BNL-65782

BIOREMEDIATION OF URANIUM CONTAMINATED SOILS AND WASTES

CONF-980939--

A. J. FRANCIS
Department of Applied Science
Brookhaven National Laboratory
Upton, New York 11973
USA

Abstract

Contamination of soils, water, and sediments by radionuclides and toxic metals from uranium mill tailings, nuclear fuel manufacturing and nuclear weapons production is a major concern. Studies of the mechanisms of biotransformation of uranium and toxic metals under various microbial process conditions has resulted in the development of two treatment processes: (i) stabilization of uranium and toxic metals with reduction in waste volume and (ii) removal and recovery of uranium and toxic metals from wastes and contaminated soils. Stabilization of uranium and toxic metals in wastes is accomplished by exploiting the unique metabolic capabilities of the anaerobic bacterium, *Clostridium* sp. The radionuclides and toxic metals are solubilized by the bacteria directly by enzymatic reductive dissolution, or indirectly due to the production of organic acid metabolites. The radionuclides and toxic metals released into solution are immobilized by enzymatic reductive precipitation, biosorption and redistribution with stable mineral phases in the waste. Non-hazardous bulk components of the waste such as Ca, Fe, K, Mg and Na released into solution are removed, thus reducing the waste volume. In the second process uranium and toxic metals are removed from wastes or contaminated soils by extracting with the complexing agent citric acid. The citric-acid extract is subjected to biodegradation to recover the toxic metals, followed by photochemical degradation of the uranium citrate complex which is recalcitrant to biodegradation. The toxic metals and uranium are recovered in separate fractions for recycling or for disposal. The use of combined chemical and microbiological treatment process is more efficient than present methods and should result in considerable savings in clean-up and disposal costs.

Keywords: *Uranium, microorganisms, reduction, citric acid, biodegradation, photodegradation.*

Introduction

Typically, the radionuclides and toxic metals in soils and wastes are present in various forms, such as elemental, oxides, coprecipitates (metals coprecipitated with iron and manganese oxides), natural minerals, carbonate complexes, and soluble and insoluble organic complexes. Unlike organic contaminants, the metals cannot be destroyed, but must either be converted to a stable form or removed. The radionuclides and toxic metals may be present initially as soluble forms or they may be formed after disposal by chemical and microbiological processes. Microorganisms catalyze the oxidation-reduction of elements; change the pH and Eh of the

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED MASTER

medium; produce specific sequestering or chelating agents; biosorb and bioaccumulate metals and radionuclides; and biodegrade radionuclide-organic complexes (1, 2). Detailed information on the characteristics of the contaminants in the waste is required for selecting or developing appropriate remediation technology; these include the chemical speciation of radionuclides and toxic metals and their mineralogical association (3-6).

I. Characterization of uranium contaminated soils and wastes

Soil samples contaminated with uranium from the Fernald site in Ohio, uranium and technetium from RMI site, Ahstabula, Ohio, and uranium-contaminated sediment and sludge samples from the West End Treatment Facility, at the U.S. Department of Energy, Oak Ridge Y-12 Plant, Oak Ridge, TN were inivestigated.

The concentrations of U in the soils varied from 473 to 1880 ppm, and Tc activities ranged from 109 to 144 pCi/g. The sludge was generated from a uranium-process waste stream after biodenitrification of nitric-acid uranium waste water, and the sediment was from a contaminated pond which received uranium-process waste water. Several million gallons of the sludge is in storage awaiting disposal. Both sediment and sludge samples contained varying levels of major elements, Al, Ca, Fe, K, Mg and Na and toxic metals, As, Cd, Cr, Co, Cu, Hg, Mn, Ni, Pb, U and Zn. The concentrations of uranium in sediment and sludge samples were 920 and 3100 ppm, respectively. The sediment contained high levels of Cr, Cu, Hg, Mn and Zn. Compared to the sediment, the sludge was high in Ca, low in Fe and Mn and contained higher amounts of Cd, Cr, Pb and Ni. Analysis of the sediment sample by X-ray fluorescence showed the presence of titanium, gallium, bromine, strontium, rubidium, yttrium, and zirconium. Selenium, a common element present in uranium mining waste, was not detected. The sediment had a high ash content, and appreciable amounts of organic carbon and nitrogen. X-ray diffraction analysis showed high levels of SiO_2. The sludge was low in organic carbon and nitrogen but high in ash and sulfate; the latter resulted from adding sulfuric acid and ferric sulfate in the waste treatment process. Chemical analysis of the sludge showed that the pH was alkaline, it was high in dissolved inorganic carbon and sulfate, and low in nitrate; the pH of the sediment was near neutral and contained high dissolved organic carbon, and low levels of the other constituents tested.

The mineralogical association of Cd, Cr, Cu, Mn, Ni, Pb, U and Zn in the sludge and sediment was determined by a selective extraction procedure (7, 8). The inert (predominantly silicates) and organic fractions of the sediment contained 301 and 293 μg/g of uranium, respectively, and the Fe-Mn oxide fraction contained 162 μg /g dry wt. Nearly half of the total uranium (1600 μg) in the sludge was associated with the carbonate fraction. The association of uranium with other fractions in the sludge was as follows: exchangeable, 400 μg; iron oxide, 216 μg; organic, 516 μg; and inert, 80 μg/g dry wt. A comparison of the total uranium obtained by digestion of the entire sample with the sum of the selective extractions showed good agreement within ±10% (±1 SEM).

Stabilization of uranium and volume reduction of wastes by anaerobic bacteria.

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, makes 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.

Stabilization means that the radionuclides and toxic metals are converted chemically or biologically to insoluble environmentally stable forms. Stabilizing the radionuclides and toxic metals and reducing the mass of the wastes facilitates their disposal. In this process, the unique metabolic capabilities of the dual-action anaerobic bacterium *Clostridium* sp. was exploited to solubilize and precipitate radionuclides (9, 10). Exposure of the sludge and the sediment to *Clostridium* sp. released a large fraction of the non-toxic waste solids into solution. The radionuclides and toxic metals were converted to a more stable and concentrated form, and, at the same time, the volume and mass of the waste was reduced. The non-hazardous materials in the solid phase, such as Ca, K, Na, Mg, and Fe were solubilized and easily removed from the waste, thus reducing its volume. The re-mobilized radionuclides and toxic metals were stabilized by precipitation and/or redistribution with stable mineralogical fractions of the waste.

Clostridia are ubiquitous in soils, sediments, and wastes, and could be very useful in pre-treating and stabilizing uranium in radioactive wastes. To determine the anaerobic microbial transformations of uranium and toxic metals in sludge and sediment samples, 5 grams of each sample were incubated anaerobically in 160 ml acid-washed sterile serum bottles with and without nutrients in an N_2 atmosphere. One hundred milliliters of deionized water, or deionized water containing glucose and NH_4Cl, were pre-reduced by boiling for 15 minutes while purging with N_2 (99.99% purity), and then added to the sludge or sediment sample; the serum bottles were sealed with butyl rubber stoppers. The samples were inoculated with *Clostridium* sp. (ATCC #55102). The treatments consisted of (i) sample plus deionized water (unamended); (ii) sample plus deionized water containing 0.5% glucose and 0.015% NH_4Cl (amended); and (iii) control (autoclaved) sample with deionized water. All samples were incubated at 24°C, in triplicate, except the control samples, which were incubated in duplicate. Periodically, all the samples were analyzed for the production of total gas, CO_2, H_2, and CH_4. An aliquot of the sample was removed and filtered through a 0.22 μm Millex filter. A portion of the filtered aliquot was analyzed for anions by ion chromatography using a conductivity detector; organic acids by HPLC, using a UV detector at 210†nm; and alcohols and glucose by HPLC, using a refractive index detector. Another portion of the aliquot was acidified with HNO_3, and analyzed for uranium and metals. The unamended samples showed neither significant microbial activity nor production of organic acid metabolic products. However, the amended samples showed an increase in total gas, CO_2, H_2, CH_4, and organic acids; pH was lowered by about 2.5 units. This change was due to the production of organic acid metabolites from glucose fermentation. The organic acids were acetic, butyric, propionic, formic, pyruvic, lactic, isobutyric, valeric, and isocaproic acids. A significant amount of gas was produced due to glucose fermentation by anaerobic bacteria, as well as from the dissolution of $CaCO_3$ in the sludge by the organic acids. A decrease in sulfate concentration was observed only in amended samples.

This treatment process removed a large fraction of soluble non-toxic metals such as Ca, K, Mg, Mn^{2+}, Na, and Fe^{2+}, and enriched Cd, Cr, Cu, Ni, Pb, U, and Zn with the remaining solid phase due to direct and indirect actions of the bacteria [11]. Metals associated with the exchangeable, carbonate, and iron oxide fractions were solubilized by indirect action due to the production of organic acid metabolites, whereas dissolution of iron oxides and metals coprecipitated with iron oxides was due to direct enzymatic reduction of iron. Uranyl ion

associated with the exchangeable, carbonate, and iron-oxide fractions was released into solution by direct and indirect actions of the bacteria, and was subsequently, reduced enzymatically to insoluble U(IV). X-ray absorption near edge spectroscopic (XANES) analysis of uranium in the treated sludge and sediment samples showed partial reduction of U(VI) → U(IV) in the sludge and complete reduction in the sediment [6]. Analysis of the mineralogical association of the metals in the wastes after microbiological action showed that many of the metals were redistributed with stable mineral phases, such as organic and silicate fractions.

Weight Loss: Substantial amounts of Ca, Fe, K, Mg, Mn, and Na were solubilized from the waste, reducing the mass ~ 15-20% in these batch studies. Further reductions in waste volume can be achieved by optimizing the process using a continuous treatment system to solubilize and remove the bulk of non-toxic waste components, particularly Ca, K, Fe, Mg, and Mn.

This biotreatment can be applied to mixed wastes containing radioactive elements and toxic metals generated from defense, energy, and industrial operations wastes to chemically convert the radionuclides and metals to more stable forms. Reducing the mass of the wastes means that more material can be stored or disposed of, can be handled easier, and can be transported. Changing the radionuclides and toxic metals to more stable forms allows the material to be processed chemically, for disposal in shallow or deep geological formations [4].

Removal and Recovery of Radionuclides and Toxic Metals.

For decontamination, both metal and radionuclide must be removed and recovered from the contaminated site, so that the site is restored. Various soil washing techniques have been developed including physical methods, such as wet-screening, attrition scrubbing, or chemical methods consisting of treating with organic and inorganic acids, salts, bases, and chelating agents. For example, the following chemicals have been used to extract radionuclide and toxic metals: nitric acid, hydrochloric acid, phosphoric acid, sulfuric acid, sodium carbonate, ammonium carbonate, sodium hydroxide, oxalic acid, citric acid, EDTA, and DTPA. Many of the inorganic chemicals used are corrosive, which irreparably damages the soil. Furthermore, all chemical extraction methods generate secondary waste streams which create further problems of hazardous waste disposal.

Among the several organic complexing agents used in extracting metals, citric acid appears to be the most preferred because it is a naturally occurring organic complexing agent, that is environmentally friendly, it shows relatively consistent removal efficiency, and is cost-effective. Citric acid forms different types of complexes with the transition metals and actinides, and has been used to extract plutonium from contaminated soils (10), to decontaminate components of nuclear reactors, to extract metals and radionuclides such as As, Ba, Cd, Cs Co, Cu, Cr, Ni, Pb, Sr, Th, U and Zn from contaminated soils, wastes, and municipal solid waste incinerator ash (12, 13).

The BNL Citric Acid Process. Although citric acid is an effective chelating agent for removing of metals and radionuclides from contaminated soils, the ultimate disposal of the citric-acid-metal extract is a concern. Francis and Dodge [13] developed a process to recover the metals and radionuclides from the extract. In this process, the extract is subjected to microbiological degradation, followed by photochemical degradation under aerobic conditions. Several metal citrate complexes are readily biodegraded, and the metals are recovered in a concentrated form, along with the bacterial biomass [14, 15]. Uranium forms a binuclear complex with citric acid and is not biodegraded [14, 16]. The supernatant containing this complex is separated, and exposed to light, whereupon it rapidly degrades forming an insoluble, stable polymeric form of uranium [17]. Uranium is recovered as a precipitate ($UO_3 \cdot 2H_2O$) in a concentrated form for recycling, or for disposal. This treatment process, unlike others which use alkaline or acidic reagents, does not create additional hazardous wastes for disposal, and causes little damage to the soil which can then be returned to normal use.

Extraction of Radionuclides and Metals. Ten grams of sludge containing uranium and several toxic metals obtained from the Oak Ridge Y-12 Plant, were extracted with 100 ml of 0.4 M citric acid, for five hours in the dark. The citric acid extract and the solids were separated and analyzed for metals by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). In this sample, Al, Be, Co, Cr, Mn, Ni, Sb, Sn, Zn, and Zr were extracted with >50% efficiency; U and Th were extracted with 87% and 94% efficiency, respectively. Ag, Cu, Pb, and V were not extracted, probably due to their type of association with stable mineral phases (8). For example, copper was predominantly associated with the organic fraction and a small amount with the iron oxide and inert fractions. The efficiency of extraction of uranium from various soils from the Fernald and RMI sites, Ohio ranged from 67 to 99% depending on the chemical speciation and the minerogical asssociation of U.

Biodegradation of Metal Citric Acid Extract. Citric-acid sludge extract was amended with 0.1% NH_4Cl. The pH was adjusted to 6.5 with NaOH, and then the extract (100 ml) was inoculated with 4 ml of an 18-hour old culture of *Pseudomonas fluorescens* (ATCC #55241). The samples were incubated for 118 hours on a shaker at 24°C. The bacterial inoculum was grown in medium containing citric acid, 2 g; NH_4Cl, 1 g; KH_2PO_4, 1 g; K_2HPO_4, 1 g; NaCl, 4†g; $MgSO_4$, 0.2 g; distilled water, 1000 ml; and pH 6.5. All samples were prepared under low light to minimize any photochemical reactions. Periodically, 5 ml aliquots were removed, filtered through a 0.22 μm filter, and analyzed for (i) pH; (ii) citric acid; and (iii) uranium. At the end of incubation, the supernatant and the solids consisting of bacterial biomass and the precipitated metals were separated by centrifugation. The dry weight was determined, and the solids were digested in a mixture of hot nitric and perchloric acids. The supernatant and the digested solids were analyzed for uranium and other metals by ICP-MS. The bacteria degraded citric acid at a rate of 0.5-0.7 mM per hour; there was little change in concentration of uranium suggesting that the uranium-citrate complex was not biodegraded. Co, Ni, Zn, and Zr were recovered with the biomass.

Photodegradation of Uranium Citrate Extract. The pH of the supernate from the biodegradation treatment primarily containing uranium citrate complex was adjusted to 3.5 with HCl, and then exposed to high output fluorescent growth lights to degrade the complex and

recover uranium. Periodically,†samples were withdrawn, filtered through a 0.22 μm filter, and analyzed for uranium, citric acid, and photodegradation products. At the end of the experiment (after 157 hours of exposure to light), the solutions were filtered and analyzed for citric-acid degradation products and metals. The uranium precipitated out of solution as a polymer soon after it was exposed to light. After 50 hours, ~ 85% of the uranium was removed from solution.

Characterization of Uranium Precipitate. The uranium precipitate was identified as ($UO_3 \cdot 2H_2O$) by X-ray Photoelectron Spectroscopy (XPS) and by X-ray Absorption Near-Edge Spectroscopy (XANES) at the National Synchrotron Light Source (NSLS) [17]. The uranium precipitate was quite insoluble at the near neutral pH, and soluble in acidic pH (<3.5).

These results show that (i) uranium was extracted from the mixed waste with >85% efficiency using 0.4 M citric acid; (ii) other metals such as Cr, Co, Mn, Ni, Sr, Th, Zn, and Zr also were extracted; (iii) the uncomplexed excess citric acid and several metal citrate complexes (Co, Ni, Zn and, Zn) except the of binuclear uranium- citrate complex, were readily biodegraded by *Pseudomonas fluorescens*, and the metals were recovered with the bacterial biomass; and (iv) the uranium citrate complex was photodegraded, allowing the uranium to form a polymer which was recovered as a concentrated solid.

The potential applications of BNL Citric Acid Process for the treating heavy metal such as lead and lead-paint contaminated soils, municipal soild waste incinerator ash and certain forms of radioactive solid and liquid wastes, looks very promising. In this process the toxic metals and uranium are recovered in a concentrated form for recycling or for disposal. This process can be applied to a variety of materials and waste forms; it does not generate secondary waste streams; it causes little damage to soil; and environmentally and economically important metals are removed in a concentrated form.

Acknowledgements

This research was performed under the auspices of the Environmental Sciences Division's Subsurface Science Program, Office of Health and Environmental Research, Office of Energy Research and in part by the Office of Technology Demonstration, EM, and U.S. Department of Energy under Contract No. DE-AC02-98CH10886.

Literature

1. Francis, A.J. 1998. Biotransformation of uranium and other actinides in radioactive wastes. J. Alloys and Compounds. (in press).

2. Francis, A. J. 1990. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. Experientia. 46:840-851.

3. Francis, A. J. 1994. Microbial transformations of radioactive wastes and environmental restoration through bioremediation. J. Alloys Compounds. 213/214:226-231.

4. Francis, A. J. 1995. Microbiological treatment of radioactive wastes. pp. 115-131. In W. W Schultz and E. P. Horwitz, (Ed.) Chemical Pretreatment of Nuclear Waste Disposal. Plenum Press, New York.

5. Francis A. J. 1997. Biotechnology of Radioactive Wastes: A General Overview. C. Ronneau and O. Bitchaeva (eds.), Biotechnology of Waste Management and Site Restoration, 19-28. Kluwer Academic Publishers. The Netherlands.

6. Francis, A.J. 1998. Bioremediation of radionuclide and toxic metal contaminated soils and wastes. In Monograph ìBioremediation of Contaminated Soils,î Soil Science Society of America/American Society for Agronomy, in press.

7. Tessier, A., P., G. C. Campbell, and M. Bisson. 1979. Sequential extraction procedure for speciation of particulate trace elements. Anal. Chem. 51:844-851.

8. Francis, A. J., C. J. Dodge, J. B. Gillow, and J. E. Cline. 1991. Microbial transformation of uranium in wastes. Radiochim. Acta. 52/53:311-316.

9. Francis, A. J., C. J. Dodge, and J. B. Gillow. 1991: Microbial stabilization and mass reduction of wastes containing radionuclides and toxic metals, U.S. Patent 5,047,152.

10. Francis, A. J., C. J. Dodge, F. Lu, G. P. Halada, and C. R. Clayton. 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp. Environ. Sci. and Technol. 28:636-639.

11. Nishita, H., R. M. Havg, and T. Rutherford. 1977. Effect of inorganic and organic compounds on the extractability of ^{235}Pu from an artificially contaminated soil. J. Environ. Qual. 6:451-455.

12. Francis, A. J. and C. J. Dodge. 1992. Reclamation with recovery of radionuclides and toxic metals form contaminated materials, soils, and wastes. No. 3189, Vol. 1:109. *Technology 2002*, NASA Conference Publication, Washington DC.

13. Francis, A. J. and C. J. Dodge. 1994a. Wastes site reclamation with recovery of radionuclides and metals, U.S. Patent No. 5,292,456.

14. Francis, A. J., C. J. Dodge, and J. B. Gillow. 1992. Biodegradation of metal-citrate complexesand implications for toxic metal mobility. Nature 356:140-142.

15. Francis, A. J. and C. J. Dodge. 1993. Influence of complex structure on the biodegradation of iron-citrate complexes. Appl. Environ. Microbiol. 59:109-113.

16. Dodge, C.J. and Francis, A.J. 1997. Biotransformation of binary and ternary citric acid complexes of iron and uranium. Environ. Sci. Technol. 31, 3062-3067.

17. Dodge, C. J. and A. J. Francis. 1994. Photodegradation of uranium citrate complex with uranium recovery. Environ. Sci.Technol. 28:1300-1306.