model system to develop a method for the removal of plutonium from solution by a *Citrobacter* sp. *J. Chem. Technol. Biotechnol.* In press.

(18) Yong, P.; Macaskie, L. E. Removal of the tetravalent actinide thorium from solution by a biocatalytic system. *J. Chem. Technol. Biotechnol.* **1995**, *64*, 89−95.

(19) Bulman, R. A. Chemistry of plutonium and the transuranics in the biosphere. *Struct. Bond.* **1978**, *34*, 39.

(20) Ockenden, D. S.; Welch, G. A. The preparation and properties of some plutonium compounds V. Colloidal quadrivalent plutonium *J. Chem. Soc.* **1956**, *653*, 3358.

(21) Wildung, R. E.; Garland, T. E. The relationship of microbial processes to the fate and behavior of transuranic elements in soils, plants and animals. In *Transuranic Elements in the Environment*: Hanson, W. C., Ed.; National Technical Information Service: Springfield, VA, 1980; U.S. Department of Commerce DOE/NTIS 22800; pp 300−335.

(22) Flowers, R. H.; Roberts, L. E. J.; Tymons, B. J. Characteristics and quantities of radioactive wastes. *Philos. Trans. R. Soc. London* **1986**, *A319*, 5.

(23) Lloyd, J. R.; Macaskie, L. E. A novel phosphorImager-based technique for monitoring the microbial reduction of technetium. *Appl. Environ. Microbiol.* **1996**, *62*, 578.
(24) Tolley, M. R.; Macaskie, L. E. Metal removal from aqueous solutions. PCT Patent Application No. GB94/00626, 1994.
(25) Macaskie, L. E.; Empson, R. M.; Lin, F.; Tolley, M. R. Enzymatically-mediated uranium accumulation and uranium recovery using a *Citrobacter* sp. immobilized as a biofilm within a plug-flow reactor. *J. Chem. Technol. Biotechnol.* **1995**, *63*, 1.
(26) Dorhout, P. K.; Kissane, R. J.; Abney, K. D.; Avens, L. R.; Eller, P. G.; Ellis, A. B. Intercalation reactions of the neptunyl (VI) dication with hydrogen uranyl phosphate and hydrogen neptunyl host lattices. *Inorg. Chem.* **1989**, *28*, 2926.

Received for review September 24, 1997. Accepted September 30, 1997. [⊗]

ES9708528

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.