Microbial transformations of radioactive wastes and environmental restoration through bioremediation

A.J. Francis

Department of Applied Science, Brookhaven National Laboratory, Upton, NY 11973 (USA)

Abstract

Bioremediation stabilizes and reclaims radionuclides and toxic metals from contaminated materials, soils, sediments, or wastes. The mechanism of microbial transformations of the radionuclides and toxic metals commonly found in energy wastes are summarized, and two processes for treating such wastes are described. In one process, anaerobic bacteria are used to concentrate, contain and stabilize the toxic metals and radionuclides in the waste, with a concurrent reduction in its volume. In the second process, the radionuclides and toxic metals are extracted from the wastes with citric acid which is then subjected to biodegradation, followed by photodegradation to recover the metals.

1. Introduction

The presence of radionuclides and toxic metals in wastes, soils and materials is a major environmental concern. Unlike organic contaminants, the metals cannot be destroyed, but must either be stabilized or removed. Microorganisms are known to catalyze the transformations of organic and inorganic compounds in nature and can be used effectively in solubilizing or immobilizing various forms of radionuclides and toxic metals present in mixed wastes [1]. The mechanisms by which microorganisms catalyze dissolution or precipitation of such contaminants include: (i) oxidation–reduction reactions which affect valence state and solubility; (ii) changes in pH and Eh; (iii) production of sequestering agents and (iv) bioaccumulation and biosorption. The radionuclides and toxic metals in wastes are often present in various forms, such as elemental, oxides (simple and complex oxides including ferrites), coprecipitates (metals coprecipitated with oxides of iron), carbonate complexes, organic and inorganic complexes, and naturally occurring minerals. 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, microbes can use nitrate, sulfate, metals, and carbon dioxide as alternate electron acceptors. The extent of dissolution and precipitation of metals can be significant, particularly under anaerobic conditions. In this paper, the mechanisms of microbial dissolution, stabilization, and recovery of radionuclides and toxic metals are discussed, with a view to exploit such processes for treating mixed wastes.

2. Microbial transformations of radionuclides and toxic metals

2.1. Dissolution of metal oxides

Oxides of metals are present in soils, ores, and residues generated from nuclear- and fossil-fuel cycles. The metal oxides are usually in crystalline forms and are insoluble in water. Microorganisms catalyze the dissolution of metal oxides by direct or indirect action [2]. Direct action involves enzymatic reductive dissolution of the metal from a higher to a lower oxidation state, wherein the metal is used as the terminal electron acceptor. Indirect action encompasses dissolution due to the production of metabolites, such as organic acids and chelating agents, as well as lowering the pH of the medium. Microbial reduction and dissolution of iron and manganese oxides under anaerobic conditions have been studied extensively.

In addition to the simple oxides, complex oxides, such as ferrites, can be solubilized by microbes. Ferrites are mixed-valence iron-oxide compounds having the general formula XY_2O_4 and a crystal structure related to spinel, $MgAl_2O_4$. They exist in a normal or an inverse structure, depending on the preference of the individual ions for octahedral coordination with oxygen [3]. In the normal spinel, iron is present solely in the octahedral coordination, with the divalent metal in a tetrahedral coordination. In the inverse spinel, iron is present only

in octahedral coordination with iron. Ferrites have semiconducting and possibly superconducting properties. Because of their unique sorption and magnetic properties, ferrite treatment of radioactive aqueous waste streams containing actinides has been proposed [4].

The dissolution of several normal and inverse ferrites by *Clostridium* sp. under anaerobic conditions occurs through enzymatic reductive dissolution of iron (direct action), and through the organic-acid metabolites (indirect action) produced by the bacteria. The iron in the octahedral coordination in normal spinel, and in tetrahedral coordination with the divalent metal in inverse spinel, was solubilized by the direct action of bacteria. There was no clear relationship between the type of ferrite (normal and inverse spinels) and the mechanism of dissolution. The inverse spinels containing cobalt and copper ferrites were solubilized by indirect and direct action, respectively. Among the normal spinels tested, manganese ferrite was solubilized by direct action, while zinc ferrite was solubilized by direct and indirect action. Similarly, there was no relationship between the type of ferrites and the extent of dissolution. For example, nickel ferrite was not solubilized at all [5].

2.2. Dissolution of metals coprecipitated with iron oxides

Iron oxides scavenge transition and heavy metals in soils, sediments, and energy wastes. Sorption and coprecipitation are the predominant means by which most of the metals are retained by iron oxide. Sorption is a process in which metals are bound to the surface of a solid by adsorption and surface precipitation, whereas coprecipitation is the simultaneous precipitation of a chemical element with other elements and includes mixed-solid formation, adsorption, and inclusion. Toxic metals, such as As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, U, and Zn from fossil- and nuclear-fuel cycle waste streams, geothermal fluids, and electroplating wastes are removed by coprecipitation with ferric iron. This method of removing the toxic metals seems to be very efficient and economically feasible, but problems remain with disposing of the coprecipitated metals. Significant dissolution of metals from the coprecipitate can be brought about by chemical and microbiological action. In general, the solubility of iron oxide depends upon its degree of crystallinity; amorphous iron oxides are orders of magnitude more soluble than a well-crystallized geothite or hematite.

We showed that the metals Cd, Cr, Ni, Pb, and Zn, coprecipitated with geothite (α-FeOOH) are solubilized by the anaerobic bacterium, *Clostridium* sp. Dissolution of the metals was caused by direct enzymatic reduction of ferric iron and the release of the associated metals,

and indirect action. The extent of dissolution depended upon the type of association of the metals with geothite. In the presence of bacteria, substantial amounts of Cd and Zn which were closely associated with iron were released due to reduction of ferric iron. The dissolution of Ni was due to direct and indirect actions, while a small amount of Cr was solubilized only by direct action [6]. Lead was solubilized predominantly by indirect action. The concentration of Pb in solution decreased due to biosorption [7, 8].

Table 1 summarizes the mechanisms of anaerobic microbial dissolution of several crystalline, water-insoluble forms of metal oxides. Disposal of wastes containing such compounds, including metals coprecipitated with iron oxide, may result in the extensive leaching of iron, toxic metals, and radionuclides due to enhanced anaerobic microbial activity [5–8].

2.3. Dissolution of radium

At many uranium mining and milling sites, soluble radium is removed as a coprecipitate with $BaSO_4$ by adding $BaCl_2$ to sulfate-rich tailing effluents. The resulting $(Ba,Ra)SO_4$ precipitate is allowed to settle, yielding a radioactive sludge and supernatant which is sufficiently low in $^{266}Ra^{2+}$ to be discharged. The disposal of radioactive sludges must ensure that $^{226}Ra^{2+}$ does not leach into groundwaters because the stabilized radioactive wastes may be transformed into mobile compounds by microbial activity. For example, Ra coprecipitated with $BaSO_4$, was solubilized by sulfate reducing bacteria under anaerobic conditions [9]. High Ba^{2+} concentrations in aquifers have been attributed to sulfate reduction by *Desulfovibrio vulgaris*. Thus, McCready and Krouse [10] showed that *D. vulgaris* could release H_2S, $^{226}Ra^{2+}$, and Ba^{2+} from a $(BaRa)SO_4$ sludge. Studies of $(Ba,Ra)SO_4$ sludges from two Canadian uranium mine and mill sites showed that after adding usable carbon, SO^{2-}_4 was reduced to S^{2-} with a concurrent release of $^{266}Ra^{2+}$, Ba^{2+}, and Ca^{2+}, due to enhanced microbial activity. Levels of dissolved $^{226}Ra^{2+}$ reached approximately 400 Bq l^{-1} after 10 weeks of incubation. These results suggest that in ultimately disposing of these sludges, conditions must be maintained that minimize the activity of the indigenous sulfate-reducing bacteria to ensure that $^{226}Ra^{2+}$ is not released. Uranium mill effluents, containing toxic amounts of Mn^{2+} and ^{226}Ra, were treated with *Arthrobacter* sp., which precipitated about 92% of the Mn^{2+} as hydrous oxide of manganese and, along with this, about 95% of the ^{226}Ra was coprecipitated [11]. However, under anaerobic conditions Mn and ^{226}Ra can be remobilized due to bacterial reduction of Mn^{4+}.

TABLE 1. Mechanism of dissolution of metal oxides by *Clostridium* sp. [2, 5]

Metal oxide	Oxidation state of metal used	Mechanism of action[a]	Observed
CdO	2^+	Indirect	Indirect
CO$_2$O$_3$	3^+	Direct $(Co^{3+} \rightarrow Co^{2+})$	?
Cr$_2$O$_3$	3^+	Indirect	None
CuO	2^+	Indirect	Indirect
Fe$_2$O$_3$	3^+	Direct $(Fe^{3+} \rightarrow Fe^{2+})$	Direct
MnO$_2$	4^+	Direct $(Mn^{4+} \rightarrow Mn^{2+})$	Direct
Mn$_2$O$_3$	3^+	Direct $(Mn^{3-} \rightarrow Mn^{2+})$	None
NiO	2^+	Indirect	None
Ni$_2$O$_3$	3^+	Direct $(Ni^{3+} \rightarrow Ni^{2+})$	None
PbO	2^+	Indirect	Indirect
PbO$_2$	4^+	Direct $(Pb^{4+} \rightarrow Pb^{2+})$	None
ZnO	2^+	Indirect	Indirect
CuFe$_2$O$_4$	$(2^+, 3^+)$	Fe, direct; Cu, indirect	Direct, indirect
α-FeOOH·M	$(2^+, 3^+)$	Fe, direct; M, indirect $M = Cd^{2+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Zn^{2+}$	Direct, indirect

[a]Indirect, dissolution due to microbial metabolites or lowering of pH of medium; direct, enzymatic reductive dissolution of metals from higher oxidation state to lower oxidation state; none, no significant dissolution of metals detected.

2.4. Immobilization due to biosorption and mineral formation

Immobilization of toxic metals and radionuclides in wastes and waste streams by microbial action is due to bioaccumulation by microbial cells and biopolymers, reduction and precipitation of metals, and formation of insoluble metal sulfides and minerals. Of the various inorganic and organic metal complexing agents, such as hydrous metal oxides, clays, and humic substances, microorganisms and their constituent polymers are among the most efficient scavengers of metallic ions. Microorganisms bioaccumulate metals such as Ag, Au, Cd, Cu, Ga, Hg, Ni, Pb, Pd, and Pt by bacteria, fungi, and algae, and also radionuclides such as ^{60}Co, ^{137}Cs, ^{85}Sr, Ra, Th, U, Pu, and Am [1].

Microbial biofilms not only bind significant quantities of metallic ions under natural conditions, but they also serve as templates for the precipitation of insoluble mineral phases. Ferris et al. [12] reported that Fe, Mn, Ni, Cu, and Co were concentrated in biofilms, and that the extracellular polymers often contained an iron oxide precipitate resembling ferrihydrite (Fe$_2$O$_3$·H$_2$O). In later papers, Ferris et al. [13, 14], describe in detail the mechanisms and the biochemistry of the interactions of metal ions with the bacterial cell walls, extracellular biopolymers, and microfossil formations as means of immobilizing toxic metals.

2.5. Immobilization due to reductive processes

Reduction of an element from a higher to a lower oxidation state or to elemental forms affects the solubility of the metals. For example, the following conversions by microorganisms have been reported: selenate, se-

linite, tellurate, and tellurite, to elemental forms; vanadate to vanadyl compound; molybdate and molybdenum trioxide to a molybdenum-blue compound; arsenate to arsenite, mercuric chloride to elemental mercury; chromate ions to chromic ions which precipitate at neutral pH; pentavalent and trivalent bismuth to an elemental form; lead dioxide to its divalent state; osmium tetroxide to osmate ion; osmium dioxide and trivalent osmium to the metal and, uranyl uranium to tetravalent state [15-20].

2.6. Transformations of uranium

X-Ray absorption near edge spectroscopy (XANES) at the National Synchrotron Light Source (NSLS) and X-ray photoelectron spectroscopy (XPS), showed that soluble U(VI) was reduced to insoluble U(IV) by the anaerobic bacterium, *Clostridium* sp. (Fig. 1). Uranium was reduced only in the presence of growing or resting cells. Organic-acid metabolites, the extracellular components of the culture medium, and heat-killed cells failed to reduce uranium under anaerobic conditions. The addition of uranyl acetate or uranyl nitrate (>210 μM U) to the culture medium retarded the growth of the bacteria as evidenced by an increase in the lag period, by decreases in turbidity and in the total production of gas and organic-acid metabolites [21, 22].

The *Clostridium* sp. solubilized ferric iron in hematite, geothite, and ferrites, and manganese(IV) in pyrolusite by enzymatic reduction; as well as the oxides of cadmium, copper, lead, and zinc due to the production of organic-acid metabolites. The change in free energy for the reduction of manganese and iron was -83.4 and -27.2 kcal mol^{-1} CH$_2$O, respectively, while for the reduction
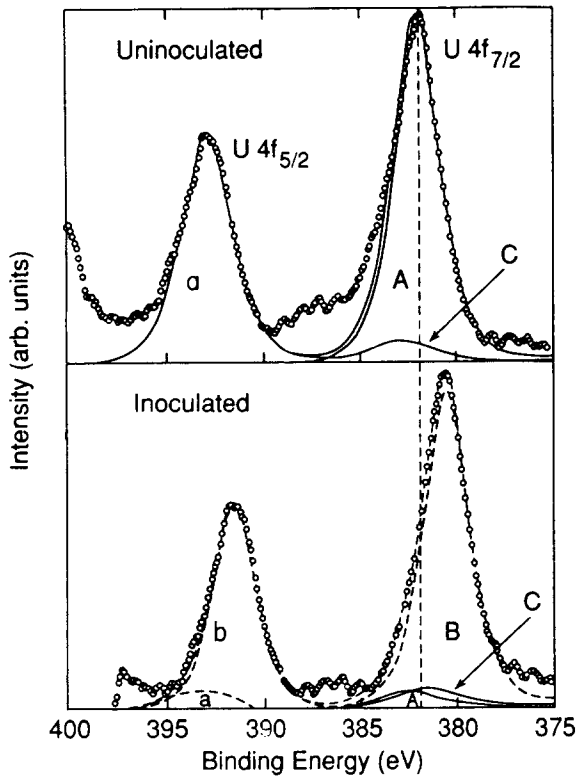
Fig. 1. XPS analysis of sample inoculated with *Clostridium* sp. showed reduction of U^{6+} (Aa) to U^{4+} (B,b); C, satellite peak [22].

of hexavalent uranium to the tetravalent state was -63.3 kcal mol^{-1}, and to the trivalent state 52.2 kcal mol^{-1}. Uranium reduction should occur in the sequence Mn(IV) > U(VI) > Fe(III).

3. Stabilization of uranium and toxic metals in wastes

Clostridia are ubiquitous in soils, sediments, and wastes, and could be very useful in pretreating and stabilizing uranium in radioactive wastes. We investigated the anaerobic microbial transformations of uranium and toxic metals present in depleted uranium wastes [20, 23]. In addition to the high concentrations of uranium (30000 ppm), these wastes contained high levels of the toxic metals As, Cd, Cr, Cu, Hg, Pb, Ni, and Zn. A substantial amount of uranium, associated with the exchangeable, carbonate, and iron-oxide fractions, was released into the medium due to enhanced anaerobic microbial activity, but little uranium was detected in solution. 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 (Fig. 2). The uranium released from exchangeable, carbonate, and iron oxide fractions was
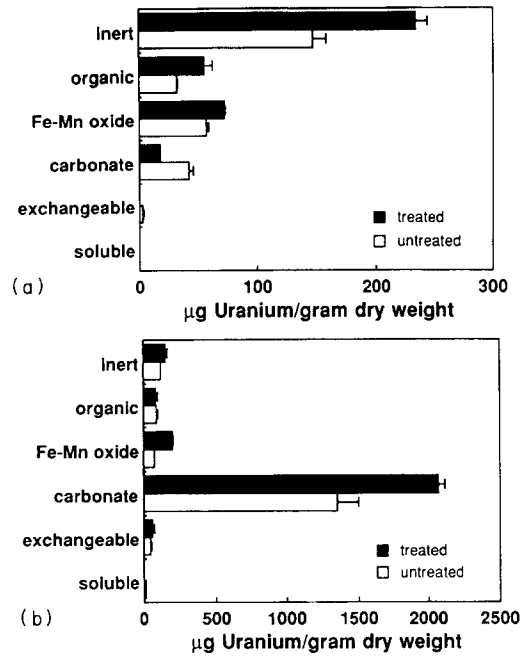

Fig. 2. Mineral association of uranium in (a) contaminated sediment and (b) sludge from uranium processing plant, both exposed to anaerobic microbiological action (treated). Untreated sample is control [23].
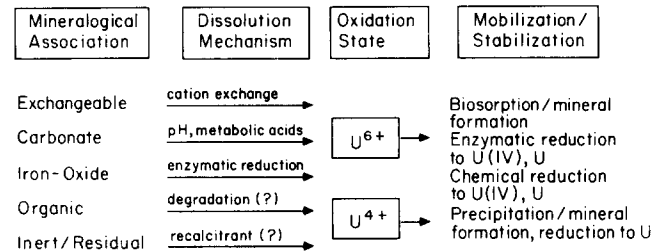

Fig. 3. Anaerobic microbial transformation of uranium in mixed wastes, mechanisms of action [23].

subsequently immobilized due to (a) biosorption by the bacterial biomass, and (b) reduction and precipitation under reducing conditions brought about by microbial action. Figure 3 illustrates the mechanisms of anaerobic microbial transformations of uranium in mixed wastes.

In this process (Fig. 4), anaerobic microbial treatment of uranium processing wastes resulted in the removal of a large fraction of soluble non-toxic metals such as Ca, K, Mg, Mn^{2+}, Na and Fe^{2+}, plus enrichment and stabilization of Cd, Cr, Cu, Ni, Pb, U, and Zn with the remaining solid phase due to direct and indirect actions of the bacteria [23]. Metals associated with the exchangeable, carbonate, and iron oxide fractions were solubilized by indirect action due to the production of organic acid metabolites. Dissolution of oxides of iron and metals coprecipitated with iron oxides was due to direct enzymatic reduction of iron. Uranyl ion associated with the exchangeable, carbonate, and iron oxide frac-
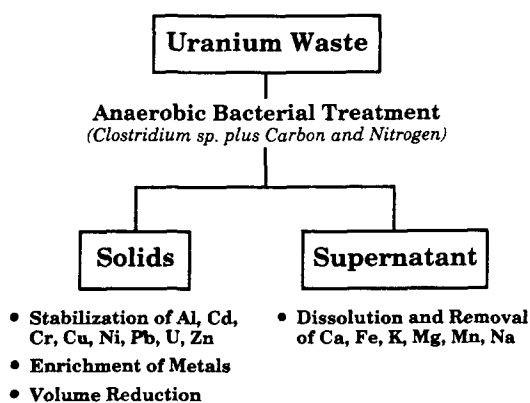
Fig. 4. Anaerobic microbial stabilization and volume reduction of uranium waste.

tions was released into solution by direct and indirect actions of the bacteria and subsequently was reduced enzymatically to insoluble U(IV). There was an overall reduction in the volume and mass of the waste due to the removal of bulk soluble non-toxic components, such as Ca, Na and Fe.

4. Removal and recovery of radionuclides and toxic metals from waste

To decontaminate the waste material, both metals and radionuclides contaminants must be removed from the contaminated site so that it can be returned to a useful condition. Chelating agents have been used extensively to remove radionuclides and toxic metals. For example, contaminated components of nuclear reactors were decontaminated using citric acid (U.S. Patent Nos. 4,839,100; 4,729,855; 4,460,500; 4,587,043; 4,537,666; 3,664,870 and 3,103,909). Metal recovery methods involve using ion-exchange columns, porous d.c. electrodes, or combusting the organics.

Many chelating agents used in decontamination are recalcitrant. Biodegradation of the metal chelates should result in the precipitation of released ions as water-insoluble hydroxides, oxides, or salts, thereby retarding the migration of metals. Citric acid, a naturally occurring organic complexing agent, is used to extract metals such as Ba, Cd, Cr, Ni, Zn, and U from solid wastes and liquid concentrates by forming water-soluble, metal-citrate complexes. Different types of complexes are formed with the transition metals and actinides, and can involve bidentate, tridentate, binuclear, or polynuclear complex species. The extract containing the radionuclide/metal complex is then subjected to microbiological degradation followed by photochemical degradation under aerobic conditions. Several metal-citrate complexes are degraded by the bacteria and the metals recovered in a concentrated form with the

bacterial biomass. The type of complex formed between the metal and citric acid plays an important role in determining its biodegradability [24]. Uranium forms a binuclear complex with citric acid which is not biodegradable; when exposed to light, the complex rapidly decomposes, resulting in the precipitation of uranium as uranium trioxide [25].

We extracted the uranium and several toxic metals from a sludge sample obtained from a uranium processing facility at Y-12 Plant, Oak Ridge, TN, with 0.40 M citric acid. Uranium was extracted with >85% efficiency. Other metals, such as chromium, cobalt, manganese, nickel, strontium, thorium, zinc, and zirconium, also were extracted from the waste [26, 27]. The citric acid extract from the sludge was amended with nutrients and inoculated with Pseudomonas fluorescens, American Type Culture Collection (ATCC) 55241. The bacteria degraded citrate at a rate of 0.5 to 0.7 mM^{-1}. There was little change in the concentration of uranium in samples subjected to biodegradation, indicating that the uranium citrate complex remained intact. The supernate after the biodegradation treatment, primarily containing the uranium complex, was exposed to light to degrade the complex and recover the uranium (Fig. 5).

This process has significant potential for commercialization because: it can be applied to various materials and waste forms; it does not generate secondary waste streams; it causes little damage to soil; and, it removes environmentally and economically important metals in a concentrated form for recovery and recycling. Using combined chemical, photochemical, and microbiological
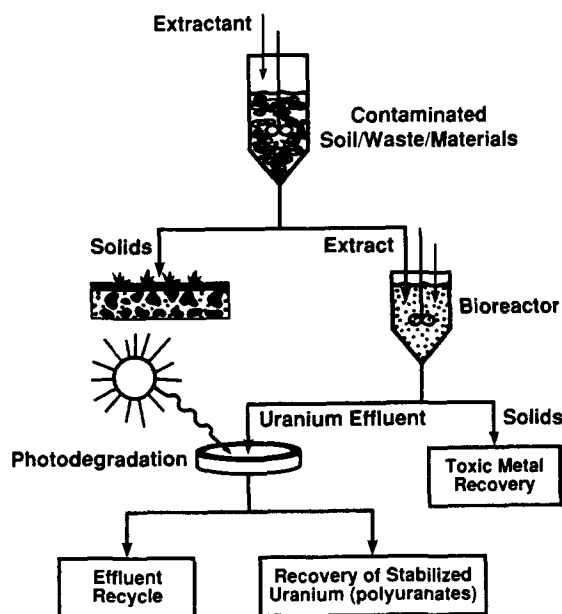

Fig. 5. Removal and recovery of uranium and toxic metals by citric acid extraction, biodegradation, and photodegradation [26, 27].

treatment, the processing of contaminated materials is efficient and will lower considerably the costs of clean-up and disposal.

References

1 A.J. Francis, *Experimenta*, *48* (1990) 849.
2 A.J. Francis and C.J. Dodge, *Appl. Environ. Microbiol.*, *54* (1988) 1009.
3 E. Prince and R.G. Trenting, *Acta Crystallogr.*, *9* (1956) 1023.
4 R.L. Kochen and J.D. Navratil, *Lanthanide Actinide Res.*, *2* (1987) 9.
5 A.J. Francis and C.J. Dodge, *Geomicrobiol. J.*, *9* (1991) 27.
6 A.J. Francis, C.J. Dodge, K. Chendrayan and H.L. Quinby, *Anaerobic microbial dissolution of lead and production of organic acids*, US Patent No. 4,758,345, July, 1988.
7 A.J. Francis and C.J. Dodge, *Environ. Sci. Technol.*, *24* (1990) 373.
8 A.J. Francis and C.J. Dodge, *Anaerobic microbial remobilization of coprecipitated metals*, AUI 92-02, patent pending, 1992.
9 C.E. Bolze, P.G. Malone and M.J. Smith, *Chem. Geology*, *13* (1974) 141.
10 R.G.L. McCready and H.R. Krouse, *Geomicrobiol. J.*, *2* (1980) 255.
11 A.K. Mathur and K.K. Dwivdey, *Uranium*, *4* (1988) 385.
12 F.G. Ferris, W.S. Fyfe and T.J. Beveridge, *Chem. Geology*, *63* (1987a) 225.
13 F.G. Ferris, W.S. Fyfe and T.J. Beveridge, *Geology*, *16* (1987b) 149.
14 F.G. Ferris, S. Schultze, T.C. Witten, W.S. Fyfe and T.J. Beveridge, *Appl. Environ. Microbiol.*, *55* (1989) 1249.
15 A. Mohaghehgi, D.M. Updegraff and M.B. Goldhaber, *Geomicrobiol. J.*, *4* (1985) 153.
16 D.R. Lovely, E.J.P. Phillips, Y.A. Gorby and E.R. Landa, *Nature*, *350* (1991) 413.
17 D.R. Lovely and E.J.P. Phillips, *Appl. Environ. Microbiol.*, *58* (1992) 850.
18 C.A. Woolfolk and H.R. Whitely, *J. Bacteriol.*, *84* (1962) 647.
19 J.W. Kauffman, W.C. Laughlin and R.A. Baldwin, *Environ. Sci. Technol.*, *20* (1986) 243.
20 A.J. Francis, C.J. Dodge, J.B. Gillow and J.E. Cline, *Radiochim. Acta.*, *52/53* (1991) 311.
21 C.J. Dodge, A.J. Francis, F. Lu, G.P. Halada, S.V. Kagwage and C.R. Clayton, in D.L. Perry, N.D. Shinn, R.L. Stockbauer, K.L. D'Amico and L.J. Terminello (eds.), *Applications of Synchrotron Radiation Techniques to Materials Science*, Materials Research Society, Pittsburgh, PA, 1993, pp. 89–93.
22 A.J. Francis, C.J. Dodge, F. Lu, G.P. Halada and C.R. Clayton, XPS and XANES studies of uranium reduction by *Clostridium* sp., *Environ. Sci. and Technol.*, *28* (1994) 636.
23 A.J. Francis, C.J. Dodge and J.B. Gillow, *Microbial stabilization and mass reduction of wastes containing radionuclides and toxic metals*, US Patent No. 5,047,152, September, 1991.
24 A.J. Francis, C.J. Dodge and J.B. Gillow, *Nature*, *356* (1992) 140.
25 C.J. Dodge and A.J. Francis, Photodegradation of uranium citrate complex with uranium recovery, *Environ. Sci. and Technol.*, *28* (1994) 1300.
26 A.J. Francis and C.J. Dodge, *Technology 20002, NASA Conference Publication No. 3189*, *Vol. 1*, *1992*, p. 109.
27 A.J. Francis and C.J. Dodge, *Wastes site reclamation with recovery of radionuclides and metals*, US Patent No. 5,292,456, March 8, 1994.