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Microbial Activity of Trench Leachates from Shallow-Land, Low-Level Radioactive Waste Disposal Sites

A. J. FRANCIS,* S. DOBBS, AND B. J. NINE

Brookhaven National Laboratory, Upton, New York 11973

Trench leachate samples collected anoxically from shallow-land, low-level radioactive waste disposal sites were analyzed for total aerobic and anaerobic populations, sulfate reducers, denitrifiers, and methanogens. Among the several aerobic and anaerobic bacteria isolated, only *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., and *Clostridium* sp. were identified. Mixed bacterial cultures isolated from the trench leachates were able to grow anaerobically in trench leachates, which indicates that the radionuclides and organic chemicals present were not toxic to these bacteria. Changes in concentrations of several of the organic constituents of the waste leachate samples were observed due to anaerobic microbial activity. Growth of a mixed culture of trench-water bacteria in media containing a mixture of radionuclides, ^{60}Co, ^{85}Sr, and $^{134,137}Cs$, was not affected at total activity concentrations of 2.6×10^2 and 2.7×10^3 pCi/ml.

Low-level radioactive wastes generated from nuclear industry, hospitals, universities, and research institutions are disposed of in shallow-land pits and trenches. The disposal sites studied are located in sparsely populated areas: at Maxey Flats (Morehead), Ky.; Beatty, Nev.; Sheffield, Ill.; Barnwell, S.C.; West Valley, N.Y.; and Richland, Wash. A comprehensive summary of the shallow-land radioactive waste burial operations, classification of wastes, and projections of burial capacities of the existing commercial sites has been presented by Holcomb (6).

Although inventories of the radioactive materials buried in the disposal sites are available, no specific records are kept on the kinds and quantities of organic wastes buried. In general, the organic wastes consist of contaminated paper, packing materials, clothing, plastics, ion-exchange resins, scintillation vials, solvents, chemicals, decontamination fluids, carcasses of experimental animals, and solidification agents. Water leachate samples collected from disposal sites at Maxey Flats, Ky., and West Valley, N.Y., contained ^{14}C, ^{3}H, ^{60}Co, ^{90}Sr, $^{134,137}Cs$, ^{241}Am, and $^{238,239,240}Pu$ (8, 9). In addition, several organic compounds consisting of straight- and branched-chain aliphatic acids, aromatic acids, alcohols, aldehydes, ketones, amines, aromatic hydrocarbons, esters, ethers, and phenols were identified in the samples (4).

Although much is known about the microbial transformations of various non-radioactive elements and organic compounds in nature, we have very little information on the microbial activities of the low-level radioactive wastes in relation to long-term storage, disposal, and possible release of radioactivity into the biosphere. This study describes the abundance, distribution, and growth of microorganisms in the waste leachates containing a variety of radionuclides and organics, to begin to evaluate the role of microorganisms in the transformation of radioactive wastes.

MATERIALS AND METHODS

Sample collection. Water samples from trenches at the disposal sites of Maxey Flats, Ky., West Valley, N.Y., Sheffield, Ill., and Barnwell, S.C., were collected under anoxic conditions (9). Approximately 5 gallons (ca. 18.9 liters) of trench leachate was flushed through the collection system before samples were taken for microbiological, inorganic, organic, and radiochemical analyses. For microbiological analyses, about 100 ml of the trench-water sample was collected anoxically in a sterile nitrogen-filled serum bottle through a 22-gauge needle connected to the main collection system through a T-valve. The samples were packed in ice and shipped to the laboratory. The bacterial populations were enumerated within 24 h of sample collection.

Population enumeration. Total aerobic and anaerobic bacteria were determined by the pour-plate technique using Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Tenfold serial dilutions of trench water were made in 90-ml sterile distilled-water blanks in stoppered serum bottles, previously flushed and filled with N_2. The dilution agar plates were incubated aerobically or anaerobically at 28°C for up to 2 weeks. Anaerobic incubation was achieved by using anaerobic jars with disposable gas generators and anaerobic indicators (BBL GasPak 150 anaerobic system).

Denitrifying bacteria were determined by the most-probable-number technique using nitrate broth as described by Focht and Joseph (3).

Sulfate-reducing bacteria were determined by the most-probable-number technique using prereduced anaerobically sterilized (7) sulfate API broth (Difco Laboratories, Detroit, Mich.) prepared in 9-ml volumes in 20-ml serum bottles. Dilutions and inoculations were carried out simultaneously as follows: with a sterile syringe and needle, 1.0 ml of trench water was injected into the first bottle of medium. The bottle was inverted several times, without removing the needle, and the syringe was rinsed by drawing medium into the syringe and injecting it back into the bottle. Next, 1 ml of the inoculated medium was drawn into the syringe, and the needle was removed from the bottle while still retaining the 1 ml of diluted inoculum. This was then injected into the second bottle, and the entire procedure was repeated until a dilution of 10^{-4} was achieved. Vials were incubated at 28°C for 1 to 3 weeks and examined daily for blackening of the medium.

Methanogenic bacteria in the leachate samples were determined by the the most-probable-number technique by measuring the production of methane in a prereduced anaerobically sterilized modified medium (1, 12). The composition of the modified medium used in this study was as follows: KH_2PO_4, 0.75 g; K_2HPO_4, 1.45 g; NH_4Cl, 0.9 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; Na_2CO_3, 2.0 g; $Na_2S \cdot 9H_2O$, 0.5 g; L-cysteine hydrochloride, 0.5 g; Trypticase peptone, 2.0 g; yeast extract, 2.0 g; sodium formate, 2.0 g; sodium acetate, 2.0 g; trace mineral solution (12), 9.0 ml; resazurin solution (0.1%), 1 ml; distilled water, 1,000 ml. Cultures were grown in 9.0 ml of medium in 20-ml aluminum sealed tubes (Bellco Glass Co., Vineland, N.J.) with butyl-rubber septum-lip stoppers (Bellco) in an atmosphere of 80% H_2 and 20% CO_2 as described by Balch and Wolfe (1). Dilutions and inoculations were carried out in the same manner as described for enumeration of sulfate-reducing bacteria. The presence of methane in the head space was determined, after 4 weeks of incubation at 28°C, by gas chromatography. A Perkin-Elmer model 3920 gas chromatograph equipped with a flame ionization detector fitted with a stainless steel column (3.7 m by 3.2 mm) packed with Porapak R (80/100 mesh) was used. The operating temperatures of the injector, column, and detector were 150, 60, and 250°C, respectively.

Growth of bacteria in trench leachate. Trench leachate samples were anoxically filter sterilized using a Millipore stainless-steel filter holder equipped with a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). A 100-ml sample of filter-sterilized leachate wathen inoculated with 0.5 ml of 7-day-old mixed culture of bacteria isolated by anaerobic incubation of trench leachate at 28°C. The inoculated cultures were incubated anaerobically at 28°C in BBL GasPak 150 anaerobic jars. At periodic intervals, 1-ml samples were removed from each culture, and the number of bacteria as colony-forming units was determined on Trypticase soy agar plates incubated under anaerobic conditions.

Microbial degradation of organic materials in anaerobic environments proceeds, albeit slowly, but which of the organics found in the leachates are further degraded, and the extent of decomposition, are not clear. For this purpose, leachate samples collected from trenches 26 and 32 from Maxey Flats, Ky., were filter sterilized. A mixed culture of bacteria isolated from each of the two trenches was used to inoculate 100 ml of the respective filter-sterilized trench waters in serum bottles filled with N_2. Uninoculated, filter-sterilized control samples were incubated under identical conditions. After 30 days of incubation at 28°C, the inoculated and the control samples were analyzed for the organic constituents by gas chromatography and mass spectrometry (4).

Effect of radionuclides on growth of trench-water bacteria. To determine the effect of radionuclides on growth of trench-water bacteria, a mixture of ^{60}Co, ^{85}Sr, and $^{134,137}Cs$ (New England Nuclear Corp., Boston, Mass.) was prepared in 0.5 M HCl and added to 50 ml of the bacterial growth medium to give total activity concentrations of 2.6×10^2, 2.7×10^3, 2.7×10^4, and 2.7×10^5 pCi/ml. The uninoculated media containing radionuclides were acidified with 10 ml of 6 M HCl and were analyzed by gamma-ray spectroscopy using a Ge(Li) detector and a multichannel analyzer system. The levels of each isotope present in the medium at a given total activity concentration are shown in Table 1.

One milliliter of a 4-day-old mixed culture of bacteria isolated from Maxey Flats trench 32, grown aerobically in filter-sterilized trench leachate, was used to inoculate 50 ml of medium in 300-ml nephelo flasks. The medium consisted of NH_4NO_3, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.025 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; $(NH_4)_2SO_4$, 0.4 g K_2HPO_4, 1.2 g; dextrose, 5.0 g; yeast extract, 2.5 g; and distilled water, 1,000 ml. Each flask was surrounded by 5-cm-thick lead bricks to act as a shield against radiation effects from the adjacent flasks. The flasks were incubated at room temperature. The growth of bacteria was monitored by measuring the optical density at 525 nm in a Spectronic-20 spectrophotometer.

RESULTS AND DISCUSSION

Bacterial population enumeration. Table 2 gives the population of total aerobic and anaerobic bacteria in leachate samples collected from four trenches and one monitoring well at Maxey Flats, Ky.; five trenches from West Valley, N.Y.; four trenches from Barnwell, S.C.; and two trenches and one well from Sheffield, Ill. The population of aerobic bacteria was greater than that of the anaerobes in most of the leachate samples analyzed. The methods employed in this study did not distinguish the facultative organisms from strict anaerobes or aerobes. The

TABLE 1. *Levels of radionuclides added to bacterial growth media*

Isotope	Activity (pCi/ml)			
^{60}Co	1.1×10^2	1.0×10^3	1.1×10^4	1.1×10^5
^{85}Sr	8.9×10^0	1.3×10^2	1.4×10^3	1.4×10^4
^{134}Cs	3.8×10^1	3.9×10^2	3.4×10^3	3.4×10^4
^{137}Cs	1.0×10^2	1.2×10^3	1.1×10^4	1.1×10^5
Total	2.6×10^2	2.7×10^3	2.7×10^4	2.7×10^5

TABLE 2. *Population of bacteria in trench leachate samples*

Sample	Collection date	Aerobic (CFU/ml)[a]	Anaerobic (CFU/ml)
Maxey Flats, Ky.			
Trench 2	7/77	1.2×10^3	1.0×10^2
Trench 26	7/77	4.7×10^3	4.1×10^2
Trench 32[b]	7/77	4.8×10^4	1.2×10^4
Trench 19	5/78	2.2×10^2	3.2×10^2
Well UBIA	5/78	3.4×10^3	ND[c]
West Valley, N.Y.			
Trench 3	10/78	5.0×10^4	4.0×10^3
Trench 4	10/78	2.3×10^3	3.3×10^3
Trench 5	10/78	1.6×10^3	3.5×10^2
Trench 8	10/78	1.4×10^3	7.6×10^2
Trench 9	10/78	5.0×10^2	7.3×10^3
Barnwell, S.C.			
Trench 8D	3/79	2.0×10^8	1.0×10^4
Trench 6D1	3/79	3.3×10^3	1.3×10^2
Trench 25/21D1 Trench 3D1	3/79	3.5×10^4	2.2×10^3
	3/79	1.5×10^5	1.2×10^3
Sheffield, Ill.			
Trench 14A	4/79	1.7×10^5	4.4×10^4
Trench 18	4/79	7.1×10^2	6.9×10^1
Well 525	4/79	6.3×10^2	4.2×10^2

[a] CFU, colony-forming units.
[b] Sample was analyzed 7 days after collection.
[c] ND, None detected.

populations of denitrifying, sulfate-reducing, and methanogenic bacteria in the leachate samples are given in Table 3.

Identification of bacteria. On the basis of differences in colony morphology, several colonies of bacteria were isolated from the aerobic and anaerobic agar plates from Maxey Flats and West Valley samples. Tentative identification of some of the isolates was made with the aid of *Bergey's Manual of Determinative Bacteriology* (2), according to Willis (11) and Holdeman and Moore (7). Of the 14 aerobic and 15 obligate anaerobic isolates, 26E1, 26E5, 32E1, 32E2, and 32E3 were identified as *Bacillus* spp.; 26E2, 26E3, 26E4, 32E6, 32E100, 2E1, 2E2, and 2E3 were identified as *Pseudomonas* spp.; 32E101 was identified as *Citrobacter* sp.; and all 15 anaerobes were identified as *Clostridium* spp. Several aerobic gram-negative isolates with different biochemical properties were present but were not identified. The radionuclides and the organic compounds present in the trench water may be acting as mutagenic agents on these bacteria.

Growth of bacteria in trench leachate. Although aerobic, anaerobic, and facultative organisms are present in trench leachates, it is not known whether organisms are capable of growth in trench leachates containing several radionuclides and a variety of organic compounds. Mixed-culture bacteria were isolated under anaerobic conditions from Maxey Flats trench 26 and 32 and West Valley trench 3 and 5 leachate samples and tested for their ability to grow in the respective filter-sterilized trench leachates under anaerobic conditions (Fig. 1). Addition of mineral salts medium without any carbon source to the trench leachates did not significantly increase the growth rates of these bacteria. The fluctuations in the growth curve may be due to the presence of mixed-culture bacteria and differences in the types and quantities of the organic constituents in the leachates. Nevertheless, these bacteria are able to grow under anoxic conditions using the nutrients present in the leachates, which indicates that the organic compounds and the radionuclides present in the leachates did not inhibit the growth of these bacteria.

Trench leachates incubated with a mixed microflora exhibited changes in concentrations of several organic compounds (Tables 4 and 5). Changes in concentrations of several acidic compounds were observed in both the leachate samples. Several of the low-molecular-weight organic acids are formed due to breakdown of complex organic materials and are further metabolized by microorganisms; hence these compounds are in a dynamic state, being both synthesized and destroyed. Microbial degradation products of the organic wastes may influence the transport of radionuclides by leaching, solubilization, and formation of organoradionuclide complexes. The chemical and biological stabilities of the synthetic (decontamination agents), naturally occurring, and microbially synthesized complexes and the existing radionuclide complexes are among the major 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. Furthermore, comprehensive information on the behavior of the contaminated organic compounds in the disposal environment may aid in the formulation of guidelines that will either restrict or allow certain kinds of compounds for shallow-land disposal.

Effect of radionuclides on trench-water bacteria. The radioactivity of the buried waste in the trenches is several orders of magnitude higher than the levels of activity detected in the trench leachates (5). In addition to the radio-

TABLE 3. *Populations of denitrifying, sulfate-reducing, and methanogenic bacteria in trench leachate samples*

Sample	Collection date	Denitrifiers (MPN/ml)[a]	Sulfate reducers (MPN/ml)	Methanogens (MPN/ml)
Maxey Flats				
Trench 19S	5/78	3.3×10^1	4.0×10^0	4.9×10^0
Well UBIA	5/78	4.6×10^2	ND[b]	1.0×10^0
West Valley				
Trench 3	10/78	1.3×10^4	7.0×10^1	2.3×10^1
Trench 4	10/78	2.3×10^3	4.9×10^2	1.7×10^0
Trench 5	10/78	3.3×10^2	1.1×10^1	ND
Trench 8	10/78	7.9×10^2	1.7×10^2	1.0×10^0
Trench 9	10/78	1.3×10^2	3.5×10^2	4.5×10^0
Barnwell				
Trench 8D2	3/79	2.3×10^5	1.1×10^0	0.8×10^0
Trench 6D1	3/79	1.1×10^3	ND	ND
Trench 25/21D1	3/79	1.3×10^4	1.3×10^2	0.2×10^0
Trench 3D1	3/79	5.4×10^4	ND	ND
Sheffield				
Trench 14A	4/79	2.4×10^5	ND	0.2×10^0
Trench 18	4/79	9.5×10^2	4.9×10^1	ND
Well 525	4/79	1.7×10^3	2.3×10^0	ND

[a] MPN, Most probable number.
[b] ND, None detected.

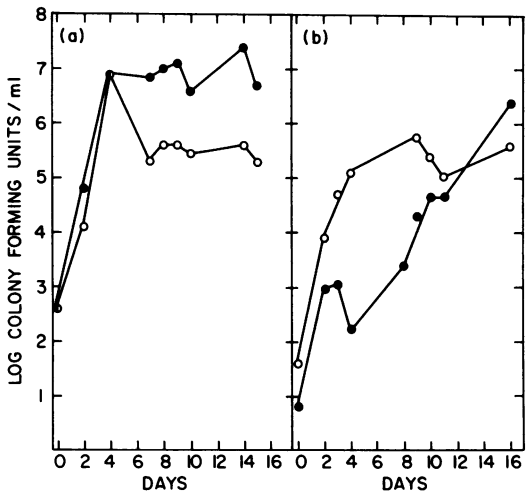

FIG. 1. *Anaerobic growth of mixed-culture bacteria in trench leachates. (a) Maxey Flats, trench 26 (○), trench 32 (●); (b) West Valley, trench 3 (○), trench 5 (●).*

TABLE 4. *Anaerobic degradation of organic compounds present in Maxey Flats trench 26 leachate sample by a mixed culture of bacteria*

Compound	Initial concn (mg/liter)	Percent change
2-Methyl propionic acid	3.5	+31
2-Methyl butanoic acid	18.7	+16
Valeric acid	4.6	−100
C_6 acid (unidentified)[a]	NQ	+5.8
C_6 acid (unidentified)[a]	NQ	+3.6
Hexanoic acid	1.8	−100
2-Methyl hexanoic acid	1.3	+8
Cresol	1.8	+11
C_8 acid (unidentified)[a]	NQ	−4
C_8 acid (unidentified)[a]	NQ	−0.5
Benzoic	1.1	0
Phenyl acetic acid	1.4	−7
Phenyl propionic acid	1.2	−100
α-Terpineol	0.16	−6

[a] Percent changes in concentration were determined on the basis of the ratio of the compound with the internal standard. NQ, Not quantified.

nuclides, a variety of low-molecular-weight organic acids and alcohols are present in the leachates. The presence of these organic compounds is primarily due to the microbial decomposition of complex organic materials under anaerobic conditions. Although aerobic and anaerobic bacteria have been detected in the leachate samples and have been shown to be metabolically active, it is not known whether these bacteria can grow at higher levels of radioactivity. Therefore, the threshold level of radioactivity beyond which the trench-water bacteria cannot survive or contribute to the degradation of pit wastes was determined.

The effect of a mixture of the radionuclides on the growth of a mixed culture of bacteria isolated from the Maxey Flats trench 32 leachate sample is shown in Fig. 2. There was no signifi-

cant difference in effect on the growth of bacteria between the control, containing no radionuclides, and the media containing 2.6×10^2 and 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 radionuclides found in Maxey Flats trench leachates (9). At a concentration of 2.7×10^4 pCi/ml, the growth of bacteria was inhibited and two distinct growth curves were observed. This is probably due to selection of radioresistant strains or mutants of bacteria. However, growth of bacteria was completely inhibited at 2.7×10^5 pCi of radioactivity per ml. At these concentration levels of radionuclides, it is possible that the inhibition of growth is due to the combined effects of radiation and metal toxicity.

Various microbial processes may bring about changes in the forms of radionuclides, such as oxidation-reduction reactions, solubilization, leaching, and formation of radionuclide complexes of microbial metabolites. For example, bacteria, fungi, and actinomycetes resistant to Pu have been isolated from soil and shown to be capable of transporting Pu into the cell and altering its form in the cell and in solution. The resulting soluble Pu complexes tend to be of higher molecular weight than simple complexes and to be negatively charged (10). The finding of active microflora in the trench leachates strongly suggests that they may play a significant role in the transformation of radionuclides and the organic constituents of the waste and thus affect the long-term storage, mobility, and migration of radionuclides from the waste disposal sites into the biosphere.

TABLE 5. *Anaerobic degradation of organic compounds present in Maxey Flats trench 32 leachate sample by a mixed culture of bacteria*

Compound	Initial concn (mg/liter)	Percent change
2-Methyl propionic acid	5.9	−2
2-Methyl butyric acid	20.6	−52
3-Methyl butyric acid	9.9	0
Valeric acid	5.5	+27
2-Methyl pentanoic acid[a]	NQ	−24
C_6 acid (unidentified)[a]	NQ	−27
Phenol	1.1	−27
Hexanoic acid	5.1	−45
2-Methyl hexanoic acid	3.0	−10
Cresol (isomers)	3.9	−21
C_8 acid (unidentified)[a]	NQ	−13
Benzoic acid	1.9	−26
Octanoic acid	1.9	−21
Phenyl acetic acid	3.8	−50
Phenyl propionic acid	9.3	−53
Phenyl hexanoic acid	NQ	−11
α-Terpineol	0.26	−12
Tributyl phosphate	0.24	0

[a] Percent changes in concentration were determined on the basis of the ratio of the compound with the internal standard. NQ, Not quantified.

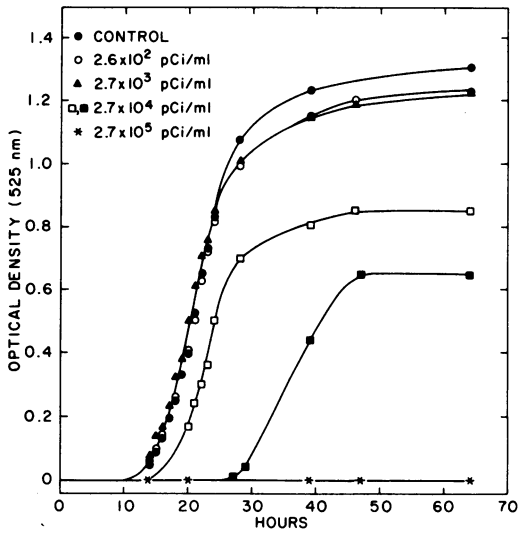

FIG. 2. *Effect of ^{60}Co, ^{85}Sr, and $^{134,137}Cs$ on growth of Maxey Flats trench 32 mixed-culture bacteria. Levels of each radionuclide in the total activity concentrations of 2.6×10^2, 2.7×10^3, 2.7×10^4, and 2.7×10^5 pCi/ml are given in Table 1.*

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