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MICROBIAL TRANSFORMATIONS OF RADIONUCLIDES AND ENVIRONMENTAL RESTORATION THROUGH BIOREMEDIATION

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Microbial Transformations of Radionuclides and Environmental Restoration Through Bioremediation

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ABSTRACT

Treatment of waste streams containing radionuclides, the remediation of contaminated materials, soils, and water, and the safe and economical disposal of radionuclides and toxic metals containing wastes is a major concern. Radionuclides may exist in various oxidation states and may be present as oxide, coprecipitates, inorganic, and organic complexes depending on the process and waste stream. Unlike organic contaminants, the metals cannot be destroyed, but must either be converted to a stable form or removed. Microorganisms present in the natural environment play a major role in the mobilization and immobilization of radionuclides and toxic metals.by direct enzymatic or indirect non-enzymatic actions and could affect the chemical nature of the radionuclides by altering the speciation, solubility and sorption properties and thus could increase or decrease the concentrations of radionuclides in solution.

Fundamental understanding of the mechanisms of microbiological transformations of various chemical forms of uranium present in wastes and contaminated soils and water has led to the development of novel bioremediaition processes. One process uses anaerobic bacteria to stabilize the radionuclides by reductive precipitation from higher to lower oxidation state with a concurrent reduction in volume due to the dissolution and removal of nontoxic elements from the waste matrix. In an another process, uranium and other toxic metals are removed from contaminated surfaces, soils, and wastes by extracting with the chelating agent citric acid. Uranium is recovered from the citric acid extract after biodegradation followed by photodegradation in a concentrated form as $UO_3{\cdot}2H_2O$ for recycling or appropriate disposal. These processes use all naturally occurring materials, common soil bacteria, naturally occurring organic compound citric acid and sunlight.

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1. MICROBIAL TRANSFORMATIONS OF RADIONUCLIDES

Large volumes of radionuclides and toxic metals containing wastes generated from nuclear fuel cycle and nuclear weapons production facilities and the contamination of the environment is a major concern. The cost of remediating contaminated sites is quite prohibitive. Because these problems are pervasive, new innovative treatment and remediation technologies, including bioremediation using microorganisms for stabilization or removal and recovery of the contaminants are being developed (1).

Although the mechanisms of interactions between metal ions with microorganisms have been extensively studied, fundamental information is lacking on specific microbial processes and the biochemical mechanisms involved in the transformations of radionuclides and toxic metals in contaminated soils and waste sites. Microorganisms can affect the solubility, bioavailability, and mobility of radionuclides and toxic metals (2). Unlike organic contaminants, metals cannot be destroyed, but must be either removed or converted to a stable form. Radionuclides and toxic metals may be present initially as soluble or insoluble forms and, after disposal, may be converted from an insoluble to a soluble form or vice versa by the actions of microorganisms. These processes can be exploited for developing biotreatment strategies, including dissolution or immobilization of various forms of radionuclides and toxic metals present in wastes. Microbial activities are influenced by the presence of electron donors and acceptors. Microorganisms use oxygen as an electron acceptor under aerobic conditions, while, in the absence of oxygen, they can use nitrate, sulfate, metals, and carbon dioxide as alternate electron acceptors.

The form of the metal (e.g., elemental, oxide, sulfide, ionic, inorganic complex, organic complex, co-precipitate), the availability of electron donors, nutrients (nitrogen, phosphorus), the presence of electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, organic compounds), and the environmental factors (pH, Eh, temperature, moisture) affect the type, rate, and extent of microbial activity, and hence, these transformations. Under appropriate conditions, dissolution or immobilization of radionuclides and metals is brought about by one or more of the following mechanisms: (i) oxidation-reduction of metals which affect their valence state and solubility; (ii) changes in pH and Eh which affect the ionic state of the metals and their solubility; (iii) solubilization, and leaching of elements by microbial metabolites and decomposition products, such as organic acid metabolites or production of specific sequestering agents; (iv) volatilization due to alkylation reactions (biomethylation); (v) immobilization leading to formation of stable minerals or bioaccumulation by microbial biomass and biopolymers; (vi) biotransformation of metal/radionuclide organic complexes, and (vi) transport of radionuclides as biocolloids (2).

A clear understanding of the mechanisms of transformation of the contaminants under specific microbial process conditions may aid in the designing and developing appropriate treatment technology; these include processes for recovering and recycling elements, waste volume reduction, minimization, and stabilization. In addition comprehensive understanding of the microbial transformations of radionuclides under different environmental conditions will be useful in predicting their fate and transport in waste repositories, as well as the long-term stewardship of the contaminated and remediated sites still containing low levels of residual radionuclides (3-6).

In this paper, (i) the basic microbial processes involved in the transformation of uranium, (ii) the potential application of such knowledge to remediate contaminated sites or decontaminate metal surfaces and, (iii) treatment of waste stream with minimization of secondary waste generation are presented.

2. BIOREDUCTION OF URANIUM BY ANAEROBIC BACTERIA

Oxidizing and reducing conditions affect the mobilization and immobilization of radionuclides and toxic metals. In anaerobic environments, certain metals can be reduced enzymatically from a higher to a lower oxidation state which affects their solubility and bioavailability. For example, reduction of Fe(III) → Fe(II), Mn(IV) → Mn(II), Pu(IV) → Pu(III) increases its solubility, while reduction of Cr(VI) → Cr(III), U(VI) → U(IV) or Pu(VI) → Pu(IV) decreases their solubility (6-9). Many organic compounds can form stable complexes with radionuclides, and increase their solubilization and leaching. Likewise, microbial metabolites and waste degradation products or intermediates may be an important source of agents that affect the long-term solubility of radionuclides.

The direct implication of microorganisms in the reduction of uranium is of considerable interest because of its potential application in bioremediating of contaminated sites, in pretreating radioactive wastes, and in processes critical to the performance of nuclear waste repositories. Although a wide variety of microorganisms are present in radioactive wastes and in natural deposits of radioactive minerals, the extent to which microbes can alter the mobility of radionuclides is not fully known. The reduction of uranium was reported in axenic cultures of iron-reducing bacteria, fermentative bacteria, sulfate-reducing bacteria, and cell-free extracts of *Micrococcus lactilyticus,* and in uranium wastes by clostridia.

$$\mathrm{U(VI)}_{aq} \xrightarrow[\text{fermenters, sulfate reducers}]{\text{dissimilatory metal reducers}} \mathrm{U(IV)}_{s}$$

Clostridia are strict anaerobic spore forming bacteria in ubiquitous in soils, sediments, and wastes. We isolated a *Clostridium* sp. (ATCC 53464), a gram-positive, rod shaped (2 to 3 by 1.0 μm), spore forming, nitrate reductase-negative, N_2-fixing bacteria from coal cleaning residues which reduced Fe(III) to Fe(II), Mn(IV) to Mn(II), Tc(VI) to Tc(IV), U(VI) to U(IV) and PU(IV) to Pu(III) (6-9). Speciation of uranium in microbial cultures by x-ray absorption near edge spectroscopy (XANES) at the National Synchrotron Light Source (NSLS) showed that soluble U(VI) added as uranyl nitrate was reduced to insoluble U(IV) by *Clostridium* sp. (Figure 1). XANES spectra of U at the M_V absorption edge shows the absorption edge for uninoculated U at 3551.1 eV, which is identical to U(VI) standard. The inoculated sample shows a peak at 3550.1 eV, which is lower than U(IV) but higher than U metal, indicating the presence of a highly reduced form of U, most probably a mixture of U(IV) and U(III).Uranium was reduced only in the presence of growing or resting cells. Organic-acid metabolites, spent culture medium, and heat-killed cells failed to reduce uranium under anaerobic

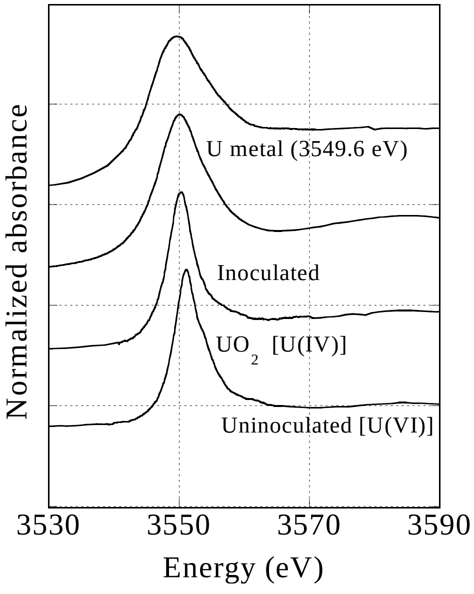

Fig. 1. XANES spectra of U reduction by *Clostridium* sp. at the M_V absorption edge (8).

conditions (8). The *Clostridium* sp. also solubilized ferric iron in hematite, goethite, and ferrites, and manganese(IV) in pyrolusite by enzymatic reduction, as well as the oxides of cadmium, copper, lead, and zinc by the production of organic-acid metabolites. The change in free energy for reducing manganese and iron was -83.4 and -27.2kcal/mol CH_2O, respectively, while for reducing of hexavalent uranium to the tetravalent state, the change was -63.3kcal/mol, and to the trivalent state, 52.2kcal/mol. Uranium reduction should occur in the sequence Mn(IV) > U(VI) > Fe(III).

3. BIOTRANSFORMATION URANIUM COMPLEXED WITH CITRIC ACID

Citric acid, a naturally occurring compound, is a multidentate ligand and forms stable complexes with various metal ions. The type of complex formed plays an important role in determining its biodegradability (Figure 2) (10). The rate and extent of biodegradation of several metal-citrate complexes varies. For example, *Pseudomonas pseudoalcaligenes* degraded Mg-citrate at a lower rate than Ca-, Fe(III)-, and Al(III)-citrate (11). Studies with *Klebsiella* sp. showed that citric acid and Mg-citrate were readily degraded, whereas Cd-, Cu-, and Zn-citrate were resistant (12). Both studies also showed that metal toxicity was not responsible for the lack of, or the lower rate of, degradation of certain metal-citrate complexes but gave no other explanation. Biodegradation studies with *P. fluorescens* showed that bidentate complexes of Fe(III)-, Ni-, and Zn-citrate were readily biodegraded, whereas complexes involving the hydroxyl group of citric acid, the tridentate Al-, Cd- and Cu-citrate complexes, and the binuclear U-citrate complex were not (Figure 3). The presence of the free hydroxyl group of citric acid is the key determinant in effecting biodegradation of the metal complex. The lack of degradation was not due to their toxicity, but was limited by the transport and/or metabolism of the complex by the bacteria (13). No relationship was observed between biodegradability and stability of the complexes. The tridentate Fe(II)-citrate complex, although recalcitrant, was readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)-citrate form, denoting a structure-function relationship in the metabolism of the complex (14).

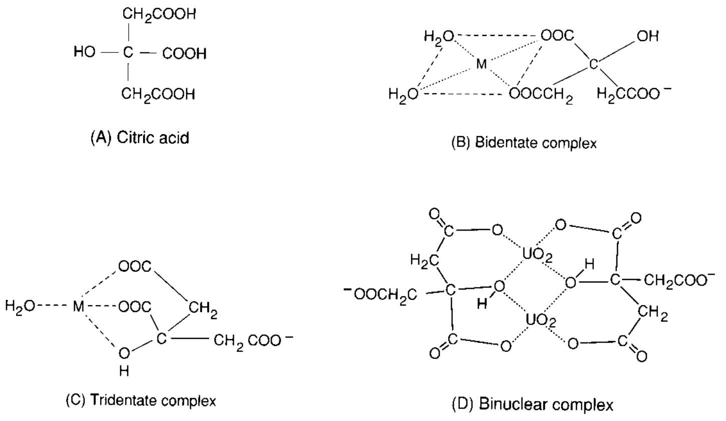
(A) Citric acid
(B) Bidentate complex
(C) Tridentate complex
(D) Binuclear complex

Fig 2. Types of metal citrate complexes. (A) citric acid; (B) bidentrate complex invovlving two carboxylic acid groups; (C) tridentate complex involving two carboxylic acids and the hydroxyl group; (D) binuclear complex with UO_2^{2+}(10).

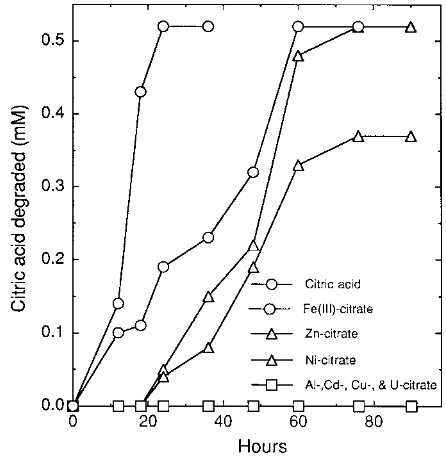

Fig 3. Biodegradation of metal-citrate complexes by *Pseudomonas fluorescens* (13).

4. BIOREMEDIATION URANIUM OF CONTAMINATED MATERIALS, SOILS AND WASTES

The presence of radionuclides and toxic metals in wastes, soils, sediments, and materials is a major environmental concern. Unlike organic contaminants, the metals cannot be destroyed, but must either be converted to a stable form or removed. Basic research in understanding the fundamental mechanisms of microbiological transformations of radionuclides and toxic metals has led to the development of two novel processes for remediating of contaminated surfaces, soils and wastes: (i) stabilization of radionuclides and toxic metals in wastes by anaerobic bacteria with a concurrent reduction in volume, and (ii) removal and recovery of radionuclides and toxic metals in a concentrated form for recycling or appropriate disposal.

4.1. Characterization of Uranium in Contaminated Soils and Wastes. The radionuclides and toxic metals most commonly found in soils and wastes include americium, cadmium, cesium, chromium, cobalt, copper, lead, manganese, nickel, plutonium, technetium, thorium, strontium, uranium, and zinc. Typically, these contaminants are present in various forms, such as elemental, carbonate complexes, oxides, coprecipitates (metals coprecipitated with iron and manganese oxides), natural minerals, and soluble and insoluble organic complexes. In soils and wastes they may be present initially as soluble forms or they may be formed after disposal by chemical and microbiological processes. In addition to the total concentrations of the radionuclides detailed information needed on the chemical speciation (oxidation state) and their mineralogical association such as exchangeable form, carbonate complex, Fe-, and Mn-oxide association, organic and inert fractions. Such information is useful in the selection of appropriate remediation technology and meeting the clean goals (15-17).

We investigated soil samples contaminated with uranium from the Fernald site in Ohio, soils contaminated with 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. The concentrations of U in the soils varied from 473 to 1880 ppm, and Tc activities ranged from 109 to 144 p ci/g (Figure 4). The sludge was generated from a uranium-process waste stream after biodenitrification of nitric-acid uranium wastewater. Several million gallons of the sludge is in storage awaiting disposal. The sludge sample contained varying levels of major elements, Al, Ca, Fe, Mg, K, and Na, and toxic metals, As, Cd, Cr, Co, Cu, Pb, Mn, Hg, Ni, U, and Zn. The sludge sample contained 3100

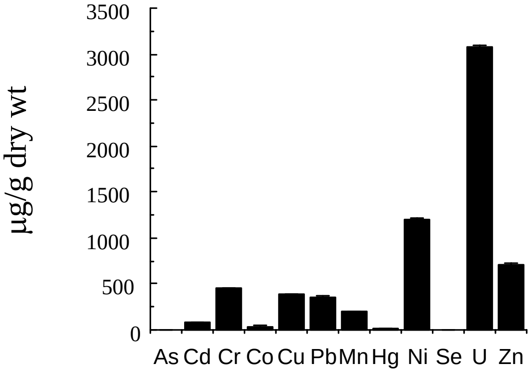

Fig 4. Analysis of metals in sludge (15).

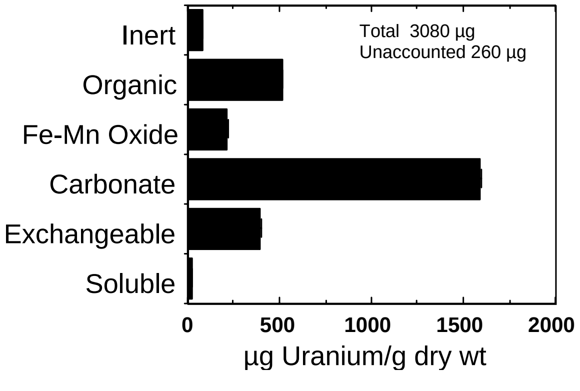

Fig 5. Minerlogical association of uranium in sludge (15).

ppm of uranium. The sediment contained high levels of Cr, Cu, Mn, Hg, and Zn. The sludge contained high levels of Ca, Cd, Cr, Pb, and Ni and low in Fe and Mn. In addition to those elements 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 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.

The mineralogical association of cadmium, chromium, copper, manganese, nickel, lead, uranium, and zinc in the sludge was determined by a selective extraction procedure. Figure 5 shows these associations of uranium in the sludge. Nearly half of the total uranium (1600 μg) in the sludge was associated with the carbonate fraction. The association of uranium with other fractions 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).

5. STABILIZATION AND VOLUME REDUCTION OF RADIONUCLIDES AND TOXIC METAL IN WASTES

In this process uranium and toxic metal containing wastes are treated with an anaerobic bacterium *Clostridium sp*. to release a large fraction of the waste solids into solution. The radionuclides and toxic metals are converted to a stable form. At the same time, their volume and mass are reduced for economical disposal. For example, anaerobic microbial treatment of uranium containing sludge from the Y-12 Plant, Oak Ridge TN, resulted in the removal of a large fraction of soluble non-toxic metals such as Ca, K, Mg, Mn^{2+}, Na, and Fe^{2+}; enrichment and stabilization of Cd, Cr, Cu, Ni, Pb, U and Zn; and overall volume and mass reduction. In this novel approach to treatment of wastes, the unique metabolic capabilities of the dual-action anaerobic bacteria are exploited to (i) solubilize radionuclides and toxic metals by direct enzymatic action as well as indirect action due to production of organic acid metabolites; and (ii) stabilization of remobilized radionuclides and toxic metals by bioprecipitation reactions in a

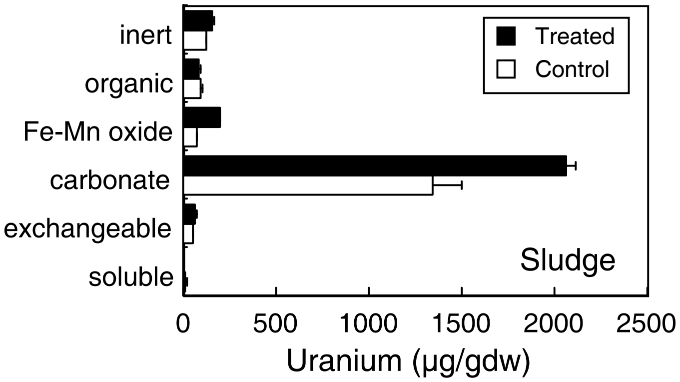

Fig 6. Mineralogical association of uranium before and after anaerobic bacterial treatment (16).

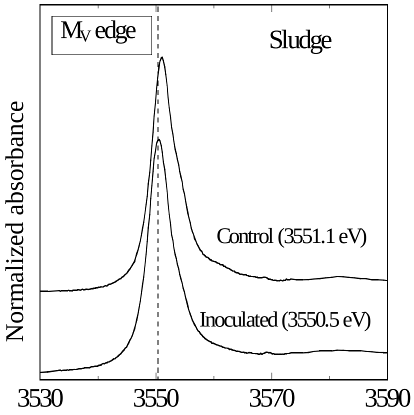

Fig 7. X-ray absorption near edge spectra (XANES) of sludge treated with anaerobic bacteria showing reduction of U(VI) to U(IV) (16).

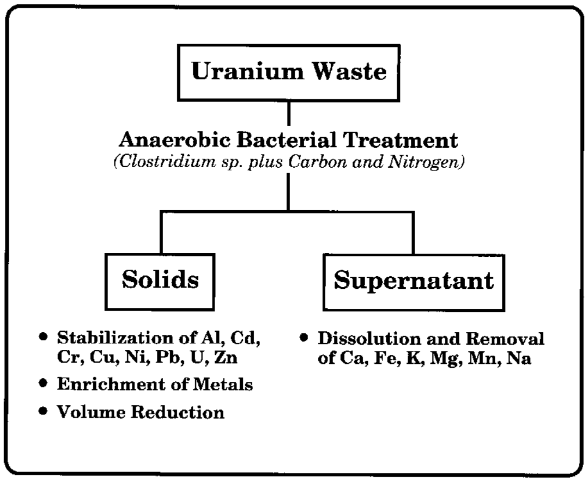

Fig 8. Anaerobic Microbial stabilization and volume reduction of uranium waste (16; U.S. Patent 5,047,152).

concentrated form in the waste for disposal. The solubilized non-hazardous bulk components Ca, K, Fe, Mg, and Mn are removed resulting in significant waste volume and weight reduction. The non-hazardous materials in the solid phase were solubilized and removed from the waste, thereby reducing its volume. The remobilized radionuclides and toxic metals are stabilized by precipitation reactions and redistributed with stable mineral phases of the waste The net weight reduction in batch studies was approximately 20%. An additional reduction in waste volume can be achieved by process optimization of process parameters. This biotreatment process can be applied to wastes generated from defense, energy, and nuclear industries. The radionuclides and toxic metals are converted to a more stable and concentrated form which allows the material to be chemically processed more readily or disposed of in shallow or deep geological formations. Anaerobic microbial pretreatment results in a waste which is less susceptible to environmental factors and can be disposed of more economically (16). Reactive barrier technology is based on the activities of these anaerobic bacteria. However, the long-term stability of the microbially immobilized uranium is not known.

6. REMOVAL AND RECOVERY OF TOXIC METAL AND RADIONUCLIDE CONTAMINATED SOILS AND MATERIALS

For remediation, 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, readily biodegradable, environmentally friendly, exhibits 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 to decontaminate components of nuclear reactors, to extract metals and radionuclides such as arsenic, barium, cadmium, cesium cobalt, copper, chromium lead, nickel, zinc, strontium, thorium, and uranium from contaminated soils, wastes, and incinerator ash.

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. A biotreatment process was developed to recover the metals and radionuclides from the extract based on the ability of bacteria to degrade metal citrate complexes (17). 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 except uranium which is recovered after biodegradation by photodegradation. 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.

6.1. Extraction of Uranium from Contaminated Materials by Citric acid. The Citric Acid Process has been successfully tested to treat uranium and toxic metals contaminated soils from Fernald (FERMCO), and Ashtabula (RMI) sites in Ohio, and sludge from uranium processing plant at Y-12, Oak Ridge, TN. Using single step batch extractions, 70 - 99% of the uranium has been effectively removed from the sludge and soil samples (Table 1). Biodegradation and photodegradation treatments of the extract resulted in >99% removal and recovery of the extracted uranium (18).

Table 1 shows citric acid's efficiency in extracting uranium and several other metals from the sludge and soils (18). The form and the mineralogical association of the metals influenced the extraction efficiency. Metals associated with organic- and inert- fractions were probably more stable and difficult to extract. Uranium was removed from the RMI and Fernald soils with an efficiency of 85% and 99%, respectively. In addition, significant amounts of Al, Ca, Co, Cr, Mg, Mn, Ni, Pb, Sr, Th, and Zn from the sludge; Ca, Cu, Mg, Mn, and Zn were extracted from the RMI soil; and Ca, Mg, Mn, and Sr from Fernald soil.

TABLE 1. Extraction of Uranium and Other Metals by Citric Acid from Sludge and Contaminated Soils[a]

	Y-12 Oak Ridge sludge		RMI soil		Fernald soil[b]	
metal	total (μg gdw^{-1})	extracted (%)	total (μg gdw^{-1})	extracted (%)	total (μg gdw^{-1})	extracted (%)
Al	30500 ± 500	59	6610 ± 1760	nd	37000	3
Ba	427 ± 25	24	nd	–	380	32
Ca	364000 ± 6000	43	22700 ± 400	72	42000	99
Cd	66 ± 6	9	nd	–	nd	–
Co	0.7 ± 0.3	75	nd	–	nd	–
Cr	342 ± 10	75	3.2 ± 2.3	nd	35	9
Cu	329 ± 18	1	336 ± 12	69	85	12
Fe	4700 ± 100	–	13900 ± 800	nd	28000	3
Mg	7510 ± 100	90	2800 ± 310	59	18000	52
Mn	234 ± 3	83	334 ± 19	60	730	66
Ni	1120 ± 10	80	nd	–	nd	–
Pb	224 ± 27	49	nd	–	nd	–
Sr	125 ± 5	59	nd	–	140	65
Th	3.1 ± 0.1	94	–	–	–	–
Ti	922 ± 95	28	2300 ± 60	17	3600	5
U	2410 ± 100	87	732 ± 94	85	449	99
Zn	839 ± 7	60	169 ± 13	51	82	16

[a] nd, none detected; –, not determined. Sludge and RMI soil were extracted with 0.4 M citric acid. [b] Fernald soil extracted with 0.3 M citric acid.

6.2. Biodegradation of Citric Acid Extract. *Pseudomonas fluorescens* (ATCC #55241), isolated from a low-level radioactive waste disposal site at West Valley, NY, was used to biodegrade the citric acid extracts. This bacterium degraded several metal citrate complexes but not uranyl citrate. The extract was diluted four-fold with deionized water, 0.1% NH_4Cl was added, and the pH was adjusted to 6.5 with NaOH. One hundred milliliters of the diluted extract, in triplicate, was inoculated with 4 ml of an 18-h old culture of *P. fluorescens* and incubated on a shaker at 24°C. All samples were prepared under low light to minimize any photochemical reactions. Citric-acid extracts of Y-12 sludge, Fernald, and RMI soil samples were biodegraded by *P. fluorescens* at the rate of 0.83, 0.63, and 1.01 mmol h^{-1}, respectively (Figure 9). The pH of the extracts increased from 6.5 to ~8.5 due to the degradation of citric acid. The uranyl-citrate complex was recalcitrant and remained in solution. However, a slight decrease in concentration of soluble uranium was noticed in all extracts when most of the citrate was degraded and the culture had reached a stationary growth phase. In contrast, the concentration of other metals in the extract generally decreased with time. The following are the rates and extent of removal of selected metals from the extract (µmol h^{-1}, %): Al (10.5, >99), Ca (41.7, >99), Cu (0.46, >99), Fe (6.2, >99), Mg (9.1, 80), Sr (0.26, >99), Ti (0.11, 89), and Zn (2.6, >99). Analysis of the solids following biodegradation showed concentration and recovery of precipitated metals with the biomass.

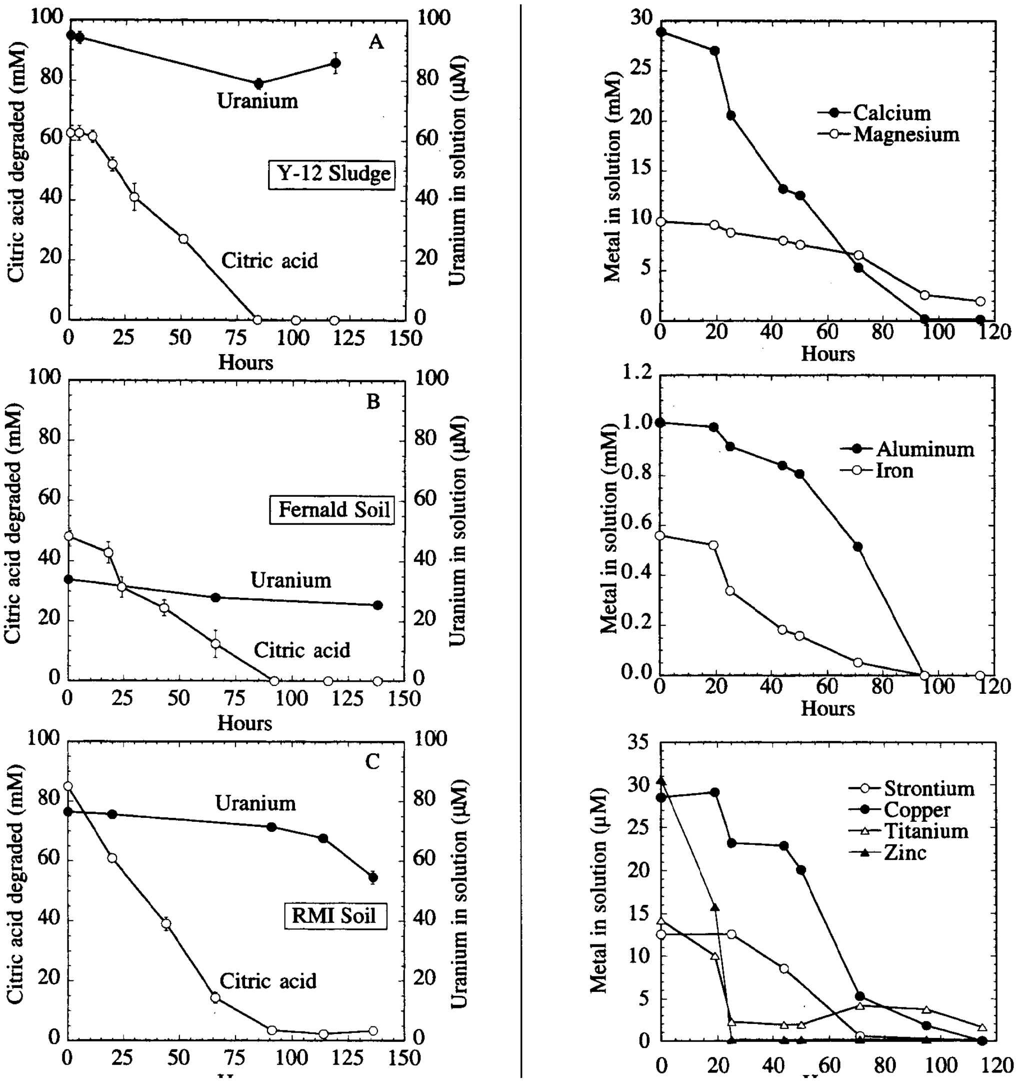

Fig 9. Biodegradation citric acid extract and fate of uranium and metals (18).

6.3. Photodegradation of Uranyl citrate after biodegradation of citric acid extract. The supernatant recovered after biodegradation of the citric acid extract was filtered through a 0.22 µm filter. The pH of the filtered supernatant was adjusted to 3.5 with HCl, and then exposed to high output fluorescent growth lights placed ~20 cm above the sample in an incubator at 26 ± 1^{o}C. The lamps displayed a broad spectrum in the visible region from 400 to 700 nm, and the total intensity at the sample was 0.27 mEinstein m^{-2} s^{-1}. The maximum absorption of the uranyl citrate complex was at 435 nm, and the light intensity at this wavelength was 0.04 mEinstein m^{-2} s^{-1}. Control samples were incubated in the dark. Periodically, 2 ml samples were withdrawn, filtered through a 0.22 µm filter, and analyzed for uranium, citric acid, and photodegradation products. The precipitate from the photodegradation of the extract was recovered by centrifugation and characterized by X-ray absorption spectroscopy. Exposure of the citric-acid extracts containing uranium to light after biodegradation precipitated the uranium, the rate and extent of which varied with the extract: Y-12 sludge (1.5 µmol h^{-1}, 85%); FERMCO soil, (0.26 µmol h^{-1}, 72%); and RMI soil (1.82 µmol h^{-1}, 100%) (Figure 6). The pH of the samples increased slightly from 3.5 to 3.6 during the photodegradation (8). The uranium precipitate was identified as ($UO_3 \cdot 2H_2O$) by x-ray Absorption Near-Edge Spectroscopy (XANES) and Extended X-ray absorption fine structure (EXAFS) at the National Synchrotron Light Source (NSLS) (19, 20).

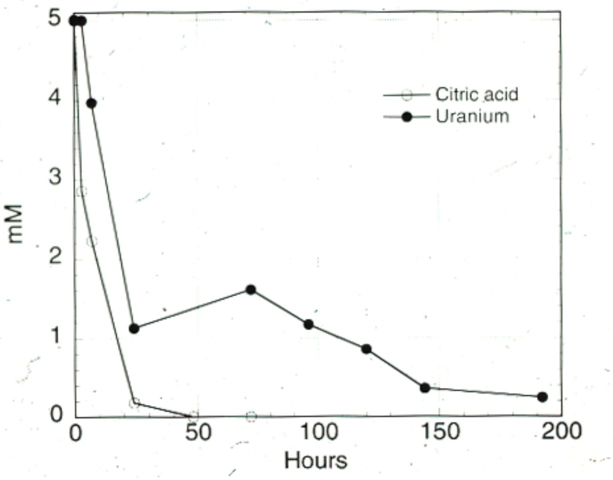

Fig 10. Photodegradation of 1;1 uranium citric acid (19).

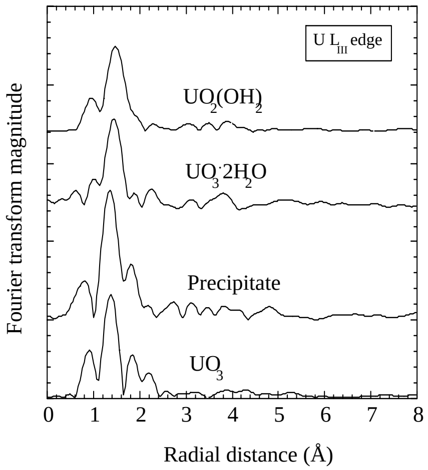

Fig 11. EXAFS analysis of uranium precipitate from phortdegradation of uranyl citrate (19).

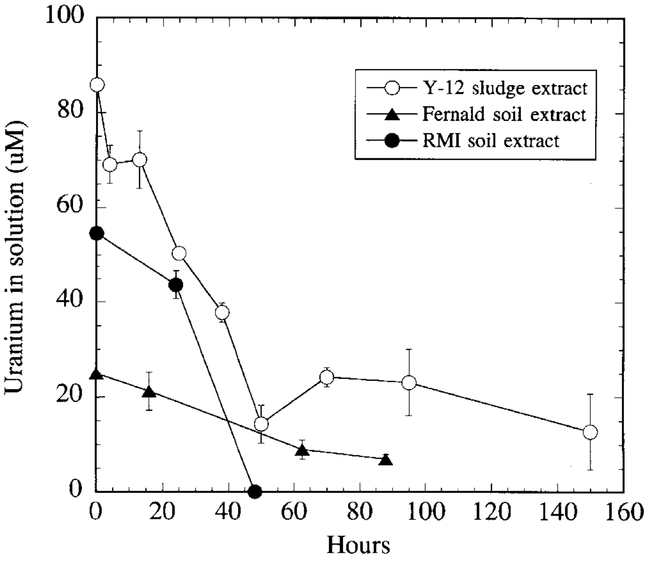

Fig 12. Photodegrdation of uranyl citrate after biodegradation citric acid soil extracts (18-20).

6.4. Decontamination of Uranium Contaminated Metallic Surfaces by Citric acid.

Based on studies with the remediation of radionuclide contaminated soils we developed a simple and safe method was developed for the removal of radioactive contaminants from slightly contaminated steel and other surfaces so that the metals can be reused (21, 22). Citric acid was used to selectively remove the low levels of radionuclides such as U from contaminated metallic surfaces and then the citric acid solution containing iron and uranium was subjected to biodegradation to remove iron followed by photodegradation to recover uranium resulting in a significant reduction in secondary waste generation. Biodegradation of citric acid solution containing iron and uranium resulted in removal of iron (96%) from solution. Uranium however, was recalcitrant due to the nature of the complex formed. Photodegradation of citric acid solution following biodegradation resulted in complete precipitation of uranium.

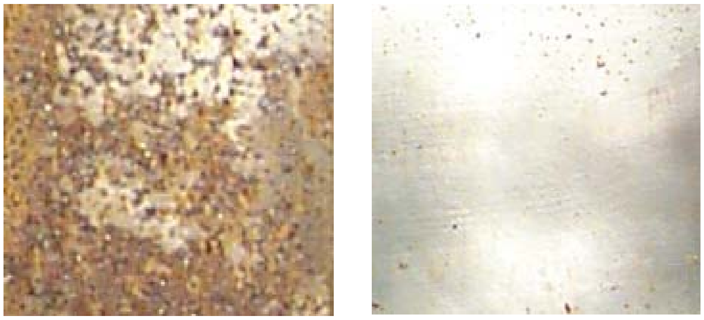

Fig 13. Uranium contamianted carbon steel coupons before and after decontramination using citric acid-peroxide-citric acid (CPC) treatment (22).

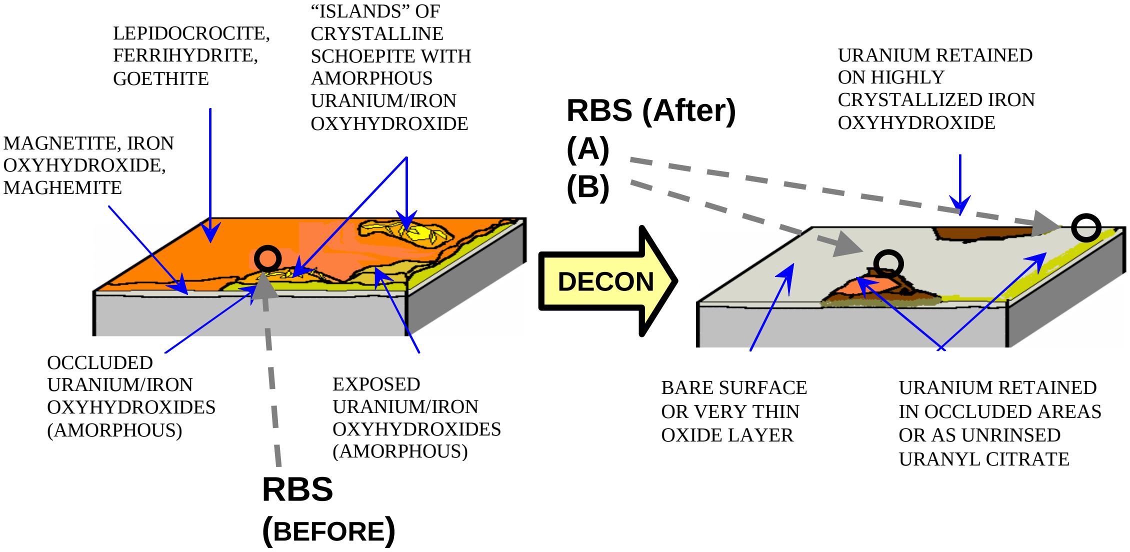

Fig 14. Nature of uranium association with corroded steel coupons before and after citic cid-peroxide -citric acid treatment. Rutherford backscattering (RBS) showing uranium retention before and after treatment (22).

6.5. Citric acid Process Description. The overall process consists of three steps: extraction, biodegradation, and photodegradation (Figure 1). Citric acid, a naturally occurring organic complexing agent, is used to extract metals and radionuclides from contaminated soils and wastes by formation of different types of complexes such as a bidentate, tridentate, binuclear and polynuclear species. The extract containing radionuclides and metals is subjected to biodegradation resulting in the bioprecipitation of metals such as Ba, Cd, Cr, Ni, Pb, Zn, and radionuclides Co, Sr, and Th which are recovered in a concentrated form. Uranium forms a binuclear complex with citric acid which is recalcitrant and remains in solution. The supernatant containing the uranium in solution after biodegradation of the extract is separated and upon exposure to light, undergoes rapid photodegradation resulting in the precipitation of uranium as a stable oxide ($UO_3 \cdot 2H_2O$). Uranium is recovered in a concentrated form after photodegradation for recycle or disposal.

These results demonstrate the potential for removing uranium and toxic metals from wastes, contaminated soils, and materials in a process involving extraction with the complexing agent citric acid, biodegradation of the extract to recover the toxic metals, followed by photochemical degradation of the extract to recover uranium as $UO_3 \cdot xH_2O$. Thus the mixed wastes containing toxic metals and uranium can be separated and recovered in a concentrated form as hazardous- and radioactive- waste fractions for recycling or for disposal.

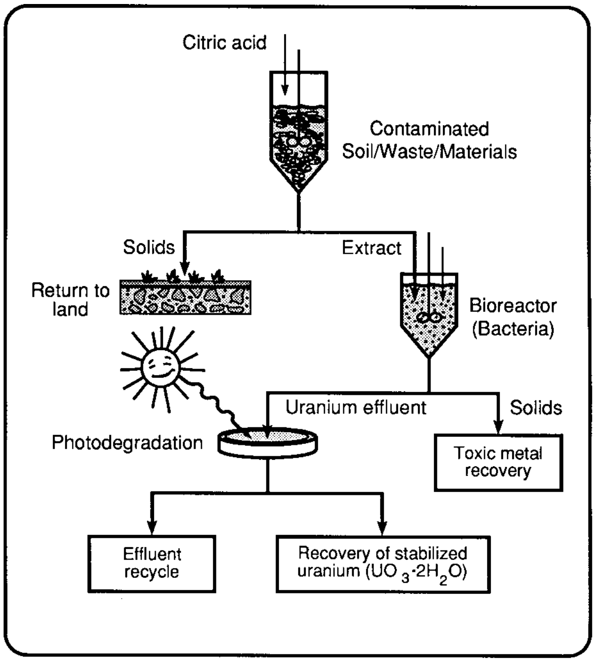

Fig 15. Citric acid treatment process (17; U.S. Patent No. 5292456).

6.6. Removal of Lead and other toxic Metals from Soil. The Citric Acid Process has also been used to remove lead, cadmium and other toxic metals from municipal solid waste incinerator ash and treatment of lead paint contaminated soils. The treatment of lead contaminated soils, however, remains difficult and costly. Restoration of these sites will not only meet the environmental regulatory compliance but also render the property usable and limits environmental exposure. Previous and currently used methods of lead remediation include stabilization using cement or precipitating agents such as phosphate which convert lead to a less water soluble form. However, these methods do not reduce the lead content of the contaminated soils. Current methods also significantly increase waste volume. The overall process consists of two steps involving extraction of contaminants by citric acid followed by biotreatment of the extract (Figure 16). Biodegradation of the extract results in the bioprecipitation of lead and degradation of citric acid to carbon dioxide and water. Lead and other metals recovered as hydroxide or phosphate along with the biomass in a concentrated form are suitable for recycle or disposal.

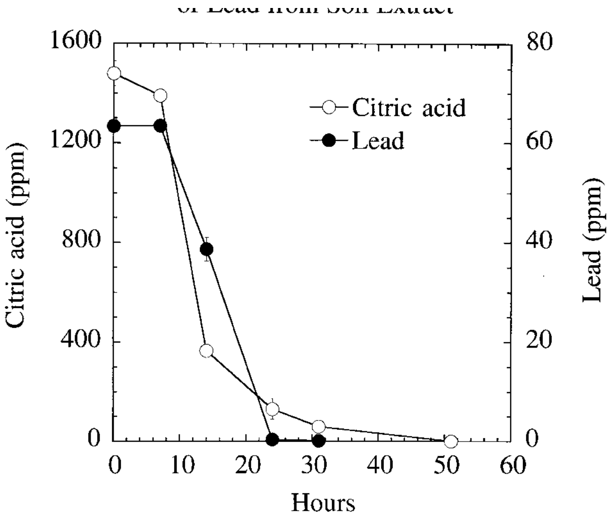

Fig 16. Biodegradation lead citric acid soil extract and bioprecipitation of lead (17, 18.

The process was tested on soil samples collected from a site which had typical lead paint applied to some of its structures. Lead concentrations in the soils tested ranged from 1750-1980 ppm. The lead was predominantly associated with the carbonate (23%), iron oxide (44%) and organic (26%) fractions of the soil. The removal efficiency of lead from the soil was >80%. The extracted lead is recovered from solution with >99% by bioprecipitation (Figure 16). The treated soil which contained 340 - 440 ppm of residual lead passed toxicity leaching procedure (TCLP) tests. The soil is then seeded with naturally occurring bacteria to biodegrade any residual citric acid with concurrent biostabilization of the lead.

7. SUMMARY

The potential applications of biotechnology in treating contaminated soils, materials, and certain forms of radioactive solid and liquid wastes, looks very promising. The mechanisms of biotransformation of radionuclides and toxic metals in contaminated soils and wastes were briefly discussed with the view of using such a knowledge-base for developing novel bioremediation strategies. Based on the information, two microbiological processes were described for treating wastes, soils, and materials contaminated with radionuclides and toxic metals. In one process, anaerobic microbes are used to concentrate, contain, and stabilize the toxic metals and radionuclides in the waste with a concurrent reduction in volume. In the second process, the radionuclides and toxic metals are removed from the waste or contaminated material and recovered in a concentrated form for recycling or for disposal.

(i) Stabilization of Radionuclides and Toxic Metals and Volume Reduction of Wastes by Anaerobic Bacterial Treatment. Stabilization of toxic metals and radionuclides in mixed wastes sludges, and contaminated soils or sediments is accomplished by exploiting the unique metabolic capabilities of the anaerobic bacterium, *Clostridium* sp. In this novel approach, the radionuclides and toxic metals are solubilized by 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 in the waste matrix by (i) enzymatic/chemical reductive precipitation processes; (ii) redistributed with stable mineral phases in the waste due to readsorption with the reactive surfaces; and (iii) biosorbed in the biomass. The re-mobilized radionuclides and toxic metals are thus stabilized by bacterial action. Iron, calcium, and other non-hazardous materials in the soluble phase are easily removed, reducing its volume. The remaining solids, which contain the hazardous components in a concentrated, but stable form, can be disposed of safely in the subterranean environment. The reduced volume of the waste also affords considerable savings in disposal costs (16).

(ii) Removal and Recovery of Uranium and Toxic Metals from Waste and Metal Surfaces. Uranium and toxic metals are removed from wastes or contaminated materials or soils by extracting with the complexing agent-citric acid. The citric-acid extract is subjected to microbiological degradation to recover the toxic metals, followed by photochemical degradation of the uranium citrate complex which is not biodegraded. The toxic metals and uranium are recovered in a concentrated form for recycling or for disposal. This process has significant potential for commercialization because (i) it can be applied to a variety of materials and waste forms; (ii) mixed waste is separated into radioactive and hazardous waste; (iii) uranium is separated from the toxic metals and recovered for recycling or disposal; (iv) it does not generate secondary waste streams; (v) it causes little damage to soil; and (vi) environmentally and economically important metals are removed in a concentrated form (17).

The use of organic acids to remove radionuclides from contaminated materials has the following advantages: (i) wet extraction which reduces dust formation, (ii) dissolution of oxides from the surface layer to remove fixed contaminants including those co-precipitated with iron oxides, (iii) the ability to penetrate porous materials to remove contamination below the surface, (iv) biodegradation of the secondary aqueous waste stream destroys most of the organic acids to carbon dioxide and water, and (v) the subsequent photodegradation of waste generated from biodegradation results in the precipitation and recovery of uranium in almost pure and concentrated form with volume reduction, which can be disposed of or recycled. We observed that the OPC process yields a mixture consisting of uranyl hydroxide and ferrihydrite, whereas pure uranium as schoepite is obtained from the CPC process. These results also suggest that, overall citrate-peroxide-citrate is an environmentally friendly green-chemistry process that uses all naturally occurring materials citric acid, common soil bacteria, and sunlight.

The use of combined chemical, photochemical, and microbiological treatments of contaminated materials will be more efficient than present methods and result in considerable savings in clean up and disposal costs.

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