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ABSTRACT

A major national concern in the subsurface disposal of energy wastes is the contamination of ground and surface waters by waste leachates containing radionuclides, toxic metals, and organic compounds. Microorganisms play an important role in the transformation of organic compounds, radionuclides, and toxic metals present in the waste and affect their mobility in subsurface environments. Microbial processes involved in dissolution, mobilization, and immobilizatin of toxic metals under aerobic and anaerobic conditions are briefly reviewed. Metal complexing agents and several organic acids produced by microbial action affect mobilization of radionuclides and toxic metals in subsurface environments. Information on the persistence of and biodegradation rates of synthetic as well as microbiologically produced complexing agents is scarce but important in determining the mobility of metal organic complexes in subsoils. Several gaps in knowledge in the area of microbial transformation of naturally occurring organics, radionuclides, and toxic metals have been identified, and further basic research has been suggested.

A major concern in the subsurface disposal of energy wastes is the contamination of ground and surface waters by waste leachates containing radionuclides, toxic metals, and organic compounds [1]. The radionuclides may initially exist in soluble or insoluble form or they may be solubilized after disposal as the result of physical, chemical, and microbiological processes [2,3]. Microorganisms, which are ubiquitous throughout nature, have long been recognized for their ability to bring about transformation of organic and inorganic compounds. To date, we have little information on the microbial processes which influence the fate and long-term transport of radionuclides and toxic metals in the subsurface environments. The form in which the metal occurs, e.g., ionic, inorganic complex, or metal organic complex, strongly influences its mobility in the environment. Many of the naturally occurring organic compounds in subsurface environments are (i) capable of forming stable complexes with radionuclides, and (ii) capable of supporting growth of aerobic and anaerobic microorganisms. Since the waste materials contain organic and/or inorganic constituents including toxic metals and radionuclides, depending on the environmental conditions, one may encounter autotrophic as well as heterotrophic microbial activities [1,4]. Stimulation of heterotrophic microbial activity may affect the transformation and transport of radionuclides in the subsurface. These include oxidation-reduction reactions, production of organic acids, synthesis of specific and nonspecific sequestering agents, biodegradation radionuclide organic complexes, bioaccumulation and remineralization of radionuclides, biomethylation, and production of radioactive gases. Several of the microbial processes involved in the mobilization and immobilization of toxic metals and radionuclides under aerobic and anaerobic conditions are summarized in Figure 1.

MICROBIAL POPULATION AND ACTIVITY IN SUBSOILS, AQUIFERS, AND ENERGY WASTES

Although the importance of microbial transformations of organic and inorganic constituents of nuclear and fossil energy wastes have been recognized, to date we have very limited information about the distribution and activities of microorganisms in subsurface environments and their effect on the mobilization or immobilization of toxic metals and radionuclides.

Recent studies with subsoils, aquifer samples [5-7], and waste core samples from a coal mining waste disposal site [8] employing aseptic sampling procedures and direct-light microscopy and electron microscopic methods indicate the presence of indigenous subsurface microorganisms. Balkwill and Ghiorse [7] reported that bacteria are the predominant forms of life in the subsurface environment such as shallow water table aquifers. The authors found that a portion of the subsurface microflora is metabolically active and that it has the potential to degrade some of the aromatic and halogenated hydrocarbon pollutants of groundwater. Shallow aquifer sediments are inhabited by stable populations of bacteria that can survive in these oligotrophic environments (<0.04% total organic matter in the sediment and <10 mg of dissolved organic carbon per liter in the groundwater) by metabolizing low levels of residual organic substances.

A survey of microbial population distributions in water and subsoil samples from deep mines designated for disposal of high-level radioactive waste in Europe was conducted by Christofi et al. [9] and West et al. [10]. The investigators found a variety of organisms representing autotrophic and heterotrophic groups which include native as well as introduced organisms from mining operations. The presence of active microbial population in deep

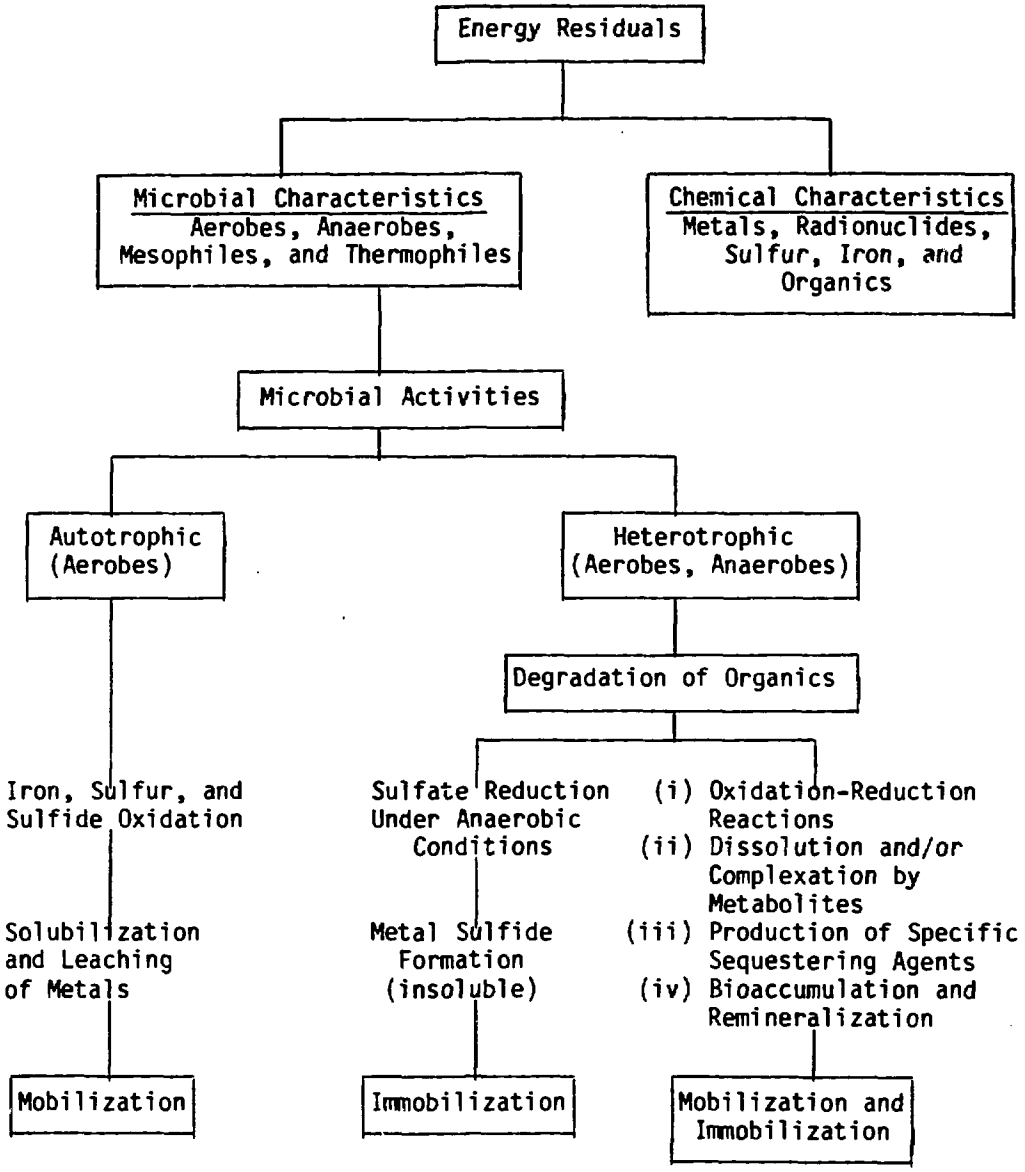

Figure 1. Microbial Transformation of Toxic Metals.

subsurface environments clearly suggests that under appropriate conditions these microbes could play a significant role in the transformation and transport of radionuclides in the subsurface environments.

The abundance and distribution of microbial populations in leachate samples collected from commercial low-level radioactive waste disposal sites at Maxey Flats, Kentucky; West Valley, New York; Barnwell, South Carolina; and Sheffield, Illinois, have been determined [11]. The population ranges of aerobic and anaerobic bacteria were 4.0×10^2 to 2.4×10^4 and 9.0×10^1 to 1.5×10^4/ml, respectively. Most of the bacteria isolated from trench waters were faculative anaerobes, although a few strict anaerobes were also present. Abundance of gas-producing organisms such as sulfate reducers, denitrifiers, and methane producers were also enumerated.

The radioactivity of buried waste within the trenches is several orders of magnitude greater than levels of activity detected in trench leachates. Francis et al. [11] therefore examined the threshold level of radioactivity above which the trench-water bacteria cannot survive or contribute to the degradation of organics in wastes. A mixture of $^{134,137}Cs$, ^{60}Co, and ^{85}Sr was added to mixed cultures of trench-water bacteria. There was no significant difference in effect on the growth of bacteria between the control, containing no radionuclides, and the media containing 9.6 Bq (2.6×10^2 pCi) and 100 Bq (2.7×10^3 pCi) of radioactivity per ml. These levels of radionuclides added to the bacterial growth media were of the same order of magnitude as those found in Maxey Flats trench leachates. At a concentration of 1×10^3 Bq/ml (2.7×10^4 pCi/ml), the bacterial growth was inhibited and two distinct growth curves were observed. It was concluded that this effect is probably due to selection of radio-resistant strains of bacterial mutants. However, at 1×10^4 Bq/ml (2.7×10^5 pCi/ml), the growth of bacteria was completely inhibited. At these radionuclide concentrations it is possible that the growth inhibition is due to the combined effects of radiation and metal toxicity. There is little evidence for enhanced migration of radionuclides from the disposal site as a result of their incorporation or bioaccumulation in bacteria. Radio resistance of soil microorganisms and bioaccumulation of radionuclides by microorganism are discussed in detail by Francis [1].

Research is currently under way at Brookhaven National Laboratory (BNL) to determine the role of anaerobic microbial processes and the biogeochemical mechanisms involved in the mobilization of toxic metals from energy wastes. An anaerobic bacterial culture isolated from coal beneficiation residue solubilized oxides of lead, copper, and manganese, but not nickel. Dissolution of lead oxide by the anaerobic bacterial isolate is due to the production of acetic, butyric, and lactic acids which form stable complexes with lead. The solubilized metal is bioavailable to the organism as evidenced by lead associated with cell biomass [12].

In a related study at BNL, coal beneficiation residues (1) high in trace metals and low in organic carbon (fines fraction) and (2) low in trace metals and high in organic carbon (filter cake) were used to determine the extent of microbial dissolution of toxic metals under aerobic and anaerobic conditions. Aerobically, native autotrophic bacteria solubilized varying amounts of As, Cr, Cu, Mn, Ni, Pb, and Zn from filter cake and fines fraction. Dissolution of the above metals was increased severalfold when inorganic nutrients N and P were supplemented. Under anaerobic conditions, native anaerobic microflora, when amended with C and N, solubilized Fe^{2+}, Cr, and Mn from filter cake and

fines fraction. Concentrations of soluble Ni and Zn from filter cake decreased under anaerobic conditions in C- and N-amended samples, presumably because of sulfate-reducing activity and formation of insoluble metal sulfides.

In conjunction with laboratory research, field research is under way at a reclaimed coal mine site with distinct aerobic and anaerobic areas [3] in order to determine the influence of anaerobic microbial processes on the mobilization of toxic trace metals from coal waste. Core profiles collected from aerobic and anaerobic locations were analyzed for physical, chemical, and microbiological characteristics. Water leachate samples from a series of wells were collected anoxically and analyzed for microbial numbers and types, trace metals, and major cations and anions. Temperature, Eh, pH, dissolved oxygen, and specific conductance were measured using the in-line system in the field. In addition to the aerobic, autotrophic microbial dissolution of toxic trace metals from core samples, anaerobic microorganisms present in the core samples contribute to mobilizations of Fe^{2+}, Mn, Zn, Cr, and Ni in subsurface environments. Addition of carbon and nitrogen to the core samples enhanced the rate of dissolution of these metals. Water leachates from anaerobic wells contain Fe^{2+}, Mn, Zn, and Cr.

ORGANIC COMPOUNDS IN WASTE LEACHATE AND IN DEEP AQUIFERS AND THEIR EFFECT IN MOBILIZATION OF RADIONUCLIDES AND TOXIC METALS

The presence of organic materials in the waste creates great concern because they are responsible for most of the problems encountered at burial sites. Many of the organic compounds are capable of forming stable complexes with radionuclides or of increasing the solubilization and leaching of buried radionuclides. The distributions of organic compounds in trench leachate and monitoring well water samples were studied by Francis et al. [13]. Water samples collected from several low-level radioactive waste disposal sites contained high levels of dissolved organic carbon (Table I). These values may be compared with the value for unpolluted groundwater, which is reported by most investigators to be between 5 and 30 ppm and usually less than 10 ppm. Means [14,15] analyzed deep aquifer samples collected from Sterno and Stripa mines from Sweden and from Hanford, Washington, for organic compounds. He identified several phenols, organic acids, low-molecular-weight fulvic acids, hydrocarbons, aldehydes, and alcohols (Table II).

Several classes of organic compounds consisting of organic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, esters, ethers, and phenols have been identified in low-level radioactive waste leachates. Further analysis of the leachate sample from low-level radioactive waste disposal sites indicates the presence of synthetic chelating agents [14,15, Toste, in these proceedings]. The radionuclide-organic complexes present in waste leachate and in microbial culture samples are listed in Table III.

In addition to the synthetic chelating agents, complexing agents are also found in decomposing organic matter introduced as waste, and water mobile organic constitutents, primarily humic and fulvic acids, present in the soil. The soil organic ligands, humic acid, fulvic acid, and decomposing organic waste contain complex mixtures composed of oxygenated organic degradation products. 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

Table I

Distribution of Water Samples from Low-Level Radioactive Wate Disposal Sites According to Dissolved Organic Carbon Content [13]

Sample source	Total number of samples	No. of samples DOC content (mg/l) 2-10	10-100	100-1000	1000-7000
Maxey Flats, KY					
Trench	46	2	10	23	11
Wells	10	10	0	0	0
West Valley, NY					
Trench	13	0	0	4	9
Wells	10	8	2	0	0
Sheffield, IL					
Trench	1	0	1	0	0
Wells	26	0	4	13	9
Barnwell, SC					
Trench	9	4	2	3	0
Wells	3	3	0	0	0

Table II

Organic Compounds Identified in Deep Aquifers [14,15]

Phenols
- Phenol
- 1,1 dimethylethyl-4-methoxyphenol
- [1,1 biphenyl]-2-ol

Organic Acids
- 2 ethylhexanoic acid
- Heptanoic acid
- Decanoic acid
- Octadecanoic acid
- Palmitic acid
- Tetradecanoic acid
- Several unidentified fatty acids
- Low-molecular-weight fulvic acid

Others
- Hydrocarbons, aldehydes and alcohols

Table III

Radionuclide-Organic Complexes Identified in Leachate and Culture Samples

Radionuclide	Organic compound	Source
Cobalt-60	EDTA	ORNL, Maxey Flats West Valley
	Mono- and dicarboxylic acids	ORNL
Strontium-90	Organic acids, hydrophilic and polar compounds	Maxey Flats
Cesium-137	Organic acids, hydrophilic and polar compounds	Maxey Flats
Plutonium	EDTA	Maxey Flats
	Several unidentified organic compounds	Maxey Flats
Plutonium, thorium, and uranium	Unidentified microbial metabolites	Laboratory study

of organic acids such as formic, acetic, benzoic, 3-hydroxy-5 methylbenzoic, p-hydroxy-benzoic, protocatechuic, vanillic, gallic, propionic, citric, fumaric, malic, oxaloacetic, butyric, glycolic, lactic, and tartaric acids [18,19]. Many of these compounds are generally excellent bi- and polydentate chelating ligands for a variety of metals.

Microbioally generated dicarboxylic acids, polyhydroxy acids, and phenolic compounds such as 2-ketogluconic acid, protocatechuic acid, and salicylic acid are effective chelating agents of heavy metals and are known to accelerate the movement of metals in soils [20,21]. Increased solubilization of heavy metal sulfides by heterotrophic bacteria under anaerobic incubations of soil or sludge [22,23] and aerobically in culture media as a result of an undescribed solubilizing agent have been reported [24]. Bolter et al. [25] 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 [26].

Chelating agents are produced by microorganisms that require iron or other essential metals for growth. Much is known about the chemistry, the biochemistry, the type of microorganisms, and the rate of production of the complexing agents which chelate iron and transport iron into the cell. When microorganisms are grown in an iron-deficient medium, they elaborate specific iron chelators in the medium. Iron exists predominantly as insoluble complexes of Fe(III) in aerobic environments. These chelating agents enhance the dissolution of the metals with which they complex and thus increase their mobility and bioavailability. The role of iron chelators such as siderochrome and siderophore has been established on the basis of promotion of microbial growth by specific iron-chelating compounds produced by the microorganism [27, 28]. As chemical and biochemical similarities have been observed between Pu(IV) and Fe(III) and between Th(IV) and Pu(IV), the iron sequestering agents

could play an important role in the complexation of Pu and other metals and thus increase their bioavailability. Recent studies show that dissolution of plutonium dioxide was enhanced in the presence of Desferol, a poly-hydroxamate chelate produced by microorganisms [29]. Wildung and Garland [30] found that microorganisms grown in the presence of Pu produced complexing agents of higher molecular weight than that of 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 [30, 31]. This clearly indicates the potential of Pu and other radionuclides present in the waste for complexation by microbially produced chelating agents and thus an increased bioavailability and mobilization of radionuclides due to microbial activity.

The significance of microbially produced chelating agents in the mobilization of toxic metals in the subsurface environments is not clearly understood. Nevertheless, the persistence of chelating agents inthe disposal environment is a major concern because of the potential of increasing the transport and bioavailability of radionuclides and toxic metals. In general, information on microbial degradation of chelating agents is scarce. Many of the chelates either are poorly biodegraded under anaerobic conditions or are found to undergo little biodegradation under anoxic conditions. Further, the extent of toxicity of the metal chelates to microorganisms is not known.

CONCLUSION

It is clear from the above discussion that microbial transformations of organics, radionuclides, and radionuclide organic complexes in the subsurface environments could be significant. On the basis of available information and in order to fully assess the significance of anaerobic microbial processes involved in the transformation and transport of toxic substances in subsurface environments, further research is recommended in the following areas: (i) identification of naturally occurring organics in aquifers and subsoils; (ii) identification of naturally occurring metal organic complexes in waste leachates, polluted groundwater; (iii) biodegradation of organics and metal organic complexes (metabolism, cometabolism, degradation rates, identification of products and pathways, minimum concentration required to effect microbial degradation, etc.); (iv) influence of environmental factors on rates of biodegradation of naturally occurring organics and metal organic complexes; (v) mobilization/immobilization of radionuclides due to enhanced microbial activity; and (vi) total biomass production.

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