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ABSTRACT

Radioactive wastes disposed of in subsurface environments contain a variety of radionuclides and organic compounds. Microorganisms play a major role in the transformation of organic and inorganic constituents of the waste and are partly responsible for the problems encountered at the waste disposal sites. These include microbial degradation of waste forms resulting in trench cover subsidence, migration of radionuclides, and production of radioactive gases such as $^{14}CO_2$, $^{14}CH_4$, HT, and CH_3T. Microbial processes involved in solubilization, mobilization, and immobilization of toxic metals under aerobic and anaerobic conditions are reviewed. Complexing agents and several organic acids produced by microbial action affect mobilization of radionuclides and heavy metals from the wastes. Microorganisms play a significant role in the transformation and cycling of tritium in the environment by (i) oxidation of tritium and tritiated methane under aerobic conditions and (ii) production of tritium and tritiated methane from wastes containing tritiated water and organic compounds under anaerobic conditions.

CONTENTS

1 INTRODUCTION

A major concern in the disposal of nuclear and nonnuclear wastes is the contamination of surface and ground waters by waste leachates containing radionuclides, toxic metals, and organic compounds. These contaminants may initially exist in soluble form or they may be formed after disposal as the result of physical, chemical, and microbiological processes. 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, particularly anaerobic microbial processes, which influence the fate and long-term transport of toxic metals and radionuclides in the subsurface environments. The form in which a metal occurs (e.g., ionic, inorganic complex, organometallic complex) strongly influences its toxicity, bioavailability, and mobility in the environment. Toxic metals in the environment may be more insidious than pollution by organic chemicals because metals, unlike organic chemicals, cannot be degraded to innocuous products such as carbon dioxide and water. In this paper the microbial processes involved in the mobilization and immobilization of toxic metals from energy wastes and the anaerobic microbial transformation of radioactive wastes in subsurface environments are discussed.

2 MICROBIAL MOBILIZATION AND IMMOBILIZATION OF TOXIC METALS

Mobilization and immobilization of toxic metals in the environment are brought about by direct or indirect action of the microorganisms. For example, various microbial processes may bring about (i) changes in pH and Eh (oxidation-reduction reactions) which affect the valence or ionic state of the radionuclides and enhance their mobility in the environment by retarding the soil-binding characteristics; (ii) chelation, solubilization, and leaching of certain elements by microbial metabolites or decomposition products; (iii) bioaccumulation and biosorption by microorganisms; (iv) biomethylation; and (v) production of gaseous compounds such as CO_2, H_2, CH_4, and H_2S. These biochemically transformed radionuclides, toxic metals, and organic compounds are indeed transported in the environment and thus increase their bioavailability to man. Several of the microbial processes involved in the mobilization and immobilization of toxic metals and radionuclides under aerobic and anaerobic conditions have been summarized in Figure 1.

2.1 Solubilization of Metals by Autotrophic Microbial Activity

Microorganisms are known to solubilize various metals from ores and in soil by production of mineral acids, organic acids, and oxidizing agents. Much of the information on the microbial contribution to leaching of metals comes from ore leaching, however. The responsible microorganisms, the chemical and the biochemical mechanisms involved in the microbial leaching or biomining of metals, have been studied intensively during the past several years.[1-4] Consequently, such microbiological processes are now being exploited on a commercial scale for extraction of copper and uranium from ores and to recover strategic metals from wastes. Since the waste materials conta' iron and sulfur, either in the oxidized or reduced form, these elements are important in driving the overall chemical and biochemical reactions at the disposal environment. In aerobic environments, solubilization and leaching of metals are principally brought about by the activities of autotrophic bacteria. Oxidizing conditions promote the oxidation of reduced forms of sulfur to sulfuric acid which, in turn, play a dominant role in the mobilization of acid-soluble metals. Further, acid pH drastically retards

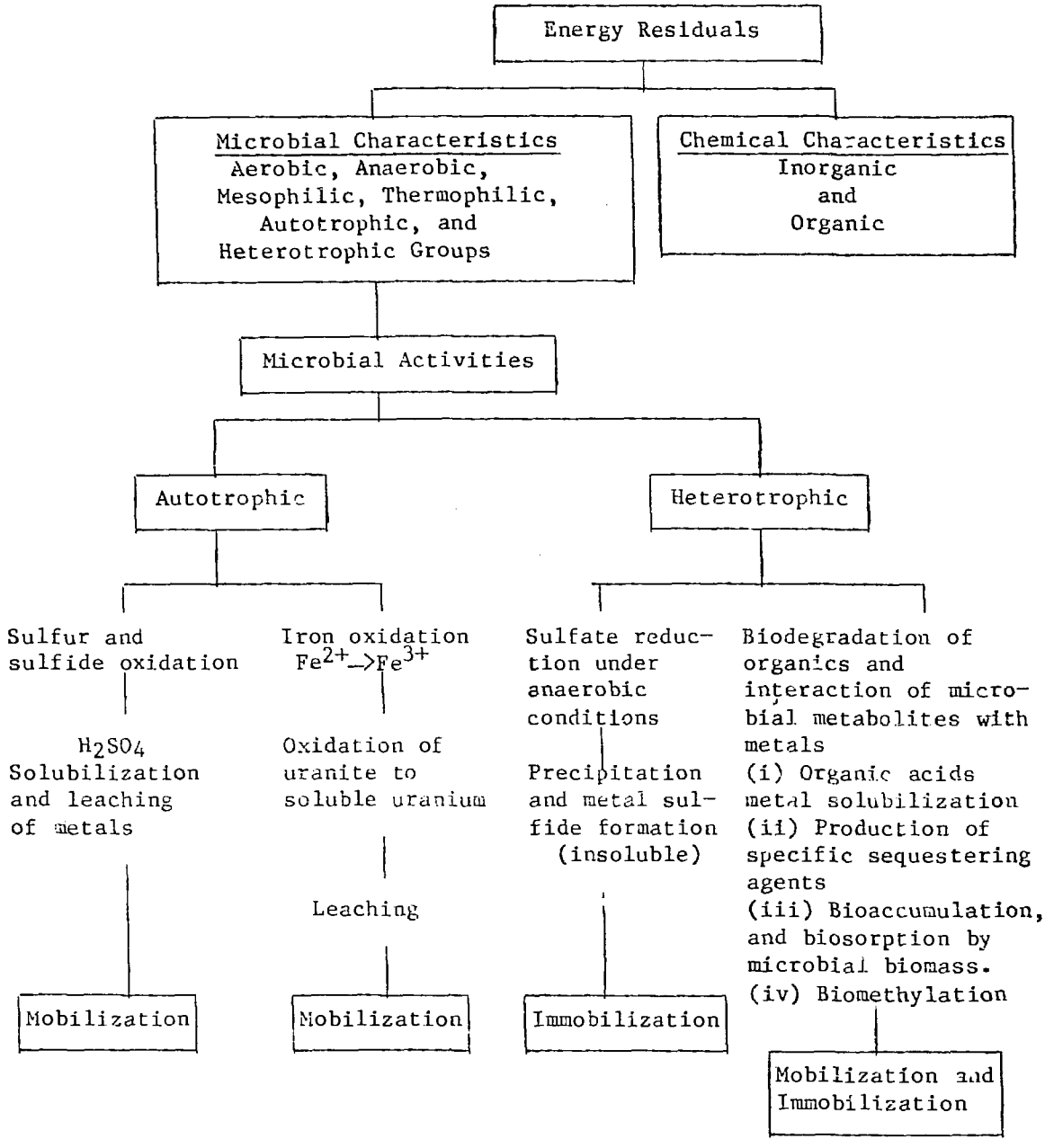

FIGURE 1 Microbial transformation of toxic metals.

the capacity of the soil or clay liner of a landfill to attenuate the movement of heavy metals from the landfill. For example, solubilization of radionuclides from radioactive wastes, uranium, and possibly thorium from mill tailings and coal wastes by sulfur- and iron-oxidizing bacteria could be significant.

2.2 Mobilization and Immobilization of Metals by Heterotrophic Microbial Activity

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 the buried radionuclides. Heterotrophic microbial utilization of the organic constituents of the waste under aerobic or anaerobic conditions may affect the transformations and transport of radionuclides in subsurface environments. These include oxidation-reduction reactions, production of organic acids, synthesis of specific and nonspecific sequestering agents, bioaccumulation of radionuclides, biomethylation, and production of radioactive gaseous products such as tritiated and ^{14}C methane.

In anaerobic environments, naturally occurring organic materials in soils can be degraded by microorganisms to simple organic acids, alcohols, aldehydes, ketones, esters, and gases such as H_2, H_2S, CO_2, and CH_4. Several aerobic and anaerobic bacteria, denitrifiers, sulfate reducers, and methanogens were found in leachate samples collected from low-level radioactive waste disposal sites.[5] The denitrifiers are responsible for the reduction of nitrate and nitrite in the presence of an adequate supply of available organic compounds under anaerobic conditions. The end products of biological denitrification are N_2O and N_2. Sulfate-reducing bacteria convert the sulfate to sulfide in anoxic environments containing sulfate. These bacteria are active in the corrosion of iron and aluminum alloys, desulfurization of oil, and deposition of mineral sulfides. The formation of metal sulfides by microbial sulfate reduction may determine the mobility of the metal, which, in several metal sulfides, is controlled by the solubility of the respective sulfides. In general, most of the metal sulfides exhibit low solubility in an aqueous solution.

2.3 Anaerobic Microbial Degradation of Organic Compounds in Radioactive Waste Leachate

Water leachate samples collected from low-level radioactive wastes contained radioactive materials such as ^{14}C, HT, ^{60}Co, ^{90}Sr, $^{134,137}Cs$, ^{241}Am, $^{238,239,240}Pu$ and organic compounds consisting of straight- and branched-chain aliphatic acids, aromatic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, ethers, and phenols.[6] These organic compounds and the microbial degradation products can influence the oxidation reduction states of radionuclides, react with radionuclides to form complexes, and influence the solubility and leachability of radionuclides. The ability of indigenous microflora of the radioactive waste to degrade the organic compounds under anaerobic conditions was examined.[7] Leachate samples collected under anoxic conditions from the radioactive waste disposal site were incubated anaerobically in sterilized stoppered 120-ml serum bottles in an atmosphere of 85% N_2, 10% CO_2, and 5% H_2 at 28°C. Acidified control samples were incubated under identical conditions. After days of incubation, samples were acidified and extracted with glass-distilled methylene chloride. The methylene chloride extracts were then concentrated and analyzed by gas chromatography and mass spectrometry.[7] The changes in concentration of several organic constituents due to anaerobic microbial action are shown in Table 1. Concentrations of several organic acids increased, probably because of breakdown of complex organic materials in the leachate. Addition of nitrogen in the form of NH_4NO_3 and $(NH_4)_2SO_4$ to leachate enhanced the degradation of several compounds. Several of the low-molecular-weight organic acids are formed as the result of the breakdown of complex organic materials and are further metabolized by microorganisms; hence these compounds are in a dynamic state, being both synthesized and destroyed. The formation and accumulation of organic acids from anaerobic decomposition of organic compounds in the waste may indeed influence the mobility of radionuclides from the disposal environments.

TABLE I. Anaerobic degradation of organic compounds present in low-level radioactive waste leachate sample.[7]

Compound	Initial concentration mg/ℓ	Percent change Addition	
		None	Nitrogen
Acetic acid[a]	NQ	+ 0	- 8
Benzene[a]	NQ	- 8	- 17
Benzoic acid	17.0	0	- 17
C_8 acid[a]	NQ	- 42	- 48
Cresol	6.5	- 7	- 44
Cyclohexanol	1.2	+ 33	+ 10
Decanoic acid	0.2	+ 66	+ 1
2-Ethylhexanoic acid	62.6	+ 20	+ 5
3-Ethyl-1-hexanol	13.2	+ 4	- 5
Hexanoic acid	78.2	+ 11	- 4
2-Methylbutanoic acid	113	+ 3	- 12
2-Methylhexanoic acid	14.0	+ 18	+ 3
2-Methylpentanoic acid	20.2	+ 7	- 5
3-Methylpentanoic acid	2.6	+ 9	- 6
2-Methylpropionic acid	36.0	- 3	- 21
Nonanoic acid	4.2	+ 52	+ 22
Octanoic acid	22.6	+ 33	+ 6
p-Dioxane[a]	NQ	- 4	- 13
Phenol	5.8	+ 13	- 16
Phenylacetic acid	13.6	0	- 16
Phenylhexanoic acid[a]	NQ	+ 1	- 17
Phenylpropionic acid	10.6	+ 1	- 15
Tributyl phosphate	0.7	0	0
Tripropylene glycol methyl ester[a]	NQ	0	- 6
Toluene	13.3	- 17	- 20
Toluic acid	2.6	+ 2	- 14
Valeric acid	79.0	+ 4	- 14

[a]Percent change in concentration was determined on the basis of the ratio of the compound to the internal standard.
NQ - Not quantified.

2.4 Microbial Production of Organic Acids and Chelating Agents

Chelation and solubilization of metals are brought about by the activities of microorganisms in nature. Several mechanisms for microbial solubilization of insoluble metals have been proposed, including organic acid production,[8] formation of chelating agents,[9] and metabolism of the metal-associated anion.[10]

2.4.1 Organic acids. Microbially generated dicarboxylic acids, polyhydroxy acids, 2-ketogluctonic acid and phenolic compounds, such as protocatechuic acid and salicylic acid, are effective chelating agents of heavy metals and are known to accelerate the movement of metals in soils.[8,11] Increased solubilization of heavy metal sulfides by heterotrophic bacteria under anaerobic incubations of soil or sludge[12,13] and aerobically in culture media as a result of an undescribed solubilizing agent have been reported.[14] Bolter et al.[15] 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.[16]

Heterotrophic organisms, bacteria and fungi, 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. Leaching by heterotrophic organisms is entirely due to the chemical reaction of excreted microbial metabolites with the materials. In many cases, a combination effect is important, for example when the organism secretes organic acids which may have the 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 (pH 6-9) with no acid production.

2.4.2 Chelating agents. 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 (siderophores) which chelate iron and transport iron into the cell. Chelating agents enhance the dissolution of the metals with which they complex and thus increase their mobility and perhaps bioavailability. 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. Dissolution of plutonium dioxide was enhanced in the presence of Desferol, a polyhydroxamate chelate produced by microorganisms.[17] This clearly indicates the potential of Pu and other metals present in the waste for complexation by microbially produced chelating agents. Wildung and Garland[18] 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 but not identified.[18,19] Pseudomonas aeruginosa (known to bioaccumulate Pu and U) produced Th and U complexing agents in culture medium.[20] In addition to phenolic and hydroxamate functional groups such as found in siderophore-type compounds, new natural products unrelated to siderophores appear to be present in the culture medium.[21] Many of the studies to date on microbial chelation of toxic metals are concerned with aerobic organisms and we have little information on anaerobic microbial production of complexing agents which could play a significant role in the mobilization of radionuclides in subsurface environments. The significance of microbially synthesized chelating agents in the mobilization of toxic metals in the environment is poorly understood and warrants further study.

3 MICROBIAL PRODUCTION OF RADIOACTIVE GASES

Microorganisms may play a significant role in the generation of radioactive gases directly through their metabolic activity or they may indirectly enhance the release of trapped gases such as radon from the radioactive decay of radium from the environment. Much attention has been given to methanogenic bacteria because of anoxic conditions that prevail in the trenches and the release of tritiated methane. Although much is known about the microbial metabolism of ^{14}C compounds and production of $^{14}CO_2$ and $^{14}CH_4$, very little is known about the microbial generation of tritiated methane.

Radioactive gaseous compounds such as CH_3T, HTO, HT, other tritiated hydrocarbons, ^{85}Kr, ^{222}Rn, $^{14}CO_2$, $^{14}CH_4$, and other ^{14}C-hydrocarbons have been detected seeping from burial trenches at West Valley, N.Y.[22] Of these, tritiated methane is among the most abundant; it has been estimated that one-tenth to two curies per year of CH_3T are released to the environment from various trenches at the West Valley disposal site.[22]

3.1 Microbial Transformation of Tritium

Water leachate samples from low-level radioactive waste disposal sites were collected anoxically and analyzed for methane bacteria by the most probable number technique; the results showed a population range of 20 to 230 per 100 ml of leachate. The ability of methanogens to produce tritiated methane from trench leachate containing tritium and other radionuclides and from synthetic media spiked with tritiated water was investigated. For this purpose, a mixed methanogenic bacterial culture was isolated from the leachate sample. Leachate aliquots of 30 ml each were transferred to sterile stoppered 60-ml serum bottles filled either with 85% N_2, 10% CO_2, and 5% H_2 or with 80% H_2 and 20% CO_2. They were incubated (a) with 10% formaldehyde to prevent bacterial growth (control) and (b) inoculated with mixed methanogenic culture. Total methane production by the control and inoculated samples is shown in Table II. The gas samples were analyzed for the presence of ^{14}C and tritium activity in the methane fraction. The samples incubated under $H_2 + CO_2$ produced more methane with higher $^{14}CH_4$ and CH_3T activity than the samples incubated under $N_2 + CO_2 + H_2$ (Table II).

TABLE II Microbial production of $^{14}CH_4$ and CH_3T from radioactive waste leachate samples.[23]

	Methane Produced (nmol)	Total Activity (pCi)	
		$^{14}CH_4$	CH_3T
Control	980	0.5	0.03
Inoculated ($N_2 + CO_2 + H_2$)	18,000	0.59	1.0
Inoculated ($CO_2 + H_2$)	68,000	12	57

Furthermore, significant quantities of tritiated methane were produced from synthetic media containing 2 mCi of tritium as tritiated water.[23] The levels of tritium used in this study had no apparent effect on methanogenesis, and the production of CH_3T increased proportionally with the increase in concentration of HTO added to the medium (Figure 2).

The low-molecular-weight ^{14}C- and tritium-containing hydrocarbons are transformed by methane bacteria to $^{14}CH_4$ and CH_3T, respectively. In addition, the methane bacteria are able to metabolize HTO in the presence of other carbon sources and produce tritiated methane. Soil microorganisms are capable of oxidation of tritium (HT) to tritiated water (HTO), and tritiated methane can be oxidized under aerobic conditions by methane-oxidizing bacteria and thus tritium can be recycled in the terrestrial environment.

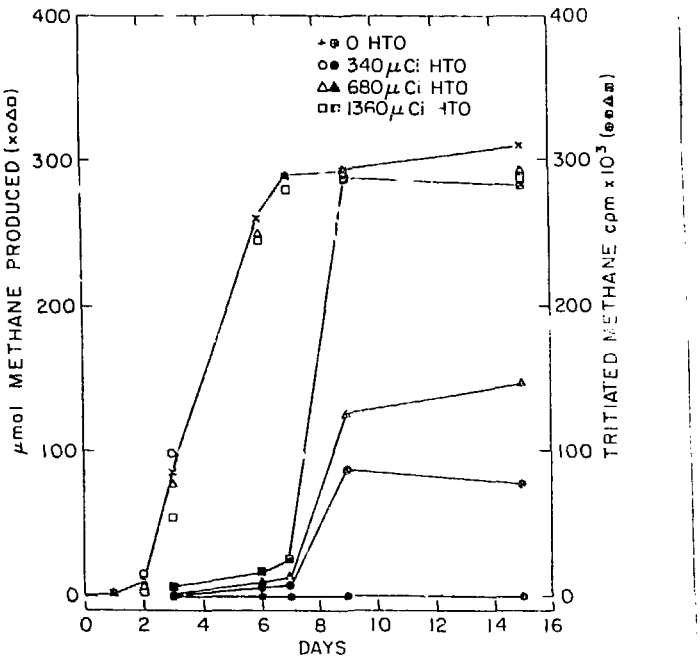

FIGURE 2 Effect of addition of tritiated water on methanogenesis and production of tritiated methane by mixed methanogenic culture.

4 ACKNOWLEDGMENTS

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