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West Valley Low-Level Radioactive Waste Site Revisited: Microbiological Analysis of Leachates

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ABSTRACT

The abundance and types of microorganisms in leachate samples from the West Valley low-level radioactive waste disposal site were enumerated. This study was undertaken in support of the study conducted by Ecology and Environment Inc., to assess the extent of radioactive gas emissions from the site. Total aerobic and anaerobic bacteria were enumerated as colony forming units (CFU) by dilution agar plate technique, and denitrifiers, sulfate-reducers and methanogens by the most probable number technique (MPN). Of the three trenches 3, 9, and 11 sampled, trench 11 contained the most number of organisms in the leachate: aerobes (5.2×10^4 CFU/ml), anaerobes (3.0×10^4 CFU/ml), denitrifiers (4.9×10^3 MPN/ml), sulfate reducers (2.2×10^4 MPN/ml), and methanogens (2.3×10^0 MPN/ml). Concentrations of carbon-14 and tritium were highest in trench 11 leachate. Populations of aerobes and anaerobes in trench 9 leachate were one order of magnitude less than in trench 11 leachate while the methanogens were three orders of magnitude greater than in trench 11 leachate. The methane content from trench 9 was high due to the presence of a large number of methanogens; the gas in this trench also contained the most radioactivity. Trench 3 leachate contained the least number of microorganisms.

Comparison of microbial populations in leachates sampled from trenches 3 and 9 during October 1978 and 1989 showed differences in the total number of microbial types. Trench 3 leachate contained more aerobes, anaerobes, and denitrifiers in 1978 than in 1989, while trench 9 contained more aerobes, denitrifiers, sulfate reducers, and methanogens in 1989. Variations in populations of the different types of organisms in the leachate reflect the changing nutrient conditions in the trenches.

1.0 INTRODUCTION

1.1 The low-level radioactive wastes disposed of at the West Valley site contain a large amount of organic material that is subject to biodegradation. Biogenic emission of radioactive gas is a critical issue in the long term performance and stabilization of the site (1). Seasonal differences in aerobic and anaerobic conditions exist in the trenches due to surface water infiltration, and release of carbon-14 and tritiated methane has been previously reported. Previous microbiological characterization of the trench leachate in 1978 revealed the presence of aerobic and anaerobic microbes and that microbial activity was not inhibited by the radionuclides and organic chemicals present in the leachate (2). Under a contract with New York State Energy Research and Development Authority (NYSERDA), Ecology and Environment, Inc. (E & E) undertook a study to evaluate the gas evolution from the trenches at the West Valley site. During October 1989, E & E collected leachate and gas samples from trenches 3, 9 and 11 at the West Valley disposal site to determine the rate and extent of radioactive gaseous emission from the site. In support of this study, Brookhaven National Laboratory (BNL) performed the microbiological characterization of trench leachate samples. In this report, microbiological analyses of three trench leachate samples collected by E & E personnel are presented.

2.0 MATERIALS AND METHODS

2.1 Sample Collection. Three leachate samples were collected in sterile 160 ml serum bottles containing 95% N_2 and 5% H_2 sealed with butyl rubber stoppers and aluminum crimp tops. They were packed in a cooler with ice and vermiculite and shipped to BNL within 24 hours of collection. The following samples for microbial analysis were received.

Date Sampled	Date Received	Trench
10/16	10/18	3
10/19	10/20	11
10/20	10/21	9

Upon receipt at BNL, the bottles were checked by the Safety and Environmental Protection Division (S&EP) for radioactive contamination with a Geiger counter, and the outside of the sample bottles were smeared for alpha and beta radiation measurements. Samples were handled carefully to avoid any contamination through spillage. They were placed on absorbant pads and aliquots were taken for the following analyses:

2.2 Direct Counts of Bacteria. The bacteria in the leachate were treated with the DNA specific stain 4'6-diamidino-2-phenylindole (DAPI) and the cells were counted by direct microscopy. The sample in the serum bottle was shaken gently, and a 2 ml aliquot was removed with a sterile syringe, and placed in a snap-cap vial. Glutaraldehyde (0.1 ml) was added for a final concentration of 5%. The aliquot was transferred into a vacuum filtration apparatus and

0.10 μl/ml of 1 mg/ml DAPI was added. The sample was covered and after seven minutes filtered through a 0.2 μm filter (Nuclepore). The UV-fluorescing organisms were counted by direct microscopy with an epi-fluorescent light source. The DAPI stain is not subject to interference and facilitates more rapid and accurate cell counts than acridine orange direct counting (AODC) method (3).

2.3 Population Enumeration of Aerobes and Anaerobes. The sample was brought to room temperature upon arrival, and shaken gently for one minute. The bottle was inverted and ten ml was withdrawn with a sterile syringe and injected into a 160 ml serum bottle containing 90 ml of sterile, prereduced deionized water under 95% N_2-5% H_2. The syringe was rinsed by drawing medium back into the syringe and injecting it back into the bottle. Ten ml of the inoculated medium was withdrawn with a sterile syringe after shaking and injected into a second dilution bottle. This procedure was repeated until a dilution of 10^{-5} was achieved.

The total number of aerobic bacteria as colony-forming units was determined by the pour plate method using trypticase soy agar (TSA) (4). The agar plates were incubated at 26±1°C for up to two weeks, and counted after four to seven days.

Anaerobes were determined by pour plate technique using Thioglycollate agar medium. The thioglycollic acid in the medium served as a reducing agent to provide a low Eh (5). The medium was prereduced by boiling and flushing with nitrogen for 15 minutes to remove the oxygen. It was then autoclaved, cooled and poured onto plates containing 1 ml of the serially diluted sample. Each dilution was plated in triplicate. The plates were placed in a GasPak 150 jar (BBL-Becton Dickinson and Co.) and flushed with nitrogen, and a GasPak CO_2/H_2 generator with palladium catalyst was placed inside the jar to achieve anaerobic conditions. The methylene blue indicator strip was also placed inside the jar and turned white indicating anaerobic conditions. The plates were incubated at 26±1°C for two weeks.

The colonies were counted on the plates that had 30 to 300 colonies. The average number of colonies on each of the three plates was multiplied by the dilution to give the number of colony forming units per milliliter. The anaerobes were counted in a glove bag filled with nitrogen to avoid exposure to air.

2.4 Enumeration of Bacteria by the Most Probable Number Technique.

2.4.1 Denitrifiers: Denitrifiers were assayed by determining the disappearance of nitrate and nitrite in nutrient medium supplemented with nitrate and by nitrous oxide accumulation in the headspace gas by acetylene blockage technique. From the stock serial dilutions, one ml was added to each of five 20 ml serum bottles containing the following medium (6):

BBL nutrient broth	8 g
Potassium nitrate (KNO_3)	0.5 g
Distilled water	1000 ml

The medium was prereduced and sterilized before inoculation with a sterile syringe. The bottles were incubated at 26±1°C for two weeks and tested for the disappearance of nitrate using diphenylamine reagent (7). Two bottles of the 10^0 and 10^1 dilution were injected with 2 ml of acetylene. This enabled accumulation of nitrous oxide in denitrifying bottles, which was then analyzed by gas chromatography (Perkin-Elmer) using a ^{63}Ni electron capture detector (Shimadzu). The presence of nitrous oxide in the headspace confirmed denitrification.

2.4.2 Sulfate Reducers: Postgate's Medium B was used to enumerate sulfate reducing bacteria (SRB) (8). The medium contained a reducing agent, thioglycollic acid, which provided a low Eh environment for the strictly anaerobic organisms. The composition of the medium was as follows:

KH_2PO_4	0.5g
NH_4Cl	1.0g
$CaSO_4$	1.0g
$MgSO_4 \cdot 7H_2O$	2.0g
Sodium lactate	3.5g
Yeast extract	1.0g
Ascorbic acid	0.1g
Thioglycollic acid	0.1g
$FeSO_4 \cdot 7H_2O$	0.5g
Deionized water	1000ml

Lactic acid was used (2.32g) instead of sodium lactate. The pH of the medium was adjusted to between 7 and 7.5 with NaOH before autoclaving. The reducing agents were added inside the anaerobic glove box after the medium was prereduced. The medium was dispensed into 20 ml serum bottles and autoclaved. The medium contained a precipitate to aid the growth of tactophilic strains (9). Five bottles of media were inoculated with one ml at each dilution and incubated for up to two weeks at 26±1°C and examined for blackening of the media indicating sulfate reduction.

2.4.3 Methanogens: The composition of the medium for enumeration of the methanogenic bacteria was as follows (2)

KH_2PO_4	0.75g
K_2HPO_4	1.45g
NH_4Cl	0.90g
$MgCl_2 \cdot 6H_2O$	0.20g
Na_2CO_3	2.00g
$Na_2S \cdot 9H_2O$	0.50g
L-cysteine HCl	0.50g
Trypticase peptone	2.00g
Yeast extract	2.00g
Sodium formate	2.00g
Sodium acetate	2.00g
0.1% resazurin	1.00ml
Trace mineral solution	9.00ml
Distilled H_2O	to 1000ml

Trace mineral salts solution (10):

Nitrilotriacetic acid	4.50g
$FeCl_2 \cdot 4H_2O$	0.40g
$MnCl_2 \cdot 4H_2O$	0.10g
$CoCl_2 \cdot 6H_2O$	0.17g
$ZnCl_2$	0.10g
$CaCl_2$	0.02g
H_3BO_3	0.019g
Sodium molybdate	0.01g
Distilled H_2O	to 1000ml

All of the ingredients were combined in a 1.2 L flask, except for the L-cysteine HCL, and boiled for 5 minutes. Carbon dioxide was bubbled through the medium, then it was cooled and placed in the anaerobic glove box. The L-cysteine HCl was added, and the pH adjusted to 7.0 with KOH. Carbon dioxide was bubbled through until the pH was 6.9 followed by nitrogen for 2 minutes. Nine ml of the medium was dispensed into 20 ml serum bottles, stoppered with butyl rubber stoppers, and 2 ml of CO_2 was injected into the serum bottles in the anaerobic glove box. The bottles were then autoclaved, cooled, and five bottles inoculated at each dilution with one ml of sample. After 3-4 weeks incubation at $26 \pm 1^{\circ}C$, the bottles were analyzed for CH_4 in the head space by gas chromatography using a flame ionization detector.

2.5 Quality Control. Aseptic technique was used for the pour plate and MPN methods. Controls were included without inoculum. Sterile deionized water was also plated and inoculated into MPN tubes to determine any contamination. Two bottles of each of the differential media were left uninoculated and assayed after the incubation period along with the samples.

2.6 Acid Producers. One ml of the leachate sample as received (undiluted) was inoculated into mineral salts medium composed of the following (11):

Glucose	5.0g
NH_4Cl	0.5g
Glycerol phosphate	0.3g
$MgSO_4 \cdot 7H_2O$	0.2g
$FeSO_4 \cdot 7H_2O$	0.0025g
$CaCl_2 \cdot 2H_2O$	0.5g
Peptone	0.1g
Yeast extract	0.1g
Distilled H_2O	1000 ml

The pH of this medium was adjusted to 6.8 ± 0.1. This medium is poorly buffered and was used to isolate anaerobic fermentative organisms from waste samples (11). The medium was prereduced and 40 ml dispensed into 60 ml serum bottles under nitrogen and hydrogen. The samples were incubated at $26 \pm 1^{\circ}C$. The production of organic acids and alcohols by the anaerobes was quantified by high performance liquid chromatography with ultra-violet and refractive index detectors after 3, 5 and 10 days of incubation.

2.7 Isolation of Bacteria. After one week of incubation, the distinct aerobic colonies were streaked onto fresh trypticase soy agar plates. The colonies were examined and their characteristics noted. Pure cultures of bacteria were transferred to agar slants and stored at 4°C.

The anaerobes were examined after two weeks of incubation inside a glove bag filled with nitrogen. The colony characteristics were noted, and the distinct colonies were stabbed into thioglycollate gel tubes and broth. They were also streaked onto thioglycollate plates and placed back into the jars.

Anaerobic growth of the organisms was prevalent deep in the tube because of the absence of oxygen. The mobility of the organism was noted in the gel by observing the growth pattern: whether it was diffuse or confined to separate colonies. The anaerobic isolates were transferred to thioglycollate agar slants and stored at 4°C.

2.8 Chemical Analyses.

2.8.1 pH: A Beckman pH meter was used to determine the pH of the leachate samples.

2.8.2 Iron: Total iron was determined colorimetrically by reduction with hydroxylamine hydrochloride and reacting with 1,10-phenanthroline. Iron (II) was determined by deleting the reduction step. Total iron was also determined by atomic absorption method.

2.8.3 Dissolved Carbon: Total carbon and inorganic carbon were analyzed on a Beckman Total Organic Carbon Analyzer model 915B on filtered and unfiltered aliquots. There was no difference between the filtered and unfiltered samples. Dissolved organic carbon was determined by subtraction of inorganic carbon from total carbon.

3.0 RESULTS AND DISCUSSION

The microbial analyses of trench 3, 9, and 11 are presented in Table 1. The pH, total Fe and Fe(II) and dissolved organic and inorganic carbon values along with other chemical data provided by E & E are presented in Table 2.

3.1 Direct Counts of Bacteria. Direct counts of bacteria in the trench leachate determined by DAPI method yielded greater numbers of cells than the combined number of aerobes and anaerobes by the plate count assay. Direct counting provides the total number of cells present which includes viable and non-viable cells. Colonies that grew on TSA plates express the number of organisms that are viable in the leachate. The direct counts for the leachate of all three trenches were higher than the total number of viable aerobes and anaerobes.

3.2 Characteristics of Leachate Bacteria.

3.2.1 Aerobes: The aerobic colonies from the leachate of trench 11 included circular red, yellow and cream colored colonies as well as large spreading irregular (shape) colonies. Circular red and yellow colonies also grew on the anaerobic plates. Aerobic bacteria were more abundant in trench 11 leachate

than the other two trenches sampled. Nine aerobic colony types were isolated from the pour plates of trench 11 leachate. Trench 9 yielded six different colony types, all of which were circular with entire margins. Two colonies were a distinctive green color; this colony characteristic was not exhibited in any other leachate. Two white and two yellow colonies were also found; these colonies were also observed on the anaerobic plates. Further identification of the organism is needed to determine if they are different bacteria. Fewer number of aerobes were found in the trench 3 leachate than in trench 9 and 11 leachate. The number of aerobes was one order of magnitude greater than anaerobes. The colonies were primarily circular red, yellow, cream and white colored. Five different aerobic and three anaerobic colony types were observed on pour plates from trench 3.

3.2.2 Anaerobes: Trench 11 leachate showed an order of magnitude more anaerobic bacteria (colony forming units) than trench 9 and two orders of magnitude more anaerobic bacteria than trench 3 leachate (Table 1).

In general, many of the colonies found on the aerobic plate were growing below the surface of the agar; many of the colonies growing on the anaerobic plates were identical in morphology to the aerobic colonies. The colonies growing below the surface of the agar, both on plates and in agar tubes, were most likely microaerophilic, or able to tolerate small amounts of oxygen. Some of the aerobic colonies may also be facultative anaerobes which are able to grow under anoxic conditions.

Anaerobes, specifically fermentative bacteria, produce organic acids, carbon dioxide and hydrogen as a result of their metabolic activity. Evidence of these metabolic by-products was found in the laboratory indicating that these processes are most likely occurring in the trench. Copious amounts of propionic, lactic, acetic and butyric acids as well as carbon dioxide and hydrogen were produced by the anaerobic bacteria isolated from the leachate samples. The natural and synthetic organic materials in the trench provide energy sources for the anaerobic bacteria. Isotopes of carbon and hydrogen, namely carbon-14 and tritium, are metabolized to yield carbon-14 CO_2, and tritium gas as well as tritiated water (12). Evolution of $^{14}CO_2$, $^{14}CH_4$ and tritiated methane (CH_3T) gas from the trenches have been observed.

3.2.3 Denitrifiers: The number of denitrifiers was much less in trench 3 leachate than in trench 9 and 11. The nitrate content of trench 3 leachate was the highest (0.8ppm). Denitrifiers were most abundant in trench 11 followed by trench 9. The nitrate content of the leachate in these trenches was below the detection limit. The lack of nitrate in the leachate sample may be due to denitrification activity, where nitrate is converted to gaseous nitrogen.

The denitrifying bacteria may be abundant but dormant in the trench leachate, exhibiting reduction of nitrate in the laboratory where ideal conditions (nitrate and organic carbon) are presented. The denitrifiers isolated from the leachate of trenches 9 and 11 provide evidence of a large denitrifying potential inside the trenches. Denitrifying bacteria are aerobic, but nitrate can be used as the primary electron acceptor in the absence of oxygen (13). Denitrifying activity may not take place in the trench leachate because of the lack of nitrate or the presence of oxygen as the preferential electron acceptor. Previous reports of denitrification

activity in deep subsurface sediments have had difficulty in correlating nitrate concentrations in the sediment pore waters and observed denitrification activity in the laboratory (6).

3.2.4 Sulfate Reducers: Sulfate undergoes dissimilatory sulfate reduction by sulfate reducing bacteria such as Desulfovibrio, Desulfomaculum, and Desulfobacter, resulting in hydrogen sulfide (H_2S) formation (9). The anaerobic bacteria utilize the sulfate ion as an electon acceptor in an anaerobic environment. These organisms are strict anaerobes, and the conditions for their growth must include sulfate source, simple organic carbon source and an anoxic environment. The number of sulfate reducers found in trenches 11 and 9 leachate (Table 1) do not correlate with the amount of sulfate found in the leachates (Table 2). The sulfate concentrations in these trenches were below the detectable limit. Trench 3 leachate contained 12 ppm of sulfate. The number of sulfate reducers found in the leachate represents the potential growth of these organisms if conditions are optimal. The sulfate reducers present in the trench may be dormant because of the lack of available sulfate as well as a metabolizable carbon source. The number of sulfate reducing bacteria in trench 9 leachate was greater than the total number of anaerobic colony forming units. This is due to the selective media used to allow growth of sulfate reducers. The medium used to enumerate the total anaerobes is a general purpose medium that encourages the growth of all types of anaerobic bacteria but not the fastidious organisms. Bacteria that utilize specific alternate electron acceptors, such as sulfate reducers, may not have had the opportunity to be adequately represented as colony forming units on media used for counting total bacteria.

A cause and effect relationship exists between the available energy sources for the anaerobes and the number of anaerobes enumerated from the leachate. Nitrate and sulfate may be depleted in the leachate because of microbial activity; likewise, the activity of the bacteria that utilize these compounds may be suppressed in the trench because of the lack of the available electron acceptor. The lack of sulfite in trenches 11 and 9 may also be evidence that sulfate reduction has depleted the sulfate. Sulfite is an intermediate in sulfate reduction and is reduced to sulfide (9).

3.2.5 Methanogens: Methane producing bacteria utilize a variety of energy sources including organic acids, alcohols, hydrogen, and simple organic compounds. Methanogens are the terminal organisms in the microbial food chain. The simple compounds produced by other organisms serve as substrates for these bacteria resulting in the production of methane (CH_4). Methanogens in the trench leachate utilize the organic compounds containing both carbon-14 and tritium and produce radioactive methane, both carbon-14 methane ($^{14}CH_4$) and tritiated methane (CH_3T) (12).

Trench 9 leachate contained the highest number of methanogens (1300 MPN/ml) (Table 1). Analysis of trench gas (Figure 1) indicated that it contained 21000 ppm of CH_4, the highest for any of the trenches sampled. Trench 3 had much less methanogens (14 MPN/ml) than trench 9, but it had 10000 ppm of methane in the trench. Trench 11 had 760 ppm of methane and the least number of active methanogens (2.3 MPN/ml). Methane content from October to November of trenches 3 and 9 differed greatly indicating high variability in methanogen activity in the trenches (P. Piciulo, personal communication).

3.3 Nutrients and Microbial Population. The leachate from trench 11 had the largest amount of dissolved organic carbon (DOC), 2440 ppm (Table 2). The aerobic and anaerobic bacteria as colony forming units (CFU) were greatest in this trench (Table 1). The dissolved organic carbon in trench 9 (1010 ppm) was 59% less than in trench 11, and 6% greater than in trench 3 (946 ppm). The large amount of DOC in the leachate is due to the nature of the waste buried in the trenches, i.e., paper and paper board, plastic, animal carcasses, etc.

Nitrogen sources were also abundant in the leachates. The total (Kjeldahl) nitrogen averaged 210 ppm for trenches 11 and 3, but was lowest in trench 9 (53 ppm) (Table 2). The carbon to total (Kjeldahl) nitrogen ratio in trench 3 was 4:1; in trench 9, 19:1; and in trench 11, 12:1. Phosphorus was below detection limit in trench 9, as was 0.36 ppm and 0.88 ppm in trench 11 and 3, respectively.

A highly reducing environment is ideal for anaerobic bacterial growth. The Eh, total Fe, and Fe(II) values are presented in Table 2. The Eh of the trench leachates ranged from a high of 57mV to a low of -67mV after the sample was taken. Trench 3 had the lowest redox potential, and the least amount of anaerobic activity observed in the laboratory. The iron in this trench was present as Fe(III) (14 ppm). The iron content was 50% and 300% less than in trenches 11 and 9, respectively. Reduced iron (Fe(II)) was not found in trench 3. The lack of iron (II) may be due to limited anaerobic activity. For example, under anaerobic conditions bacteria reduce iron (III) to iron (II) by the production of organic acids and by specific enzymatic action (12). Anaerobic bacterial activity may also be limited by the lack of available iron (III) to serve as an electron acceptor in the anoxic environment of the trench. The low Eh of the leachate should provide conditions ideally suited to iron reduction and anaerobic activity.

3.4 Radioactive Gas Evolution.

3.4.1 Microbial Numbers and Radioactivity in Leachate: The levels of tritium and carbon-14 in leachate from trench 11 are both an order of magnitude greater than leachate from trenches 3 and 9 (Figure 2). Trench 11 had the highest amount of microbial numbers in the leachate. The levels of radioactivity in the leachate seem to have no effect on the bacteria. Bacteria isolated from trench leachates have shown resistance to radioactivity up to 2.7×10^7 pCi/L due to ^{60}Co, ^{85}Sr and $^{134,137}Cs$ (2). The bacteria growing in the presence of high levels of radioactivity may be selected for radioresistant strains. Mutations of the bacteria due to radiation and chemical effects may also be prevalant in the buried waste undergoing decomposition.

3.4.2 Trench Gas: Analysis of gas samples from trench 3 show very little radioactivity (Table 3). There is a correlation between the small amount of microbial activity in trench 3 leachate and radioactive gas production.

A correlation was also evident between the microbial activity in trench 9 leachate and the radioactive gas production from the trench. Gas samples from trench 9 were the most radioactive, e.g., specific activity 320 pCi/ml and total tritium 230 pCi/ml (Table 3). This leachate was not the most microbially active overall, however, the number of methanogens isolated from

the leachate were 2 to 3 orders of magnitude greater than the other leachates. The methane and carbon dioxide concentration from trench 9 was also the highest (Figure 1). Increased methanogenic bacterial activity in trench 9 may account for the higher radioactivity of the gases produced.

The microbiological characteristics of trench 11 leachate coupled with the radiological analysis suggest that this trench has the greatest potential for radioactive gas production (Figure 2). The potential for gas evolution in trench 9 is supported by the data, whereas the radioactive gas data for trench 11 does not validate this potential (Table 3). One possibility is that the gases produced from this trench are metabolized by other bacteria such as methane oxidizing bacteria as evidenced by more CO_2 than CH_4 in the trench gas (Figure 1). Further research is required to assess the extent to which the various bacteria present in the trench contribute to overall gas production as well as gas consumption.

3.5 Comparison of Chemical and Microbiological Characteristics of Leachate from Trench 3 and Trench 9 Sampled in 1978 and 1989. In Table 4, the results of the chemical, microbiological and radiochemical analyses of the leachates collected in October 1978 and in October 1989 are presented.

Temperature and pH are similiar for both trenches at the two sampling periods. The Eh of the leachate from trench 3 was higher in 1978 although conditions in the trench were still reducing. Dissolved organic carbon in trench 3 leachate was notably higher (1.8 fold) in 1978. The tritium content of the leachate was relatively unchanged. The redox conditions in trench 9 were oxidizing in October 1978 and 1989. The tritium content was relatively unchanged in trench 9 leachate as well and does not appear to be a factor in determining population differences in 1978 and 1989 in both of the trenches.

Comparison of microbiological populations during October 1978 and 1989 in trench 3 reveal some variation in numbers of total anaerobes and denitrifiers (Figure 3). The populations found in the leachate fluctuate due to available carbon, nitrogen and electron acceptors. A change in the metabolizable organic carbon content of the leachate could be a factor for the decrease in anaerobes during October 1989.

The presence of larger numbers of sulfate reducers in trench 9 suggests that conditions favoring sulfate reduction activity existed during October 1989 (Figure 3). The nutrient data presented for 1989 does not provide evidence of optimal conditions for sulfate reduction, i.e., a sulfate source. The presence of these organisms in the leachate is determined by the prevailing nutrient conditions in the trench which provide for their existence. Changing hydrogeological conditions in the trenches such as precipitation and groundwater flow can influence nutrient availability. Conditions in trench 3 in October 1978, favored denitrification activity as evidenced by the large number of denitrifiers indicating a probable increase of nitrogen compounds and nitrate in the leachate. The populations of sulfate reducers and methanogens did not differ much at the two sampling times in trench 3.

3.5.1 Comparison of the Isolates: Bacteria isolated from pour plates of trench 3 in 1978 included five aerobic and five anaerobic colony types. Five aerobic and three anaerobic colony types were isolated in 1989. Three of the

ive aerobic colony types from the two sampling dates had the same orphological characteristics. A black anaerobic colony was noted in 1978 but his colony type was not noted in 1989. The difference in the population of naerobes present in trench 3 at the two sampling dates may be due to a ombined effect of a greater diversity of anaerobes in 1978 and the types of arbon and nitrogen sources available.

Four aerobic colony types were isolated from trench 9 in 1978, and six ifferent aerobic colony types were isolated in 1989. There were distinct orphological differences in the colony types from the two sampling dates. wo green aerobic colonies were noted in 1989 but not in 1978. Four anaerobic olony types were isolated in 1978, and five in 1989. The growth of different acterial types in 1989 due to changing conditions in the trench may account or the increased populations seen at this sampling date. Isolates identified rom both trenches in 1978 included Citrobacter sp., Pseudomonas sp., Bacillus p., and Clostridium sp.

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5.0 References

1. West Valley Trench Gas Evaluation. Expanded Statement of Work. May 1989. Prepared by Ecology and Environment for NYSERDA.

2. Francis, A. J., S. Dobbs, and B. J. Nine. 1980. Microbial activity of trench leachates from shallow-land, low-level radioactive waste disposal sites. Appl. Environ. Microbiol. 40(1):108-113.

3. Porter, K. G. and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25(5):943-948.

4. Wollum II, A. G. 1982. Cultural methods for soil microorganisms. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI, pp. 789-801.

5. Kaspar, H. F. and J. M. Tiedje. 1982. Anaerobic Bacteria and Processes. In A. L. Page, R. H. Miller and D. R. Keeney (Eds.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI, pp. 989-1009

6. Francis, A. J., J. M. Slater, and C. J. Dodge. 1989. Denitrification in deep subsurface sediments. Geomicrobiol. J. 7:103-116.

7. Tiedje, J. M. 1982. Denitrification. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, WI, pp. 1011-1026.

8. Jones, R. E., R. E. Beeman, and J. M. Suflita. 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. Geomicrobiol. J. 7:117-130.

9. Postgate, J. R. 1984. The Sulfate-reducing Bacteria, 2nd ed. Cambridge University Press, London, pp. 30-50, 132-138.

10. Zeikus, J. G. 1977. The biology of methanogenic bacteria. Bact. Rev. 41:514-541.

11. Francis, A. J. and C. J. Dodge. 1988. Anaerobic microbial dissolution of transition and heavy metal oxides. Appl. Environ. Microbiol. 54(4):1009-1014.

12. Francis, A. J., S. Dobbs, and R. F. Doering. 1980. Biogenesis of tritiated and carbon-14 methane from low-level radioactive waste. Nuc. and Chem. Waste Mgt. 1:153-159.

13. Alexander, M. 1977. Introduction to Soil Microbiology. John Wiley and Sons, Inc. New York, NY.

14. Weiss, A. J. and P. Colombo, (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.

Table 1. Microbiological Characterization of West Valley Trench Leachate - October 1989

Trench	Direct Counts (cells/ml)	Aerobes	Anaerobes	Denitrifiers	Sulfate reducers	Methanogens
		--------CFU/ml--------		----------MPN/ml----------		
3	1.8×10^5	3.2×10^3	5.8×10^2	4.9×10^2	4.0×10^1	1.4×10^1
9	1.6×10^5	6.7×10^3	4.4×10^3	3.3×10^3	1.7×10^4	1.3×10^3
11	2.8×10^5	5.2×10^4	3.0×10^4	4.9×10^3	2.2×10^4	2.3×10^0

CFU - Colony forming units
MPN - Most probable number
Direct counts: DAPI method

Table 2
Chemical Characteristics of Leachates[1]
October, 1989

Analysis	Trench 3	Trench 9	Trench 11
Redox potential (Eh) after sample	-62	61	15
Conductivity (micro-mho) after sample	7200	3040	4470
Temperature (°C)	10	11	11
pH (laboratory) *	7.6	7.4	7.2
Total carbon (ppm) *	1280	1260	2650
Dissolved organic carbon (ppm)*	946	1010	2440
Dissolved inorganic carbon (ppm)*	334	253	212
Chemical oxygen demand (COD) (ppm)	2400	2600	7800
Biological oxygen demand (BOD) (ppm)	840	29	2000
Total alkalinity (calcium carbonate) (ppm)	2600	1200	830
Total Kjeldahl nitrogen (TKN) (ppm)	220	53	200
Ammonia nitrogen (ppm)	430	140	510
Nitrate (ppm)	0.8	<0.1	<0.1
Total phosphorus (ppm)	0.88	<0.01	0.36
Sulfate (ppm)	12	<1	<1
Sulfite (ppm)	2	<2	<2
Total iron (ppm) *	14.1	60.6	33.1
Total iron (II) (ppm) *	<0.1	43.9	24.3
Total carbon-14 (pCi/L)	6.6×10^3	7.2×10^3	4.0×10^4
Total tritium (pCi/L)	2.7×10^8	4.8×10^8	1.9×10^9

[1]Data provided by Ecology and Environment Inc. except where indicated by an asterisk.

Table 3. Radioactivity in Trench Gas Samples[1]

Analysis (pCi/ml)	Trench 3	Trench 9	Trench 11
Specific Activity	5.0	320	88
Total C-14	0.2	5.6	1.7
Total H-3	3.9	230	81
CO_2 (C-14)	0.06	4.0	1.2

[1]Data supplied by Ecology and Environment Inc.

Table 4. Comparison of Microbiological and Radiochemical Characteristics of Leachate Samples Collected in 1978 and 1989

	Trench 3	
	Date Sampled	
	October 1978 (2,14)	October 1989
Chemical		
Temperature (°C)	10.5	10
pH	7.3	7.7
Eh (mV)	-3.4	-62
DOC (mg/L)	1700	946
DIC (mg/L)	95	33
Nitrate (mg/L)	1.6	0.8
Sulfate (mg/L)	26	0.88
Gross Alpha (pCi/L)	7.3×10^2	na
Gross Beta (pCi/L)	2.9×10^6	na
Carbon-14 (pCi/L)	na	6.6×10^3
Tritium (pCi/L)	4.8×10^8	2.7×10^8
Microbiological		
Aerobes (CFU/ml)	5.4×10^3	3.2×10^3
Anaerobes "	4.0×10^3	5.8×10^2
Denitrifiers (MPN/ml)	1.3×10^4	4.9×10^2
Sulfate reducers "	7.0×10^1	4.0×10^1
Methanogens "	2.3×10^1	1.4×10^1

	Trench 9	
	Date Sampled	
	October 1978 (2,14)	October 1989
Chemical		
Temperature (°C)	13.5	11
pH	6.7	7.4
Eh (mV)	18	61
DOC (mg/L)	1700	1010
DIC (mg/L)	120	253
Nitrate (mg/L)	<0.1	<0.1
Sulfate (mg/L)	<1	<1
Gross Alpha (pCi/L)	2.7×10^2	na
Gross Beta (pCi/L)	1.2×105	na
Carbon-14 (pCi/L)	na	7.2×10^3
Tritium (pCi/L)	4.6×10^8	4.8×10^8
Microbiological		
Aerobes (CFU/ml)	5.0×10^2	6.7×10^3
Anaerobes "	7.3×10^3	4.4×10^3
Denitrifiers (MPN/ml)	1.3×10^2	3.3×10^3
Sulfate reducers "	3.5×10^2	1.7×10^4
Methanogens "	4.5×10^0	1.3×10^3

na - not available.

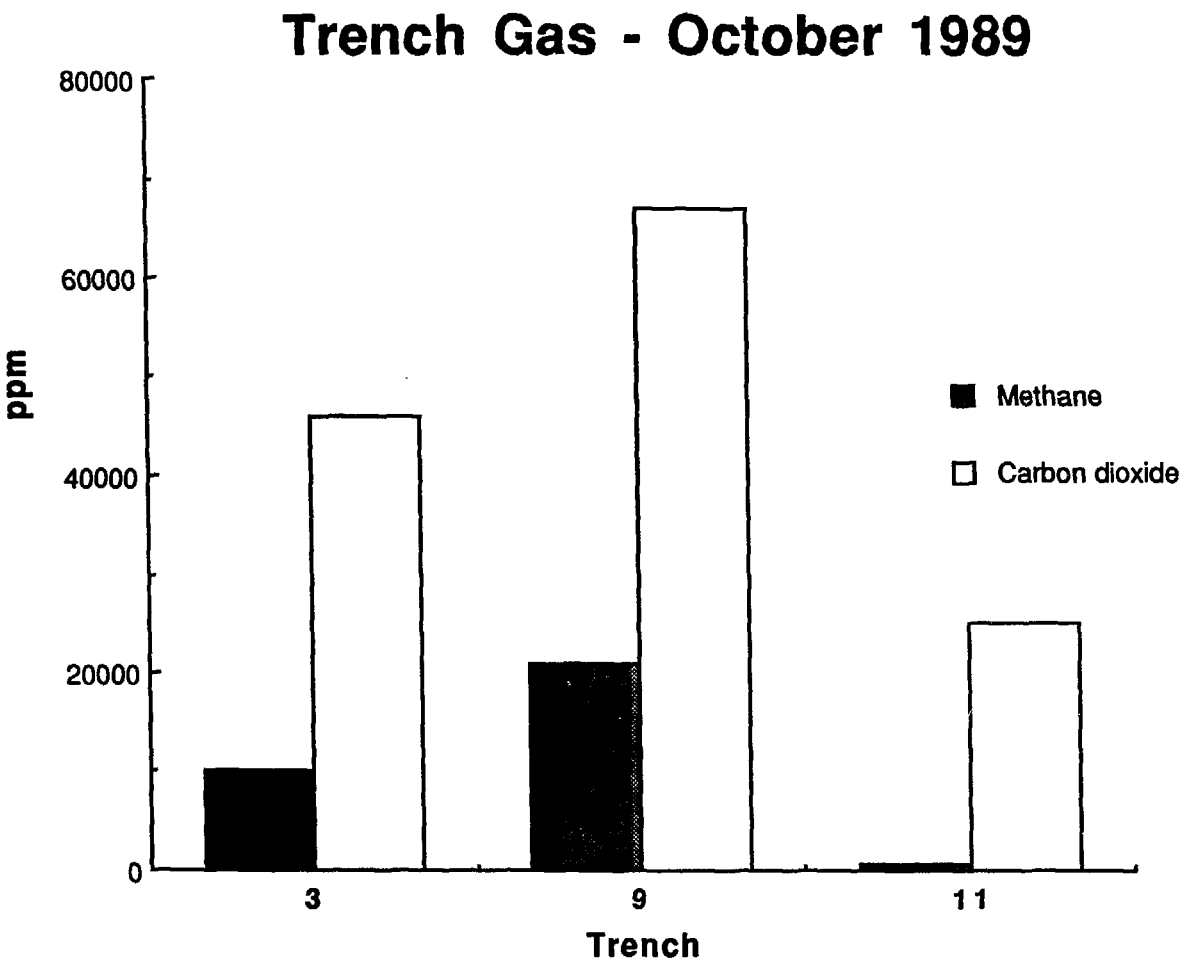

Figure 1. Carbon dioxide and methane in trench.
(Data provided by Ecology and Environment Inc.)

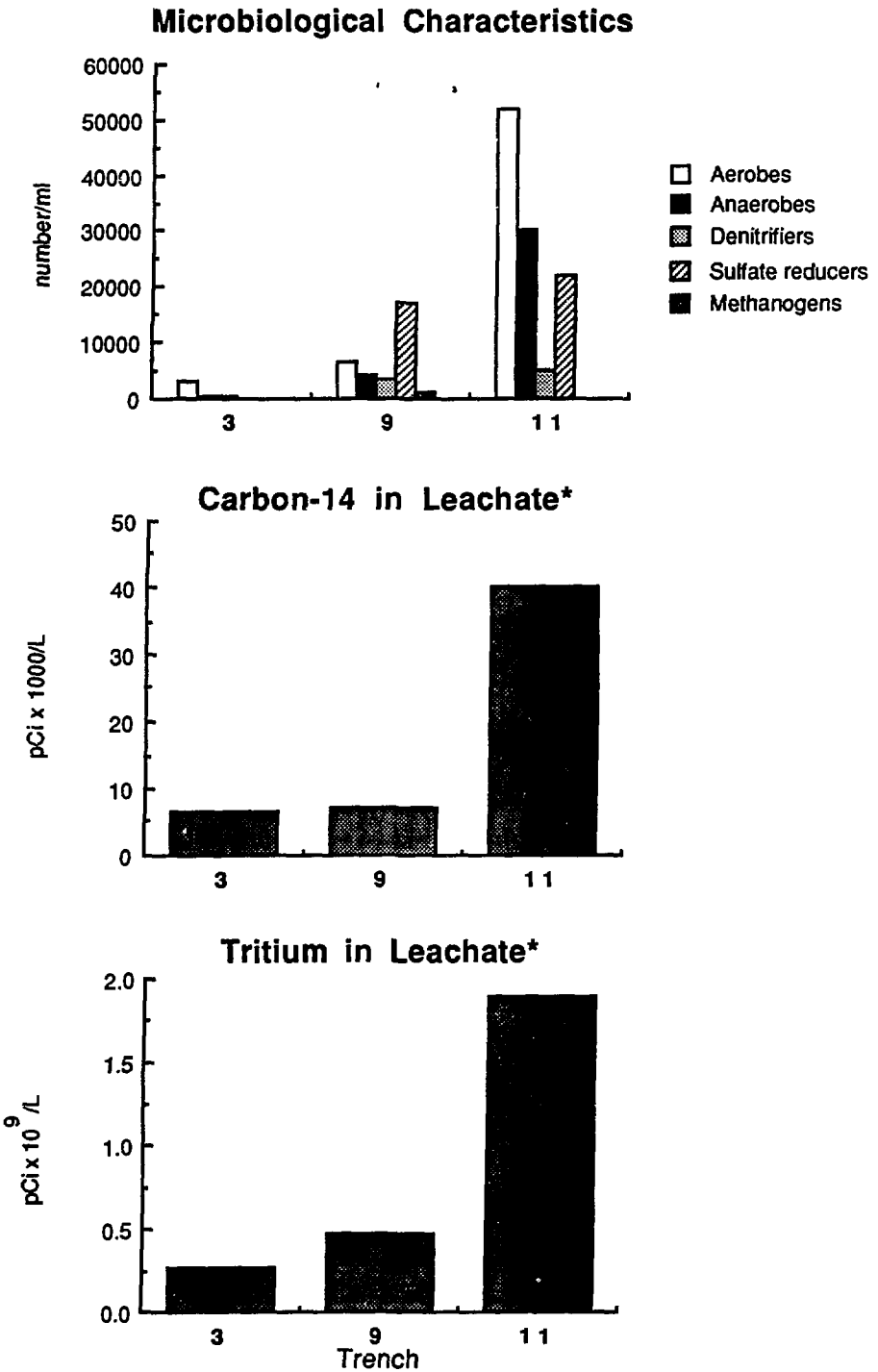

Figure 2. Microbiological and radiochemical components of leachate.
(*Data provided by Ecology and Environment Inc.)

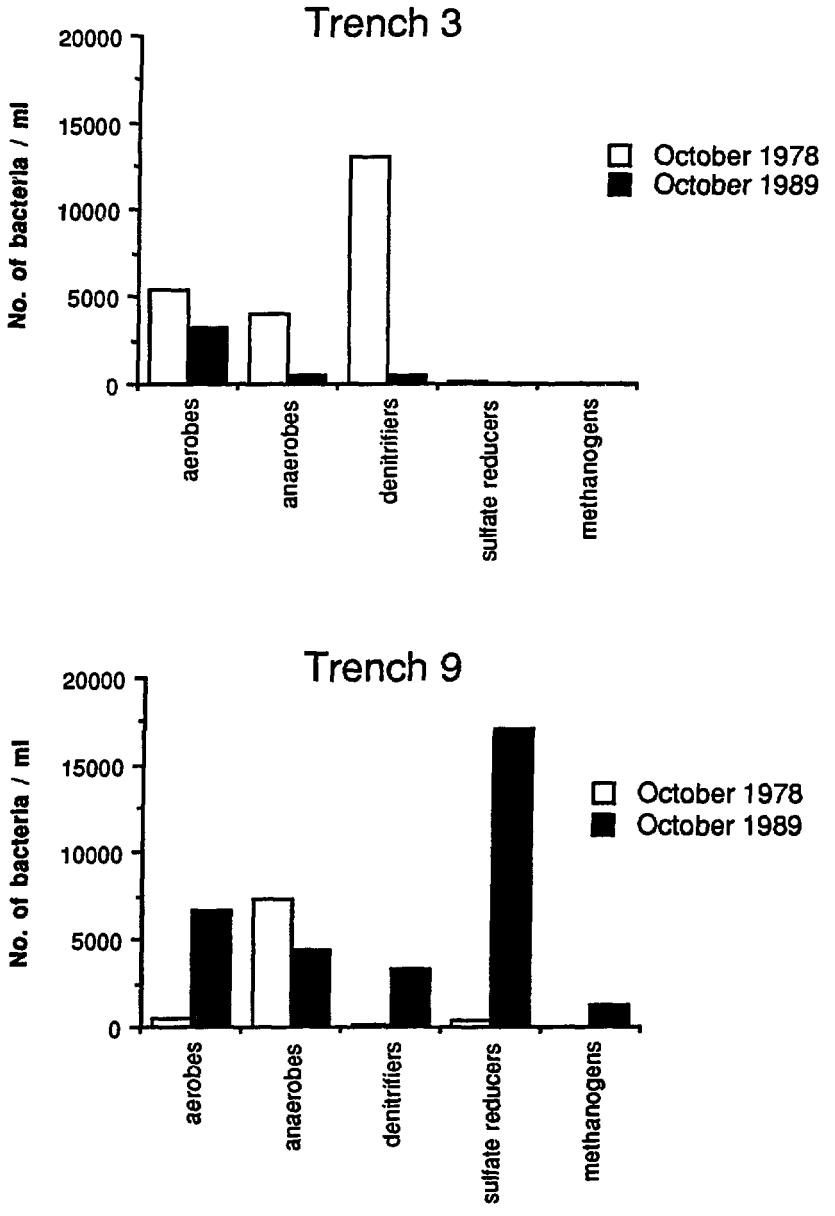

Figure 3. Comparison of microbial populations in trench leachate collected in October 1978 and 1989.