34 Trevors, J. T., Stratton, G. W., and Gadd, G. M., Cadmium transport, resistance and toxicity in bacteria, algae and fungi. Can. J. Microbiol. *32* (1986) 447–464.

35 Tsezos, M., Recovery of uranium from biological adsorbents-desorption equilibrium. Biotechnol. Bioeng. *26* (1984) 973–981.

36 Tsezos, M., Absorption by microbial biomass as a process for removal of ions from process or waste solutions, in: Immobilization of Ions by Bio-sorption, pp. 201–218. Ellis Horwood, Chichester 1986.

37 Tsezos, M., and Volesky, B., Biosorption of uranium and thorium. Biotechnol. Bioeng. *22* (1981) 583–604.

38 Tsezos, M., and Volesky, B., The mechanism of uranium biosorption by *Rhizopus arrhizus*. Biotechnol. Bioeng. *24* (1982) 385–401.

39 Wainwright, M., and Grayston, S. J., Accumulation and oxidation of metal sulphides by fungi, in: Metal-Microbe Interactions, pp. 119–130. IRL Press, Oxford 1989.

Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes

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Summary. Microbial activity in mixed wastes can have an appreciable effect on the dissolution or precipitation of toxic metals and radionuclides. Fundamental information on microbial dissolution and stabilization (immobilization) of toxic metals and radionuclides, in particular actinides and fission products, in nuclear wastes under various microbial process conditions, e.g., aerobic, denitrifying, iron-reducing, fermentative, sulfate-reducing, and methanogenic conditions is very limited. Microbial transformations of typical waste components such as metal oxides, metal coprecipitates, naturally occurring minerals, and metal organic complexes are reviewed. Such information can be useful in the development of 1) predictive models on the fate and long-term transport of toxic metals and radionuclides from waste disposal sites, and 2) biotechnological applications of waste treatment leading to volume reduction and stabilization as well as recovery and recycling of radionuclides and toxic metals.

Key words. Toxic metals; radionuclides; natural radioactive mineral deposits; metal oxides; carbonate complexes; organic complexes; coprecipitates; uranium; plutonium; low-level radioactive wastes; transuranic wastes; coal wastes.

Introduction

Microorganisms, which are ubiquitous throughout nature, have long been recognized for their ability to bring about transformations of organic and inorganic compounds. Such microbial processes have not been fully exploited in the treatment and management of nuclear and fossil-energy wastes, particularly in regulating the mobility of toxic metals and radionuclides or in the biodegradation of organic constituents to innocuous products. The contaminants in wastes may be present initially as soluble forms or they may be formed after disposal by chemical or microbiological processes. The organic compounds and inorganic elements (major and minor metals and radionuclides), depending upon their chemical forms, may react with each other to varying degrees in the waste. These include organic-inorganic complex formation, precipitation reactions, coprecipitation of metals and radionuclides with Fe- and Mn-oxides, and formation of minerals. In figure 1, the interactions between the organics, inorganics, radionuclides and microbes are depicted. Because of the complexity of the system, it is often difficult to elucidate clearly the exact mechanisms involved in the transformation of mixed wastes. Nevertheless, studies with pure or model compounds under defined conditions should provide information on the mechanisms involved in the transforma-
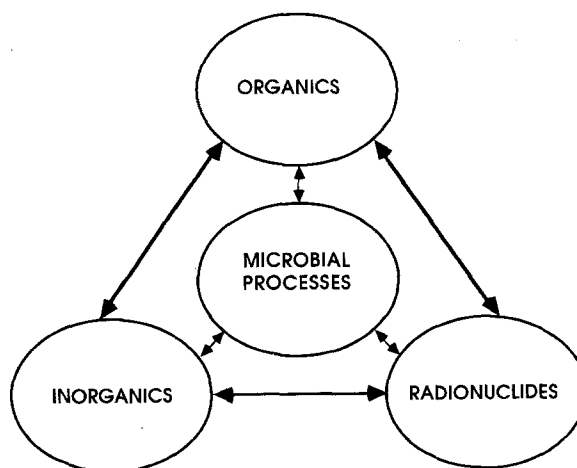

Figure 1. Interactions of organics, inorganics, radionuclides, and microbes in mixed wastes.

tions of such mixed wastes. There is a paucity of information on the fate and long-term transport of toxic metals and radionuclides present in energy-related wastes disposed of in subsurface environments. Of particular concern is the lack of information on specific microbial processes and the biochemical mechanisms involved in the dissolution (mobilization) or stabilization (immobi-

lization) of toxic metals and radionuclides in mixed wastes.

The microbial effects on low-level radioactive wastes disposed of in shallow-land burial grounds[43, 75] and the potential effects in the deep geological formations[119, 120] have been previously reviewed. A wide variety of microorganisms have been identified in coal wastes[48, 55, 56, 85, 90, 95] and in low-level radioactive wastes[45, 46] which can affect the integrity and the long-term stability of wastes. Leachate generation is a complex process involving physical, chemical, and biological actions. Microbial transformations of the waste at the disposal site may significantly influence the composition of leachates. Comprehensive information of the transformations of wastes under different microbial process conditions will be useful in developing predictive models on the fate and transport of toxic metals and radionuclides in the environment[52, 53, 76]. It will also provide useful information for biotechnological applications in waste treatment such as recovery and recycling of elements, waste volume reduction and minimization, and waste stabilization as well as better waste management methods. The purpose of this paper is to review the relevant information available on the microbial processes involved in mobilization and immobilization of toxic metals and radionuclides present in mixed wastes.

1. Microbial processes

Mobilization and immobilization of radionuclides and toxic metals under aerobic conditions, by the activities of autotrophs in the case of inorganic wastes, and heterotrophs in mixed wastes containing organics, could be significant. Similarly, under anaerobic conditions heterotrophic microbial activity which is influenced by the presence and type of electron donors and acceptors in the waste can affect the mobility of radionuclides and toxic metals. Several of the key microbial processes which affect mobilization or immobilization of toxic metals and radionuclides under aerobic and anaerobic conditions are summarized in figure 2. Microorganisms under appropriate conditions bring about metal dissolution and mobilization or immobilization by one or more of the following mechanisms: 1) oxidation-reduction of metals which affect solubility; 2) changes in pH and Eh (which affect the valence or ionic state of the metals and enhance or retard their mobility in the subsurface environment); 3) solubilization and leaching of certain elements by microbial metabolites or decomposition products, alkylation, chelation or production of specific sequestering agents; and 4) immobilization leading to formation of stable minerals or biosorption by cells and biopolymers and, 5) release of biosorbed metals due to remineralization elsewhere in the environment.
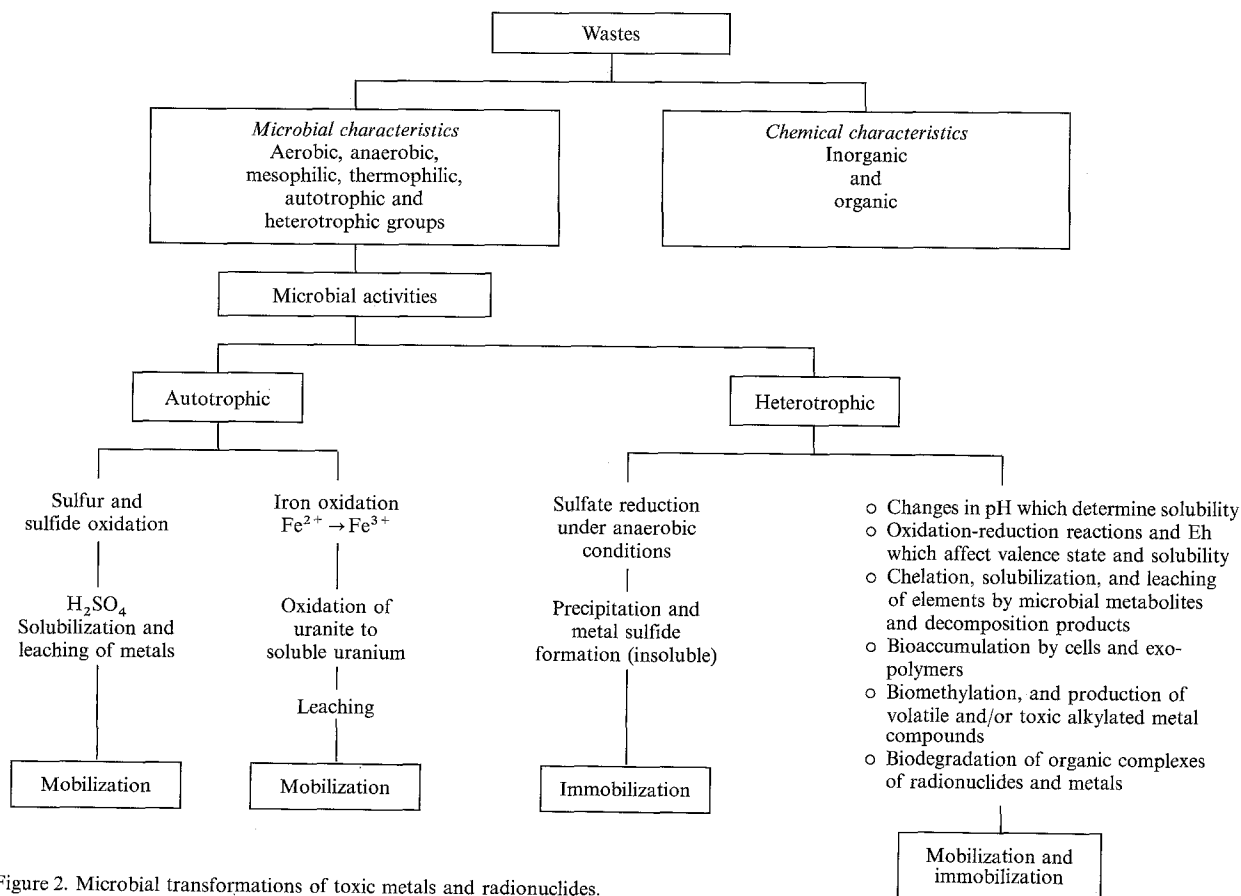

Figure 2. Microbial transformations of toxic metals and radionuclides.

The form in which the metal occurs (e.g., elemental, oxide, sulfide, ionic, inorganic complex, organic complex, or organometallics), the availability of electron donors and nutrients (carbon, nitrogen, phosphorus), the presence of alternate electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-} organic compounds), and the environmental factors (pH, Eh, temperature, moisture) will affect the types, rates, and extent of microbial activity and microbial transformation of metals. The oxidizing and reducing conditions which prevail at the disposal sites to a large extent influence the mobilization and immobilization of radionuclides and metals. Eh levels have little direct influence on the mobility of the metal, but if the environment becomes reduced due to increased microbial activity, then certain metals can undergo reduction either chemically or enzymatically from higher oxidation state to lower oxidation state. For example, reduction of Fe^{3+} to Fe^{2+} increases the solubility of iron while reduction of U(VI) to U(IV) or Cr(VI) to Cr(III) decreases their solubility. Further, the mobility of soluble metal-organic complexes in the environment is dependent upon the biological stability of these complexes. Biodegradation of the metal organic complexes and subsequent release and fate of the metal are important but least understood[21,43,44].

Transformations of toxic metals and radionuclides in mixed wastes containing organic compounds by heterotrophic aerobic and anaerobic microorganisms could be significant. We have little information on either leachate production or the extent and mechanism of metal transformations due to the activities of the heterotrophs in mixed wastes. The presence of organic materials in low-level radioactive waste is a major concern because they are responsible for most of the environmental contamination problems encountered in and around the disposal areas. Many of the organic compounds are capable of forming stable complexes with heavy metals and radionuclides or increasing their solubilization and leaching[42,43]. Likewise, microbial metabolites and waste degradation products or intermediates may be an important source of agents that affect the long-term solubility characteristics of heavy metals and radionuclides.

There are a few reported studies on microbial activity associated with the disposal of radioactive waste[75]. For example, Francis et al.[45] studied the microbial activity of trench leachates collected from the commercial low-level radioactive waste disposal sites. Aerobic, anaerobic, denitrifying, methane-producing, and sulfate-reducing bacteria were detected in leachate samples (table 1). About 75 organic compounds consisting of several straight and branched chain aliphatic acids, aromatic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, esters, ethers, and phenols were identified in the leachate samples[46]. Some of the organic compounds were biodegraded under anaerobic conditions[44]. At the shallowland, low-level radioactive waste disposal sites, in addition to synthetic chelating agents, the presence of solubilized organoradionuclide complexes, most probably of microbial origin, is a possible contributing factor to mobilization of radionuclides which might otherwise be expected to be precipitated or absorbed on clay and soil particles. Further, radioactive (tritiated and carbon-14) methane was produced by anaerobic microbial activity in the leachate[47]. The levels of radioactivity and radio-

Table 1. Chemical and microbiological analyses of leachate samples collected from low-level radioactive waste disposal sites[45,122]

Sample	Chemical pH	Eh (mV, NHE)	DOC (mg/l)	Gross alpha (pCi/l)	Gross beta (pCi/l)	Tritium (pCi/l)	Microbiological Aerobes (CFU/ml)	Anaerobes (CFU/ml)	Denitrifiers (MPN/ml)	Sulfate-reducers (MPN/ml)	Methanogens (MPN/ml)
Maxey Flats (5/78)											
Trench 19S	6.9	25	500	1.7×10^5	6.4×10^5	6.8×10^7	2.2×10^2	3.2×10^2	3.2×10^2	4.0×10^0	4.9×10^0
Well UB1-A	6.6	274	8	< 10	3.1×10^2	5.8×10^6	3.4×10^3	ND	4.6×10^2	ND	1.0×10^0
West Valley (10/78)											
Trench 3	7.3	−3.4	1700	7.3×10^2	2.9×10^6	4.8×10^8	5.4×10^3	4.0×10^3	1.3×10^4	7.0×10^1	2.3×10^1
Trench 4	6.5	54	630	1.4×10^3	1.7×10^7	3.0×10^8	2.3×10^3	3.3×10^3	2.3×10^3	4.9×10^2	1.7×10^0
Trench 5	6.7	40	2900	8.9×10^2	4.7×10^5	2.3×10^9	1.6×10^3	3.5×10^2	3.3×10^2	1.1×10^1	ND
Trench 8	6.9	−6.3	2900	1.3×10^5	4.7×10^5	3.7×10^9	1.4×10^3	7.6×10^2	1.7×10^2	1.0×10^0	1.0×10^0
Trench 9	6.7	18	1700	1.2×10^5	1.2×10^5	4.6×10^8	5.0×10^2	7.3×10^3	1.3×10^2	3.5×10^2	4.5×10^0
Barnwell (3/79)											
Trench 8D2	NA	308	170	< 9	7.9×10^2	1.0×10^2	2.0×10^8	1.0×10^4	2.3×10^5	1.1×10^0	0.8×10^0
Trench 6D1	5.9	358	2	1.6×10^1	< 28	5.7×10^5	2.3×10^3	1.3×10^2	1.1×10^3	ND	ND
Trench 25/21-D1	5.9	538	12	< 14	1.0×10^2	3.7×10^5	3.5×10^4	1.2×10^3	1.3×10^4	1.3×10^2	0.2×10^0
Trench 3D1	5.8	225	7	< 14	< 28	1.2×10^4	1.5×10^5	1.2×10^3	5.4×10^4	ND	ND
Sheffield (4/79)											
Trench 14A	5.0	143	100	8.6×10^2	5.6×10^4	6.2×10^5	1.7×10^5	4.4×10^4	2.4×10^5	ND	0.2×10^0
Trench 18	6.8	181	50	< 9	4.5×10^2	2.0×10^5	7.1×10^2	6.9×10^1	9.5×10^2	4.9×10^1	ND
Well 525	7.5	1.9	3	< 14	< 28	$<6.2 \times 10^2$	6.3×10^2	4.2×10^2	1.7×10^3	2.3×10^0	ND

ND, not detected; NA, not analyzed. The radionuclides detected in the leachate include: ^{90}Sr, ^{238}Pu, $^{239,240}Pu$, ^{241}Am, ^{60}Co, ^{134}Cs, ^{137}Cs, ^{60}Co. Field measurements of redox potential (Eh) are reported relative to the normal hydrogen electrode.

nuclides present in the leachate samples did not have any significant effect on bacteria. These bacteria were able to bioaccumulate ^{60}Co, ^{134}Cs, ^{137}Cs, and ^{90}Sr to varying degree[43,45].

Scientific information on the microbially catalyzed transformations (dissolution and immobilization) of radionuclides and metals in wastes in the subsurface environments is, however, very limited.

2. Dissolution of metals and radionuclides by autotrophs under aerobic conditions

Much of the information on the microbial dissolution of toxic metals deals with oxidation of inorganic compounds, primarily from ore leaching by autotrophic microorganisms under aerobic conditions. The responsible microorganisms, the chemical and the biochemical mechanism involved in the microbial leaching or biomining of metals, have been extensively studied[18,32,64,83,108,128]. Consequently, such microbiological processes are now being exploited on a commercial scale for extraction of copper and uranium from ores and recovery of strategic metals from wastes.

In many respects, the microorganisms and the basic principles involved in bioleaching would be operative in wastes containing inorganic compounds[69]. In general, solubilization and leaching of metals from ores are principally brought about by the activities of autotrophic iron and sulfur-oxidizing bacteria. Bacterial oxidation of pyrite and metal sulfide minerals by *T. ferrooxidans* and *T. thiooxidans* has been extensively studied[32,128]. Torma and Sakaguchi[112] investigated the oxidation of several metal sulfides by *T. ferrooxidans* and found that the rate of metal dissolution was directly proportional to the initial surface area of the substrates. The highest rate was obtained with the substrate having the highest solubility product and they reported the following order of the rate of metal dissolution NiS > CoS > ZnS > CdS > CuS > Cu$_2$S.

Inorganic components in coal and nuclear wastes, including uranium mill tailings, may undergo a series of biochemical and chemical reactions which result in the solubilization of many minerals. This dissolution process may be brought about by direct or indirect bacterial action. Direct bacterial action entails an enzymatic attack by the bacteria on components of the mineral that are susceptible to oxidation. In the process of obtaining energy from the inorganic material, the bacteria cause electrons to be transferred from iron or sulfur to oxygen. In many cases, the more oxidized product is more soluble. On the other hand, indirect leaching is the oxidation of soluble ferrous iron to ferric iron which is a powerful oxidizing agent that reacts with other metals, transforming them into the soluble oxidized form in a sulfuric acid solution. In this reaction, ferrous iron is again produced and is reoxidized by the bacteria. Bacteria (*T. ferrooxidans*) can accelerate such an oxidation reaction by a fac-

tor of more than a million[18]. In nature, both direct and indirect leaching by bacteria take place.

2.1 Dissolution of metals from coal waste

Microbial dissolution of toxic metals from two types of coal wastes, one high in pyrite and trace metals and low in organic carbon (fines fraction), and a second lower in trace metals and higher in organic carbon (filter cake) were investigated[38]. Under aerobic conditions, native autotrophic bacteria solubilized varying amounts of As, Cr, Cu, Mn, Ni, Pb, and Zn from the filter cake and fines fraction. Dissolution of the above metals was increased by severalfold when the inorganic nutrients N and P were supplemented. Selective chemical extraction analyses indicated that most toxic metals were associated with pyrite, ferric oxides and a soluble phase, possibly ferric sulfate. The predominant mechanism of dissolution of metals from coal wastes under aerobic conditions was due to bacterial oxidation of pyrite. In a similar study, As, Cr, Cu, Mn, Ni and Zn were solubilized from core samples collected from a reclaimed coal mine site in West Virginia by aerobic autotrophic microbial activity. The concentration of solubilized metals varied with depth of the core sampled (Francis et al., unpublished results). Reduced sulfur (0.3 to 4.1) present in the ash/slag of the coal gasification waste were readily oxidized by autotrophic microorganisms but the extent of metal dissolution from the waste was not investigated[107].

Anaerobically or microaerophilically, *Sulfolobus* and *T. ferrooxidans* can oxidize sulfur with the coupled reduction of ferric to ferrous iron as an alternative to the reduction of oxygen. No anaerobic iron-oxidizing bacteria have yet been found. The chemoautotrophic thiobacilli, the facultative *T. denitrificans*, can oxidize sulfide, sulfite and thiosulfate anaerobically with nitrate as the electron acceptor. In leach dumps, the internal temperature rises as a result of bacterial and chemical mineral oxidation to between 54 °C and 80 °C.
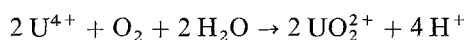
2.2 Dissolution of uranium

The iron and sulfur oxidizing bacteria play a significant role in the solubilization of uranium from ores, in mill tailings and coal wastes. The biogeochemistry of uranium has been intensively studied in light of the recovery of U from ores and bacterial leaching of pyritic uranium ore is both feasible and economical[17,32,37]. The role of *Thiobacillus ferrooxidans* in the extraction of uranium from ore is primarily indirect and confined to the generation of the oxidizing agent, ferric sulfate, and the solvent sulfuric acid. The indirect involvement of Fe^{2+}/Fe^3 in cyclically mediating the oxidation of the insoluble uranium oxide (UO$_2$) has been documented:

$$UO_2 + Fe^{3+} \rightarrow UO_2^{2+} + 2\, Fe^{2+}$$
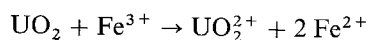
It has now been demonstrated that *T. ferrooxidans* can directly oxidize reduced compounds of uranium (uranous sulfate and UO$_2$) to its hexavalent form without the

involvement of extraneous Fe^{3+}/Fe^{2+} complex as the chemical electron carrier[29,116].

$$2\,U^{4+} + O_2 + 2\,H_2O \rightarrow 2\,UO_2^{2+} + 4\,H^+$$

In these studies, uranium sulfate which is soluble as tetravalent uranium was used as a substrate.

Jayram et al.[58] investigated the possible role of the microflora in the mobilization of uranium from rocks and its subsequent immobilization. The presence of bacteria (2.3×10^6 cells/g dry wt), higher radon values, elemental sulfur, soluble sulfates, and high leachable uranium led the authors to conclude that uranium was mobilized from the carbonaceous phyllites and was redeposited. Iron and sulfur oxidizing bacteria *T. ferrooxidans* and *T. thiooxidans* were isolated from several uranium ores[10,66], and it has been suggested that microbial oxidation of pyrite is involved in the mobilization of U from the mine at Pocos de Caldas, Brazil[121]. The role of microorganisms in the solubilization of thorium is not known.

3. Dissolution of metals and radionuclides by heterotrophs under aerobic conditions

An increase in heterotrophic microbial activity due to biodegradation of organic constituents of the waste can affect the mobilization of toxic metals and radionuclides. Heterotrophic bacteria and fungi under aerobic and anaerobic conditions are able to release metals from various materials including copper-nickel concentrates, low-grade copper ore, uranium from granites, manganese ore and potassium from leucite. Several mechanisms for heterotrophic aerobic microbial solubilization of insoluble metals have been proposed. These include organic acid production[30], formation of chelating agents[100], and metabolism of the metal-associated anion[99]. Leaching by heterotrophic organisms is entirely due to chemical reaction of excreted microbial metabolites and decomposition products[13]. In many cases, a combination effect is important, for example, when the organism secretes organic acids which may have a dual effect of increasing metal dissolution by lowering pH and increasing the load of soluble metal by complexation. Heterotrophic leaching could occur in an acid environment (pH 2–4) because of organic acid production or in an alkaline environment with no acid production.

3.1 Organic acid metabolites and complexing agents

Complexes of radionuclides and toxic metals may also be formed before waste burial as a result of laboratory procedures, process operation, decontamination activities due to microbial action, or at the burial site when species interact with buried or soil-derived organic matter. Microbially generated dicarboxylic acids, ketogluconic acid, polyhydroxy acids, and phenolic compounds such as protocatechuic acid, and salicylic acid are effective chelating agents of heavy metals and are known to accel-

erate the movement of metals in soils[30,96]. Cole[27] reported that several heterotrophic bacteria under aerobic conditions solubilized PbS, ZnS, CdS, and CuS but had no activity against $PbSO_4$, PbO, or Pb_3O_4. However, the solubilizing agent produced in the culture media was not identified. Bolter et al.[14] found that organic acids from decaying leaf litter in soil increased the solubility of heavy metals deposited from smelters. Complexation of cadmium by organic components of sanitary landfill leachates was attributed to low- and high-molecular-weight compounds representing simple carboxylic acids and compounds containing hydroxyl groups[65]. Wildung et al.[124] reported that many fungi produced low-molecular-weight ligands and extracellular complexes that substantially increased the solubility of Ni in soil. Similarly, Chanmugathas and Bollag[25] found that mobilization of strongly bound or fixed Cd was due to microbially mediated processes and was affected by soil environmental factors. Extraction of Zn from industrial waste by citric acid produced by a *Pencillium* sp. has been reported, suggesting use of such systems for recovery of metals from wastes[92].

Several heterotrophic bacteria *Bacillus* sp., *B. luteus*, *B. subtilis*, *B. cerens*, *B. pumilis*, *Pseudomonas striata*, *P. viscosa*, *P. perolens*, *P. chlororaphis*, *Achromobacter xerosis*, *A. stoloniferum*, *A. healii*, and *Desulfovibrio desulfuricans* were identified in uranium ores[10]. Heterotrophic microorganisms are able to solubilize uranium from rock materials (granitic rock) where uranium is generally present as oxide[6,11]. Such solubilization is due to production of organic acid metabolites. Munier-Lamy[82] has shown that microbial solubilization of uranium from rocks (syenite and aplite) was due to the formation of soluble low-molecular-weight organo-uranyl compounds. The metal-organic complexes were formed under aerobic (Eh + 300 to + 500 mv), acidic (pH 2.5–5) conditions by an association of metallic cations (UO_2^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}) with simple organic acids, i.e., oxalic, isocitric, citric, succinic, hydroxybenzoic, and coumaric acids via their carboxylic and phenolic groups.

In addition to the use of synthetic chelating agents, complexing agents are also found in decomposing organic matter introduced as waste, and soil organic matter. The soil organic ligands humic and fulvic acids, and decomposing organic wastes contain complex mixtures composed of oxygenated organic degradation products. Humic substances have an appreciable exchange capacity due primarily to carboxyl and phenolic hydroxyl groups, and can form stable water soluble and insoluble complexes with metals. Soil organics are generally composed of amino acids, polysaccharides, polyfunctional aromatic compounds, and porphyrins. The most abundant and well-characterized fraction of soil fulvic acid is composed of a mixture of organic acids such as formic, acetic, benzoic, 3-hydroxy-5 methylbenzoic, p-hydroxybenzoic, protocatechuic, vanillic, gallic, propionic, citric, fumaric, malic, oxaloacetic, butyric, glycolic, lactic, and tartaric

acids[77, 93]. These compounds are generally excellent bi- and polydentate chelating ligands for a variety of metals.

3.2 Siderophores and other specific chelators

Specific chelating agents are produced by microorganisms that require iron or other essential metals for growth. When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators such as siderochrome and siderophore in the medium[28, 91]. Siderophore production by soil bacteria in soils has been reported[67, 87, 94]. Since a variety of microbial types are active in the waste and waste leachate, it is possible that these microorganisms may elaborate specific or nonspecific compounds capable of complexation with the radionuclides. Iron sequestering agents could play an important role in the complexation of Pu and other metals and thus increase their bioavailability. For example, dissolution of plutonium dioxide was enhanced in the presence of Desferol, a polyhydroxamate chelate produced by microorganisms[3]. Wildung and Garland[123] found that microorganisms grown in the presence of Pu produced complexing agents of higher molecular weight than that of diethylentriaminepentaacetic acid (DTPA). Many of the cultures tested were capable of transporting Pu into the cell, and the role of complexing agents with such a transport has been suggested but was not identified[5, 123]. Likewise, *Pseudomonas aeruginosa* isolated from a plutonium-contaminated pond[59] known to bioaccumulate uranium[105] elaborated several new chelating agents for thorium and uranium when grown in the presence of these metals[88, 89]. This clearly indicates the potential of U, Th and Pu and other metals present in the waste for complexation by microbially produced chelating agents, and thus an increased bioavailability and mobilization of radionuclides and toxic metals due to microbial activity. Additional information on microbial complexing agents is discussed by Birch and Bachofen, this issue.

In addition to the metabolites, microbial exopolymers may form strong complexes with metals and radionuclides. Microbial polysaccharides which are present in biofilms often play a major role in the removal of metals from waste streams and in some instances form soluble complexes and enhance mobilization. The persistence of complexing agents in the disposal environment is a major concern because of the potential of increasing the transport and bioavailability of radionuclides and toxic metals.

4. Dissolution of metals and radionuclides under anaerobic conditions

Anaerobic conditions are common at most of the waste disposal sites and anaerobic microbial activities may significantly affect the transport and transformations of organic compounds, radionuclides, and toxic metals. There is a paucity of information on the anaerobic micro-

bial transformations of toxic metals and radionuclides under various process conditions such as denitrification, iron reduction, fermentation, sulfate-reduction, and methanogenesis. The behavior of radionuclides and toxic metals is significantly affected by anaerobic bacteria in organic matter-rich flooded soils and sediments, but little is known of the processes and mechanisms involved, in particular, of the range and nature of bacterial effects in anoxic environments[12, 57, 62, 63, 86, 126]. Increased solubilization of heavy metals from soils and sludges incubated under anaerobic conditions has been reported[12, 63]. Under anaerobic conditions, fermentation products such as organic acids and amino acids accumulate and may serve as ligands.

The predominant forms of toxic metals and radionuclides found in low-level radioactive and fossil-energy wastes can be divided into the following general categories: oxides (simple and complex oxides including ferrites), coprecipitates (metals coprecipitated with oxides of iron, aluminum), carbonate complexes, naturally occurring minerals, and organic and inorganic complexes. There is, however, very limited information on the specific characteristics of wastes disposed of in the subsurface. The available information on the anaerobic microbial transformations of the various forms of the metals and radionuclides is summarized in the following sections.

4.1 Dissolution of lead compounds

Lead is present in nuclear and coal wastes[109], and is also released into the atmosphere from fossil-fuel combustion and from lead smelters. Although the speciation of lead is not fully known, it is believed to be in the oxidized form, most probably as lead oxide, because of the oxidizing conditions. It has been estimated that 20 % of the emitted lead is in the oxide form[84]. Lead oxide is quite insoluble in water and the fate of PbO in wastes is not fully understood. Bruland et al.[20] and Burrows and Hulbert[23] noted that contaminated sediments released a greater fraction of their lead content under anaerobic conditions than unpolluted sediments. Wong et al.[125] reported that microorganisms in sediments from several Canadian lakes could transform certain inorganic and organic lead compounds into tetramethyl lead. Kee and Bloomfield[62] found that lead dioxide (PbO_2) was mobilized from soils under anaerobic conditions when amended with plant residues. Francis and Dodge[41] reported that the anaerobic bacterium *Clostridium* sp., isolated from coal cleaning waste, solubilized a significant amount of lead oxide (PbO) and to a lesser extent $PbSO_4$ but not Pb^0, PbS, and galena. Dissolution of PbO by the bacteria was due to production of organic acids and lowering of pH of the growth medium. The solubilized metal was bioavailable to the organism as evidenced by lead associated with cell biomass as well as immobilization by a polymer-like substance produced by the organism. The solubilized lead affected the growth of *Clostrid-*

ium sp., as well as the metabolic end products of glucose fermentation shifting from acetic and butyric acid fermentation to predominantly lactic acid production. Soluble lead in the culture medium interfered with the iron metabolism of the organism, possibly affecting the synthesis of iron-containing proteins involved in the electron transport system[40].

4.2 Dissolution of metal oxides

Oxides of metals are present in soils, ores, and residues generated from fossil- and nuclear-fuel cycles. The metal oxides are usually in crystalline forms and are insoluble in water; their fate after disposal in the environment is not known. Organic compounds present in the energy residues and the natural environment can have a significant effect on the solubility and mobility of the metal oxides due to chemical and microbiological action.

Under anaerobic conditions, organic compounds can bring about reductive dissolution of metal oxides from a higher to a lower oxidation state[101, 104]. This reduction has a dramatic impact on the solubility and speciation of metals. For example, oxides of Mn(III, IV), Fe(III), Co(III), and Ni(III), when reduced to divalent ions under anoxic conditions, show an increase in solubility by several orders of magnitude[101, 102]. Humic substances, catechols, hydroxyquinones, methoxyphenols, resorcinols, ascorbate, pyruvic acid, oxalic acid, amines, anilines, and other naturally occurring organic compounds, including microbial metabolites, have been shown to have redox reactivity[101–103, 110, 117]. Dissolution of cobalt, copper, lead, nickel, and zinc oxides by organic compounds from decomposing plant materials has been reported[62].

Microorganisms also play an important role in the dissolution of metal oxides by direct or indirect action. Direct action involves enzymatic reductive dissolution of the metal oxide, wherein the oxide is used as the terminal electron acceptor; whereas indirect action involves dissolution due to production of metabolites, such as organic acids and chelating agents, and lowering of the pH of the medium. Microbial reduction and dissolution of iron and manganese oxides under anaerobic conditions have been extensively studied[51]. Most studies deal with the microbial dissolution of amorphous forms of oxyhydroxides of Fe and Mn, and studies with crystalline forms have been very limited[60, 68, 80, 81, 115]. Anaerobic microbial dissolution of several crystalline, water-insoluble forms of metal oxides are summarized in table 2. An anaerobic N_2-fixing *Clostridium* sp. solubilized Fe_2O_3 and MnO_2 by direct enzymatic reduction; CdO, CuO, PbO, and ZnO were solubilized by indirect action due to the production of metabolites and the lowering of the pH of the growth medium. Although Mn_2O_3, Ni_2O_3, and PbO_2 may undergo reductive dissolution from a higher to a lower oxidation state, dissolution by direct or indirect action was not observed. Also, Cr_2O_3 and NiO were not solubilized by direct or indirect action. Significant amounts of

Table 2. Mechanism of dissolution of metal oxides by *Clostridium* sp.[39]

Metal oxide	Oxidation state of metal used	Mechanism of action[a] Expected	Observed
CdO	2^+	Indirect	Indirect
Co_2O_3	3^+	Direct ($Co^{3+} \rightarrow Co^{2+}$)	?
Cr_2O_3	3^+	Indirect	None
CuO	2^+	Indirect	Indirect
FE_2O_3	3^+	Direct ($Fe^{3+} \rightarrow Fe^{2+}$)	Direct
MnO_2	4^+	Direct ($Mn^{4+} \rightarrow Mn^{2+}$)	Direct
Mn_2O_3	3^+	Direct ($Mn^{3+} \rightarrow Mn^{2+}$)	None
NiO	2^+	Indirect	None
Ni_2O_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

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

solubilized Cd, Cu, and Pb were immobilized by the bacterial biomass.

4.3 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 processes by which most of the metals are retained by iron oxide. 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. The removal of toxic metals from waste streams by coprecipitation with iron seems to be a very efficient and economically feasible method, but problems remain with the disposal of the coprecipiated metals in the environment. Significant dissolution of metals from the coprecipitate can be brought about by chemical and microbiological action. In general, solubility of iron oxide depends upon the degree of crystallinity. Amorphous iron oxides are orders of magnitude more soluble than a well-crystalized geothite or hematite.

Metals Cd, Cr, Ni, Pb, and Zn coprecipitated with goethite (α-FeOOH) were solubilized by *Clostridium* sp.[49]. Dissolution of the metals was caused by a) direct enzymatic reduction of ferric iron and the release of metals associated with iron, and b) indirect action due to the production of metabolites. The extent of dissolution depended upon the nature of the association of the metals with goethite. 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. Lead was solubilized predominantly by indirect action. In the presence of bacteria, the concentration of Pb decreased due to biosorption.

Silver and Ritcey[98] studied the effects of sulfur-oxidizing bacteria on the release of $^{226}Ra^{2+}$ from uranium mine mill wastes which contained 0.72 % pyrite. They showed

that bacterial oxidation of the pyrite increased the amount of sulfate and decreased the amount of $^{226}Ra^{2+}$ in the effluent. At many uranium mining and milling sites, soluble radium is removed as a coprecipitate with $BaSO_4$ by the addition of $BaCl_2$ to sulfate-rich tailing effluents. The resulting $(Ba,Ra)SO_4$ precipitate is allowed to settle, yielding a supernatant which is sufficiently low in $^{226}Ra^{2+}$ for discharge to the environment and a radioactive sludge. 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 due to microbial activity. For example, radium coprecipitated with barium sulfate was solubilized by sulfate reducing bacteria under anaerobic conditions[33]. Bolze et al.[15] showed that under anaerobic conditions, $BaSO_4$ can be dissolved by microbial action. High Ba^{2+} concentrations in aquifers have been attributed to sulfate reduction by *Desulfovibrio vulgaris*[74]. McCready et al.[73] prepared a $(BaRa)SO_4$ sludge and showed that *D. vulgaris* could release H_2S, $^{226}Ra^{2+}$, and Ba^{2+} from the sludge. Studies of $(Ba,Ra)SO_4$ sludges from two Canadian uranium mine and mill sites showed that with the addition of usable carbon, SO_4^{2-} was reduced to S^{2-} with a concurrent release of $^{226}Ra^{2+}$, Ba^{2+}, and Ca^{2+} due to enhanced anaerobic microbial activity. Levels of dissolved $^{226}Ra^{2+}$ reached approximately 400 Bq/l after 10 weeks of incubation. These results suggest that the ultimate disposal of these sludges must maintain conditions to minimize the activity of the indigenous sulfate-reducing bacteria to ensure that $^{226}Ra^{2+}$ is not released to the environment.

4.4 Dissolution of metals coal wastes

The extent of dissolution of metals from coal waste under aerobic and anaerobic conditions by the indigenous microflora is shown in figure 3. The metals As, Cd, Cr, Cu, Mn, Ni, Pb and Zn were associated with carbonates, Fe-Mn oxides, coal-organics, pyrite, and silicates to varying degrees. The mechanisms of dissolution of metals under aerobic conditions by autotrophic bacteria

□ AEROBIC, INITIAL/FINAL pH = 4.53/2.15
▨ ANAEROBIC, INITIAL/FINAL pH = 4.80/5.53
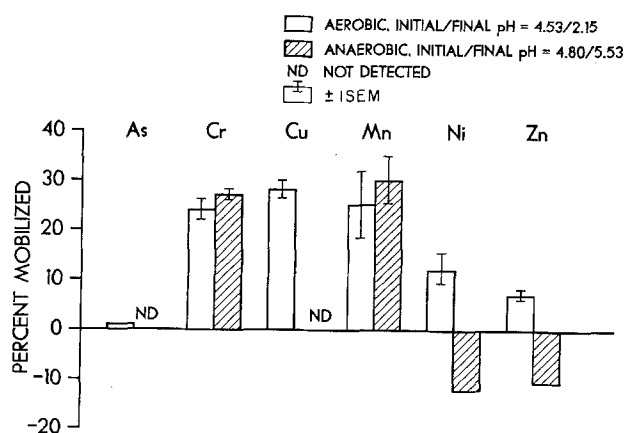ND NOT DETECTED
⊥ ± 1SEM

Figure 3. Microbial mobilization and immobilization of toxic metals from coal waste.

have been discussed in the previous section. Under anaerobic conditions, concentrations of Fe, Cr, and Mn increased due to native anaerobic bacterial activity from filter cake amended with carbon and nitrogen. Concentrations of soluble Ni and Zn in filter cake decreased due to sulfate-reduction and formation of insoluble metal sulfides. Also, dissolution of As, Cu, and Pb was not observed under anaerobic conditions. Similarly, core samples collected from a reclaimed coal mine site in West Virginia showed that, anaerobic microorganisms present in the core samples solubilized Fe^{2+}, Cr, Mn, and Zn. Addition of C and N to the cores enhanced the rate of dissolution of these metals (Francis et al., unpublished results). Under anaerobic conditions, dissolution of metals was due to the direct bacterial reduction of iron and manganese oxides and the release of trace metals coprecipitated with the oxides[38].

5. Immobilization of toxic metals and radionuclides

Immobilization of toxic metals and radionuclides in wastes and waste streams by microbial action is primarily due to bioaccumulation by microbial cells and biopolymers, reduction and/or precipitation of metals, formation of insoluble metal sulfides and minerals. There is a vast body of literature available on this topic and only the relevant information is summarized in this section. Of the various inorganic and organic metal complexing agents such as hydrous metal oxides, clays, and humic substances, microrganisms and their constituent polymers are among the most efficient scavengers of metallic ions. Bioaccumulation by microorganisms has been reported for such metals as lead[1, 41, 113], silver[26], platinum, palladium, gold, mercury[24], gallium[79], cadmium, copper, nickel by bacteria, fungi and algae; and radionuclides such as ^{60}Co, ^{137}Cs, ^{85}Sr[43, 54], uranium[97, 105, 106, 114], thorium[114], radium[106], americium[50], and plutonium[5, 59]. The potential use of microorganisms in metals and radionuclide removal from waste process streams has been described[19, 97, 106]. Such a biotreatment process has not received much attention at the commerical scale, most probably because of higher operating costs.

Microbial biofilms are not only capable of binding significant quantities of metallic ions under natural conditions but they also serve as templates for the precipitation of insoluble mineral phases. Ferris et al.[36] reported that Fe, Mn, Ni, Cu and Co were concentrated in biofilms and that the extracellular polymers often contained iron oxide precipitate resembling ferrihydrite ($Fe_2O_3 \cdot H_2O$). The mechanisms and the biochemistry of the interactions of metal ions with the bacterial cell walls, extracellular biopolymers, and microfossil formations as means of immobilization of toxic metals, are described in detail by Beveridge and coworkers[7–9, 34, 35].

The microflora in the waste disposal sites and contaminated areas can indeed preferentially bioaccumulate cer-

tain radionuclides and toxic metals[43]. It is possible then that these microorganisms, when transported by water and in the subsurface, may deposit the contaminants elsewhere in the environment upon cell lysis. Microbial transport processes in the subsurface environments are receiving much attention in light of the in situ bioremediation of contaminated aquifers.

Reduction of an element from higher oxidation state to lower oxidation state or to elemental forms affects the solubility of the metals. For example, conversion of selenate, selenite, tellurate, tellurite, to elemental forms, vanadate to a 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 precipitates at neutral pH, pentavalent and trivalent bismuth to elemental form, lead dioxide to divalent state, osmium tetroxide to osmate ion; osmium dioxide and trivalent osmium to the metal and uranyl uranium to tetravalent state by microbes have been reported[4, 16, 127].

Microbial reduction of sulfate in wastes containing toxic metals and radionuclides may result in the formation of metal sulfides. In general, most of the metal sulfides exhibit low solubility in aqueous medium. Reduction of uranium (UO_2^{2+} to UO_2) under anaerobic conditions by sulfate-reducing bacteria has been reported[31].

$$UO_2^{2+} + H_2S \rightarrow UO_2 + 2H^+ + S^0$$
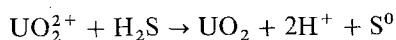
West et al.[121] noted that immobilization of uranium was due to reprecipitation of uranium in the reducing front of a uranium mine in Pocos de Caldas, Brazil. They attributed this to the activities of sulfate-reducers. Whether iron reducers play a role in the reduction of uranium is not known. The important parameters for the solubility of uranium species are Eh, pH, temperature, pressure, and uranium concentration. A change in redox potential can result in a change in valence of uranium. Percolation of uranium mine discharge water through soil lowered selenium, uranium, molybdenum and sulfate concentrations in the mine waters by the activities of *Clostridium* sp. and sulfate-reducing bacteria. The hydrogen sulfide produced by sulfate-reduction by *Desulfovibrio* reacted with uranyl and molybdate ions to form insoluble uranium and molybdenum species[61]. Uranium mill effluents containing toxic amounts of Mn^{2+} and ^{226}Ra were treated with *Arthrobacter* sp., which precipitated Mn^{2+} as hydrous oxide of manganese and along with it, ^{226}Ra was coprecipitated. About 92 % of Mn^{2+} and 95 % of ^{226}Ra were removed from the effluent by this biogenic treatment[72]. However, Mn and ^{226}Ra can be remobilized due to bacterial reduction of Mn^{++} under anaerobic conditions.

Francis et al.[38] investigated anaerobic microbial transformations of uranium and toxic metals present in depleted uranium wastes. In addition to the high concentrations of uranium (3000 ppm), the wastes contained high levels of 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 indigenous anaerobic microbial activity, but little uranium was detected in solution. The authors suggest that the uranium released from exchangeable, carbonate, and iron oxide fractions was subsequently immobilized due to a) biosorption by bacterial biomass, and b) reduction and precipitation of uranium under reducing conditions brought about by microbial action. Under reducing conditions, U(VI) can be reduced to insoluble U(IV) by chemical reduction or by microbial enzymatic action. The mechanisms of chemical reduction of U(VI) have been documented in the literature, while microbial enzymatic reduction of U(VI) has not been clearly established.

6. Biodegredation of organic complexes of radionuclides and metals

Chelating agents are widely used in the nuclear industry for decontamination of reactors and equipment, and in cleanup operations. Several metal complexing agents, along with solvents such as decalin, diisopropylbenzene, are used in the separation chemistry of the radionuclides and metals. Examples of the types of complexing agents used are polyphosphates, mono-, di-, and tributyl-phosphates, amino carboxylic acids such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotriacetic acid (NTA), and N-hydroxyethylenediaminetriacetic acid (HEDTA), dihexyl-N,N-diethylcarbamyl methylene phosphonate (DHDECMP), and carboxylic acids such as citric, hydroxy-acetic, oxalic, and tartaric acids. Although these multidentate chelates are effective in the decontamination of radionuclides by complexation, by virtue of the chemical reaction, they increase the solubility of the metals, are easily transported in the environment, and are readily bioavailable.

In general, information on microbial degradation of chelating agents is scarce. Many of these chelates either are poorly biodegraded under aerobic conditions or are found to undergo little biodegradation under anaerobic conditions[22, 78, 111, 118]. Leachates from sanitary landfills incubated aerobically and anaerobically with clay loam, calcareous clay loam, and $CaCO_3$, showed a significant reduction in chelation potential only in samples incubated under aerobic conditions. The chelating agents which had accumulated in the landfill as by-products of anaerobic fermentation seem to be stable in anaerobic systems, while under aerobic conditions relatively rapid decomposition of these compounds occurred[2]. The degradation of citrate complexes of Cd^{2+}, Cu^{2+}, Mg^{2+} and Zn^{2+} by a *Klebsiella* sp. at naturally occurring low carbon (10 µg/l) concentrations was investigated by Brynhildsen and Rosswall[21]. A marked inhibition was observed when Cd^{2+}, Cu^{2+} or Zn^{2+} was bound to the organic anion. These results suggest that metals com-

plexed with low-molecular weight organic acids, such as citric acid, are persistent in the environment.

In addition to the organic acids, many of the microbial polymers form strong complexes with metals and radionuclides. Microbial polysaccharides which are present in biofilms often play a major role in the removal of metals from waste stream and in some instances form soluble complexes and enhance mobilization. Degradation of microbial polysaccharides has been studied under aerobic conditions[71]. The rate of decomposition of the metal-polysaccharide complexes or salts and the bacterial polysaccharide were determined in surface soils under aerobic conditions. Some or all of the metals exerted marked effects on the decomposition rate and percent decomposition of the polysaccharide tested. Specific effects of the metals depended on the polysaccharide. Zinc and Al exerted little influence on decomposition of *Azotobacter chroococcum* polysaccharide, for example, while Cu and Fe reduced decomposition by about 50 %[70]. Since these compounds can be utilized by microorganisms as carbon source and recycled, the metals associated with the polymers may also be affected.

Degradation of metal complexing agents may result in precipitation of the released ion as water-insoluble hydroxides, oxides, or salts, thereby retarding metal migration. The chemical and biological stability of the synthetic (decontamination agents), naturally occurring and microbiologically synthesized complexing agents of radionuclides and toxic metals are among the critical factors which determine the mobility of the radionuclides from the burial environment into the biosphere. Lack of such information often complicates studies that deal with prediction of soil retention characteristics of radionuclides.

1 Aickin, R. M., and Dean, C. R., Lead accumulation by microorganisms. Microb. Lett. 5 (1978) 129–133.

2 Avnimelech, Y., and Raveh, A., Decomposition of chelates leached from waste-disposal sites. J. envir. Qual. 11 (1982) 69–72.

3 Barnhart, B. J., Campbell, E. W., Martinez, E., Caldwell, D. E., and Hallett, R., Potential microbial impact on transuranic wastes under conditions expected in the waste isolation pilot plant (WIPP). Los Alamos National Laboratory, LA-8297-PR. 1980.

4 Bautista, E. M., and Alexander, M., Reduction of inorganic compounds by soil microorganisms. Soil Sci. Soc. Am. Proc. 36 (1972) 918–920.

5 Beckert, W. F., and Au, F. H. F., Plutonium uptake by a soil fungus and transport to its spores, in: Transuranium Nuclides in the Environment, pp. 337–345. IAEA-SM-199/72, 1976.

6 Berthelin, J., and Munier-Lamy, C., Microbial mobilization and preconcentration of uranium from various rock materials by fungi, in: Environmental Biogeochemistry. Ed. R. Hallberg. Ecol. Bull. (Stockholm) 35 (1983) 395–401.

7 Beveridge, T. J., The immobilization of soluble metals by bacterial walls. Biotechnology and Bioengineering Symp. No. 16, 127–139. John Wiley and Sons, Inc., New York, 1986.

8 Beveridge, T. J., Ultrastructure, chemistry and functions of the bacterial wall. Int. Rev. Cytol. 72 (1981) 229–317.

9 Beveridge, T. J., and Fyfe, W. S., Metal fixations by bacterial cell walls. Can. J. Earth Sci. 22 (1985) 1892–1898.

10 Bhurat, M. C., Dwivedy, K. K., Jayaram, K. M. V., and Dar, K. K., Some results of microbial leaching of uranium ore samples from Narwepahar, Bhatin and Keruadungri, Singhbhum District, Bihar. NML Technical J. 15 (1973) 47–51.

11 Bloomfield, C., and Kelso, W. I., The mobilization and fixation of molybdenum, vanadium, and uranium by decomposing plant matter. J. Soil Sci. 24 (1973) 368–379.

12 Bloomfield, C., and Pruden, G., The effects of aerobic and anaerobic incubation on the extractabilities of heavy metals in digested sewage sludge. Envir. Pollut. 8 (1975) 217–232.

13 Bloomfield, C., Kelso, W. I., and Piotrowska, M., The mobilization of trace elements by aerobically decomposing plant material under simulated soil conditions. Chemistry Industry 9 (1971) 59–61.

14 Bolter, E., Butz, T., and Arseneau, J. F., Mobilization of heavy metals by organic acids in the soils of a lead mining and smelting district, in: Trace Substances in Environmental Health, vol. IX, pp. 107–112. Ed. D. D. Hemphill. Univ. of Missouri, Columbia, Missouri 1975.

15 Bolze, C. E., Malone, P. G., and Smith, M. J., Microbial mobilization of barite. Chem. Geol. 13 (1974) 141–143.

16 Bopp, L. H., and Ehrlich, H. L., Chromate resistance and reduction in Pseudomonas aeruginosa strain LB300. Arch. Microbiol. 150 (1988) 426–431.

17 Brierley, C. L., Biogenic extraction of uranium from ores of the Grants region, in: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena, pp. 345–363. Eds L. E. Murr, A. E. Torma and J. A. Brierley. Academic Press, New York 1978.

18 Brierley, C. L., Microbiological mining. Sci. Am. 242 (1982) 44–53.

19 Brown, M. J., and Lester, J. N., Metal removal in activated sludge: The role of bacterial extracellular polymers. Water Res. 13 (1979) 817–837.

20 Bruland, K. W., Bertine, K., Koide, M., and Goldberg, E. D., History of metal pollution in Southern California coastal zone. Envir. Sci. Technol. 8 (1974) 425–432.

21 Brynhildsen, L., and Rosswall, T., Effects of cadmium, copper, magnesium, and zinc on the decomposition of citrate by a Klebsiella sp. Appl. envir. Microbiol. 55 (1989) 1375–1379.

22 Bunch, R. L., and Ettinger, M. B., Biodegradability of potential organic substitutes for phosphates. Proc. 22nd Ind. Waste Conf., Purdue Eng. Extn. Ser. No. 129, pp. 393–396, 1967.

23 Burrows, K. C., and Hulbert, M. H., Release of heavy metals from sediments: Preliminary comparison of laboratory and field studies, in: Marine Chemistry in the Coastal Environment, pp. 382–393. Ed. T. M. Church. ACS Symposium Series. American Chemical Society, Washington, DC 1975.

24 Chakrabarty, A. M., Plasmids in Pseudomonas. A. Rev. Genet. 10 (1976) 7–30.

25 Chanmugathas, P., and Bollag, J.-M., Mobilization of cadmium in soil under aerobic and anaerobic conditions. J. envir. Anal. 16 (1987) 161–167.

26 Charley, R. C., and Bull, A. T., Bioaccumulation of silver by a multispecies community of bacteria. Arch. Microbiol. 123 (1979) 239–244.

27 Cole, M. A., Solubilization of heavy metal sulfides by heterotrophic soil bacteria. Soil Sci. 127 (1979) 313–317.

28 Cox, C. D., and Graham, R., Isolation of an iron-binding compound from Pseudomonas aeruginosa. J. Bact. 127 (1979) 357–364.

29 DiSpirito, A., and Tuovinen, O. H., Oxygen uptake coupled with uranous sulfate oxidation by Thiobacillus ferrooxidans and T. acidophilus. Geomicrobiol. J. 2 (1981) 272–291.

30 Duff, R. P., Webley, D. M., and Scott, R. O., Solubilization of minerals and related materials by 2-ketogluconic acid-producing bacteria. Soil Sci. 95 (1963) 105–114.

31 Edvardsson, U. G., Precipitation and fixation of uranium in reduced environment simulation in a continuous culture of sulfate-reducing bacteria. Contributions in Microbial Geochemistry, Department of Geology, University of Stockholm, No. 5, 1984. ISSN 0348-9302, ISBN 91-7146-622-3.

32 Ehrlich, H. L., Geomicrobiology. Marcel Dekker Inc., New York 1981.

33 Fedorak, P. M., Westlake, D. W. S., Anders, C., Kratochvil, B., Motkosky, N., Anderson, W. B., and Huck, P. M., Microbial release of $^{226}Ra^{2+}$ from $(Ba,Ra)SO_4$ sludges from uranium mine wastes. Appl. envir. Microbiol. 52 (1986) 262–268.

34 Ferris, F. G., Fyfe, W. S., and Beveridge, T. J., Bacteria as nucleation sites for anthigenic minerals in a metal-contaminated lake sediment. Chem. Geol. *63* (1987) 225–232.

35 Ferris, F. G., Fyfe, W. S., and Beveridge, T. J., Metallic ion binding by *Bacillus subtilis:* Implications for the fossilization of microorganisms. Geology *16* (1987) 149–152.

36 Ferris, F. G., Schultze, S., Witten, T. C., Fyfe, W. S., and Beveridge, T. J., Metal interactions with microbial biofilms in acidic and neutral pH environments. Appl. envir. Microbiol. *55* (1989) 1249–1257.

37 Fisher, J. R., Bacterial leaching of Elliot Lake uranium ore. Annual Western Meeting, Winnipeg. Trans. Canadian Institute of Mining and Metallurgy LXIX (1966) 167–171.

38 Francis, A. J., Dodge, C. J., Rose, A. W., and Ramirez, A., Aerobic and anaerobic microbial dissolution of toxic metals from coal waste: Mechanism of action. Envir. Sci. Technol. *23* (1989) 435–441.

39 Francis, A. J., and Dodge, C. J., Anaerobic microbial dissolution of transition and heavy metal oxides. Appl. envir. Microbiol. *54* (1988) 1009–1014.

40 Francis, A. J., and Dodge, C. J., Effects of lead oxide and iron on glucose fermentation by *Clostridium* sp. Arch. envir. Contam. Toxic. *16* (1987) 491–497.

41 Francis, A. J., and Dodge, C. J., Anaerobic bacterial dissolution of lead oxide. Arch. envir. Contam. Toxic. *15* (1986) 611–616.

42 Francis, A. J., Microbial transformations of natural organic compounds and radionuclides in subsurface environments. Proc. of the Workshop on the Effects of Natural Organic Compounds and of Microorganisms on Radionuclide Transport, OECD, RWM-6, pp. 39–49. Nuclear Energy Agency, Paris, France 1986.

43 Francis, A. J., Low-level radioactive wastes in subsoils, in: Soil Reclamation Processes: Microbiological Analyses and Applications, pp. 279–331. Eds R. L. Tate and D. Klein. Marcel Dekker, Inc., New York 1985.

44 Francis, A. J., Microbial transformation of low-level radioactive waste, in: Environmental Migration of Long-lived Radionuclides, pp. 415–429. IAEA-SM-257/72. Vienna 1982.

45 Francis, A. J., Dobbs, S., and Nine, B. J., Microbial activity of trench leachates from shallow-land, low-level radioactive disposal sites. Appl. envir. Microbiol. *40* (1980) 108–113.

46 Francis, A. J., Iden, C. R., Nine, B. J., and Chang, C. K., Characterization of organics in leachates from the low-level radioactive waste disposal sites. Nucl. Technol. *50* (1980) 158–163.

47 Francis, A. J., Dobbs, S., and Doering, R. F., Biogenesis of tritiated and carbon-14 methane from low-level radioactive waste. Nucl. chem. Waste Management *1* (1980) 153–159.

48 Francis, A. J., Dodge, C. J., Gillow, J. B., and Cline, J. E., Microbial transformations of uranium in wastes. Second International Conference on Chemistry and Migration Behavior of Actinides and Fission Products in the Geosphrere. Monterey, California, November 6–10, 1989.

49 Francis, A. J., and Dodge, C. J., Anaerobic microbial remobilization of toxic metals coprecipitated with iron oxide. Envir. Sci. Technol. *24* (1990) 373–378.

50 Giesy, J. P., and Paine, D., Effects of naturally occurring aquatic organic fractions on ^{241}Am uptake by *Scenedesmus obliquus* (Chlorophyceae) and *Aeromonas hydrophila* (Pseudomonadaecae). Appl. envir. Microbiol. *33* (1977) 89–96.

51 Ghiorse, W. C., Microbial reduction of manganese and iron, in: Biology of Anaerobic Microorganisms, pp. 305–331. Ed. A. J. B. Zehnder. John Wiley and Sons, Inc., New York 1988.

52 Grogan, H. A., The significance of microbial activity in a deep repository for low and intermediate level wastes. 78 p. Nagra Report Interner Bericht 87-OS. Nagra, Switzerland 1987.

53 Grogan, H. A., and West, J. M., Integration of laboratory and modelling studies of microbial processes relevant to nuclear waste management. 36 p. Nagra Report Interner Bericht 88-54. Nagra, Switzerland 1987.

54 Grossbard, E., and Stranks, D. R., Translocation of cobalt-60 and cesium-137 by fungi in agar and soil cultures. Nature *184* (1959) 310–314.

55 Harrison, A. P., Microbial succession and mineral leaching in an artificial coal spoil. Appl. envir. Microbiol. *36* (1978) 861–869.

56 Harrison, A. P., The acidophilic thiobacilli and other acidophilic bacteria that share their habitat. A. Rev. Microbiol. *38* (1984) 265–292.

57 Hassler, R. A., Klein, D. A., and Meglen, R. R., Microbial contributions to soluble and volatile arsenic dynamics in retorted oil shale. J. envir. Qual. *13* (1984) 466–470.

58 Jayaram, K. M. V., Dwivedy, K. K., Bhurat, M. C., and Kulshrestha, S. G., A study of the influence of microflora on the genesis of uranium occurrences at Udaisagar, Udaipur District, Rajasthan. IAEA-SM-183/31, 89–98, 1974.

59 Johnson, J. E., Svalberg, S., and Paine, D., The study of plutonium in aquatic systems of the Rocky Flats environs. Final Technical Report to Dow Chemical Co., Contract No. 41493-F. Department of Animal Sciences and the Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO 1974.

60 Jones, J. G., Gardener, S., and Simon, B. M., Reduction of ferric iron by heterotrophic bacteria in lake sediments. J. gen. Microbiol. *130* (1984) 45–51.

61 Kauffman, J. W., Laughlin, W. C., and Baldwin, R. A., Microbiological treatment of uranium mine waters. Envir. Sci. Technol. *20* (1986) 243–248.

62 Kee, N. S., and Bloomfield, C., The solution of some minor element oxides by decomposing plant materials. Geochim. cosmochim. Acta *24* (1961) 206–225.

63 Kee, N. S., and Bloomfield, C., The effect of flooding and aeration on the mobility of certain trace elements in soils. Plant Soil *16* (1962) 108–135.

64 Kelley, D. P., Norris, P. R., and Brierley, C. L., Microbiological methods for the extraction and recovery of metals. Soc. gen. Microbiol. Symp. *29* (1979) 263–308.

65 Knox, K., and Jones, P. H., Complexation characteristics of sanitary landfill leachates. Water. Res. *13* (1979) 839–846.

66 Kulshrestha, S. C., Jayaram, K. M. V., and Dar, K. K., A study of the physiology of sulfur-oxidizing bacteria and its effect on uranium mobilization and leaching at Udaisagar, Udaipur district, Rajasthan. NML Techn. J. *15* (1973) 42–46.

67 Leong, J., Siderophores: Their biochemistry and possible role in the biocontrol of plant pathogens. A. Rev. Phytopath. *24* (1986) 187–209.

68 Lovley, D. R., and Phillips, E. J. P., Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl. envir. Microbiol. *51* (1986) 683–689.

69 Lundgren, D. G., Valkova-Valchanova, M., and Reed, R., Chemical Reactions Important in Bioleaching and Bioaccumulation Biotechnology and Bioengineering Symp. No. 16, 7–22. John Wiley and Sons, Inc., New York 1986.

70 Martin, J. P., Ervin, J. O., and Shepherd, R. A., Decomposition of iron, aluminum, zinc, and copper salts as complexes of some microbial and plant polysaccharides in soil. Soil Sci. Soc. Am. Proc. *30* (1966) 196–200.

71 Martin, J. P., and Richards, S. J., Decomposition and binding action of a polysaccharide from *Chromabacterium violaceum* in soil. J. Bact. *85* (1963) 1288–1294.

72 Mathur, A. K., and Dwivedy, K. K., Biogenic approach to the treatment of uranium mill effluents. Uranium *4* (1988) 385–394.

73 McGready, R. G. L., Bland, C. J., and Gonzales, D. E., Preliminary studies on the chemical, physical and biological stability of Ba/Ra-SO$_4$ precipitates. Hydrometallurgy *5* (1980) 109–116.

74 McCready, R. G. L., and Krouse, H. R., Sulfur isotope fractionation by *Desulfovibrio vulgaris* during metabolism of BaSO$_4$. Geomicrobiol. J. *2* (1980) 55–62.

75 McGahan, D. J., Survey of microbiological effects in low-level radioactive waste disposed of to land, in: Proceedings of the International Conference on Environmental Contaminations, pp. 1–24. CEP Consultants Ltd., Edinburgh, UK 1984.

76 McKinley, I. G., West, J. M., and Grogan, H. A., An analytical overview of the consequences of microbial activity in a Swiss HLW repository. 107 p. Technical Report 85-43. Nagra, Switzerland 1985.

77 McLaren, A. D., and Peterson, G. H., Soil Biochemistry. Marcel Dekker, Inc., New York 1967.

78 Means, J. L., Kucak, T., and Crerar, D. A., Relative degradation rates of NTA, EDTA, and DTPA and environmental implications. Envir. Pollut. (Ser. B) *1* (1980) 45–60.

79 Menon, S. H., Wagner, N. Jr, and Tsan, M.-F., Studies on gallium accumulation in inflammatory lesions. I. Uptake by *Staphylococcus aureus:* Concise communication. J. nucl. Med. *19* (1978) 44–47.

80 Munch, J. C., and Ottow, J. C. G., Preferential reduction of amorphous to crystalline iron oxides by bacterial activity. Soil Soc. *129* (1980) 15–21.

81 Munch, J. C., and Ottow, J. C. G., Reductive transformation mechanism of ferric oxides in hydromorphic soils. Ecol. Bull. NFR (Naturvetensk. Forskningradet) *35* (1983) 383–394.

82 Munier-Lamy, C., and Berthelin, J., Formation of polyelectrolyte complexes with the major elements Fe and Al and the trace elements

U and Cu during heterotrophic microbial leaching of rocks. Geomicrobiol. J. *5* (1987) 119–147.

83 Murr, L. E., Torma, A. E., and Brierley, J. A., (Eds), Metallurgical Applications of Bacterial Leaching and Related Phenomenon. Academic Press, New York 1978.

84 Nriagu, J. O., Lead in the atmosphere, in: The Biogeochemistry of Lead in the Environment, Part B, pp. 137–184. Ed. J. O. Nriagu. North-Holland Biomedical Press, New York 1978.

85 Olson, G. T., McFeters, G. A., and Temple, K. L., Occurrence and activity of iron- and sulfur-oxidizing microorganisms in alkaline coal strip mine spoils. Microb. Ecol. *7* (1981) 39–50.

86 Ponnamperuma, F. N., The chemistry of submerged soils. Adv. Agronomy *24* (1972) 29–95.

87 Powell, P. E., Cline, G. R., Reidd, C. P. P., and Szaniszlo, P. J., Occurrence of hydroxamate siderophore iron chelaters in soils. Nature *287* (1980) 833–834.

88 Premuzic, E. T., Francis, A. J., Lin, M., and Schubert, J., Induced formation of chelating agents by *Pseudomonas aeruginosa* grown in the presence of Thorium and Uranium. Arch. envir. Contam. Toxic. *14* (1985) 759–768.

89 Premuzic, E. T., Lin, M., Francis, A. J., and Schubert, J., Production of chelating agents by *Pseudomonas aeruginosa* grown in the presence of Thorium and Uranium, in: Speciation of Fission and Activation Products in the Environment, pp. 391–397. Eds R. A. Bulman and J. R. Cooper. Elsevier Applied Science Publishers, New York 1986.

90 Radway, J. C., Tuttle, J. H., Fendinger, N. J., and Means, J. C., Microbially mediated leaching of low-sulfur coal in experimental coal columns. Appl. envir. Microbiol. *53* (1987) 1056–1063.

91 Rodgers, G. C., and Neilands, J. B., Microbial iron transport compounds, in: Handbook of Microbiology, vol. 2, pp. 823–830. Eds A. I. Laskin and H. A. Lechevalier. Chemical Rubber Co., Cleveland, Ohio 1973.

92 Schinner, F., and Burgstaller, W., Extraction of zinc from industrial waste by a *Penicillium* sp. Appl. envir. Microbiol. *55* (1989) 1153–1156.

93 Schnitzer, M., and Kahn, S. U., Humic Substances in the Environment. Marcel Dekker, Inc., New York 1972.

94 Schroth, M. N., and Hancock, J. G., Disease-suppressive soil and root-colonizing bacteria. Science *216* (1982) 1376–1381.

95 Schubert, J. P., and Miller, R. M., Subsurface oxidation of pyritic coal cleaning wastes by chemoautotrophic bacteria, in: 1982 Symposium on Surface Mining Hydrology, Sedimentology and Reclamation, December 5–10, 1982, pp. 623–634. University of Kentucky, Lexington, Kentucky 1982.

96 Shindo, H., and Kuwatsuka, S., Behavior of phenolic substances in the decaying process of plants. V. Elution of heavy metals with phenolic acids from soil. Soil Sci. Plant. Nutr. *23* (1977) 185–193.

97 Shumate, II, S. E., Strandberg, G. W., and Parrott, J. F. Jr, Biological removal of metal ions from aqueous process streams. Biotechnology and Bioengineering Symp. No. 8, 13–20. John Wiley and Sons, Inc., New York 1978.

98 Silver, M., and Ritcey, G. M., Lysimeter investigations on uranium tailings at CANMET. CIM Bull. *75* (1982) 134–143.

99 Silver, M., and Torma, A. E., Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates. Can. J. Microbiol. *20* (1974) 141–147.

100 Snow, G. A., Mycobactins: Iron-chelating growth factors from Mycobacteria. Bact. Rev. *34* (1980) 99–125.

101 Stone, A. T., Adsorption of organic reductants and subsequent electron transfer on metal oxide surfaces, in: Geochemical Processes at Chemical Surfaces, pp. 446–461. Eds J. A. Davis and K. F. Hayes. American Chemical Society, Washington, DC 1986.

102 Stone, A. T., Microbial metabolites and the reductive dissolution of manganese oxides. Oxalate and pyruvate. Geochim. cosmochim. Acta *51* (1987) 919–925.

103 Stone, A. T., and Morgan, J. J., Reduction and dissolution of manganese(III) and manganese(IV) oxides by organics. 2. Survey of the reactivity of organics. Envir. Sci. Technol. *18* (1984) 617–624.

104 Stone, A. T., and Morgan, J. J., Reductive dissolution of metal oxides, in: Aquatic Surface Chemistry: Chemical Processes at the Particle Water Interface, pp. 221–254. Ed. W. Stumm. John Wiley and Sons, Inc., New York 1987.

105 Strandberg, G. W., Shumate, II, S. E., and Parrott, J. R. Jr, Microbial cells as biosorbants for heavy metals: Accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. Appl. envir. Microbiol. *41* (1981) 237–245.

106 Strandberg, G. W., Shumate, II, S. E., Parrott, J. R. Jr, and North, S. E., Microbial accumulation of uranium, radium, and cesium, in: Environmental Speciation and Monitoring Needs for Trace Metal-Containing Substances from Energy-Related Processes, pp. 274–283. Eds F. E. Brinckman and R. H. Fish. NBS Special Publication 618, November 1981.

107 Strayer, R., and Davis, E. C., Reduced sulfur in ashes and slags from the gasification of coals: Availability for chemical and microbial oxidation. Appl. envir. Microbiol. *45* (1983) 743–747.

108 Summers, A. O., and Silver, S., Microbial transformations of metals. A. Rev. Microbiol. *32* (1978) 637–672.

109 Summers, R. V., Rupp, G. L., and Gherni, S. A., Physical-chemical characteristics of utility solid wastes. EA-3236, RP1487-12. Final Report. Electric Power Research Institute, Palo Alto, California 1983.

110 Sunda, W. G., Huntsman, S. A., and Harvey, G. R., Photoreduction of manganese oxides in sea water and its geochemical and biological implications. Nature *301* (1983) 234–236.

111 Tiedje, J. M., Influence of environmental parameters on EDTA biodegradation in soils and sediments. J. envir. Qual. *6* (1977) 21–26.

112 Torma, A. E., and Sakaguchi, H., Relations between the solubility product and the rate of metal sulfide oxidation by *Thiobacillus ferrooxidans*. J. Ferment. Technol. *56* (1978) 173–178.

113 Tornabene, T. G., and Edwards, H. W., Microbial uptake of lead. Science *176* (1973) 1334–1335.

114 Tsezos, M., and Volesky, B., Biosorption of uranium and thorium. Biotechnol. Bioeng. *23* (1981) 583–604.

115 Tugel, J. B., Hines, M. E., and Jones, G. E., Microbial iron reduction by enrichment cultures isolated from estuarine sediments. Appl. envir. Microbiol. *52* (1986) 1167–1172.

116 Tuovinen, O. H., Acid leaching of uranium ore materials with microbial catalysis. Biotechnology and Bioengineering Symp. No. 16, pp. 65–72. John Wiley and Sons, Inc., New York 1986.

117 Waite, T. D., and Morel, F. M. M., Photoreductive dissolution of colloidal iron oxides in natural waters. Envir. Sci. Technol. *19* (1984) 860–868.

118 Warren, C. B., Biodegradation of nitrilotriacetic acid and NTA-metal ion complexes, in: Survival in Toxic Environments. Eds M. A. Q. Khan and J. P. Beberka Jr. Academic Press, New York 1974.

119 West, J. M., Christofi, N., and McKinley, I. G., An overview of recent microbiological reserach relevant to the geological disposal of nuclear waste. Radioactive Waste Management and the Nuclear Fuel Cycle *6* (1985) 79–95.

120 West, J. M., McKinley, I. G., and Chapman, N. A., Microbes in deep geological systems and their possible influence on radioactive waste disposal. Radioactive Waste Management and the Nuclear Fuel Cycle *3* (1982) 1–15.

121 West, J. M., McKinley, I. G., and Vialta, A., The influence of microbial activity on the movement of uranium at Osamu Utsumi mine, Pocos de Caldas, Brazil, in: Scientific Basis for Nuclear Waste Management *12* (1988) 1–7.

122 Weiss, A. J., and Colombo, P., Eds, Evaluation of isotope migration – land burial water chemistry at commercially operated low-level radioactive waste disposal sites. Status Report through September 30, 1979. NUREG/CR-1289, BNL-NUREG-51143. March 1980.

123 Wildung, R. E., and Garland, T. R., The relationship of microbial processes to the fate and behavior of transuranic elements in soils, plants, and animals, in: Transuranic Elements in the Environment, pp. 300–335. Ed. W. C. Hanson. DOE/TIC-22800, Technical Information Center/U.S. Dept of Energy, Washington, DC 1980.

124 Wildung, R. E., Garland, T. R., and Drucker, H., Nickel complexes with soil microbial metabolites – mobility and speciation in soils, in: Chemical Modeling in Aqueous Systems, pp. 181–200. Monograph No. 93, American Chemical Society, Washington, DC 1979.

125 Wong, P. T. S., Chan, Y. K., and Luxon, P. L., Methylation of lead in the environment. Nature *253* (1975) 263–264.

126 Wood, J. M., Biological cycles for toxic elements in the environment. Science *183* (1974) 1049–1052.

127 Woolfolk, C. A., and Whiteley, M., Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. 1. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. J. Bact. *84* (1962) 647–658.

128 Zajic, J. E., Microbial Biogeochemistry. Academic Press, New York 1969.