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EVALUATION OF ISOTOPE MIGRATION - LAND BURIAL
WATER CHEMISTRY AT COMMERCIALLY OPERATED LOW-LEVEL RADIOACTIVE WASTE DISPOSAL SITES

PROGRESS REPORT NO. 7

OCTOBER - DECEMBER 1977

P. COLOMBO, A.J. WEISS, AND A.J. FRANCIS

NUCLEAR WASTE MANAGEMENT RESEARCH GROUP

DEPARTMENT OF NUCLEAR ENERGY BROOKHAVEN NATIONAL LABORATORY
UPTON, NEW YORK 11973

MASTER

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NOTICE

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BROOKHAVEN NATIONAL LABORATORY
ASSOCIATED UNIVERSITIES, INC.
UPTON, NEW YORK 11973

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Foreword

The burial of low-level radioactive wastes at commercially operated disposal sites began in 1962. At the present time, disposal sites exist in Maxey Flats, Kentucky; Beatty, Nevada; Sheffield, Illinois; Barnwell, South Carolina, West Valley, New York; and Richland, Washington. Projections have been made of the available capacity of these sites and the results indicate that the current burial capacity of all six sites may be exceeded by 1990 when existing sites may have to be closed or enlarged or new sites will have to be established.

The U.S. Geological Survey (USGS) is collecting data for developing site selection criteria for the disposal of solid low-level radioactive waste. In support of USGS investigations, the U.S. Nuclear Regulatory Commission is funding Brookhaven National Laboratory (BNL) to perform analyses of soil, natural waters, and waste leachates that have accumulated in the trenches at the disposal sites.

The ability to make predictions of the rate of movement of various radionuclides along the ground water flow path is one of the significant factors in developing site selection criteria. Towards this goal BNL is conducting experiments to (a) define the source term of radionuclides and other solutes in the trench waters, (b) detect and characterize radionuclides along the flow paths of the ground water, and (c) describe physical, chemical, and biological processes that control the movement of buried radionuclides at the disposal sites.

Summary

Trench water samples from the commercial low-level radioactive waste disposal sites at Maxey Flats, Kentucky, and West Valley, New York, were collected, and the bacterial populations were enumerated. The range of bacterial populations in six trench water samples were 400 to 24,000 aerobic and 90 to 15,000 anaerobic bacteria/ml. Most of the bacteria isolated from the anaerobic culture plates were facultative anaerobes, although a few strict anaerobes were also present. Mixed bacterial populations isolated from the trench waters were able to grow anaerobically utilizing the carbon and nitrogen sources present in the trench waters. Trench waters supplemented with mineral salts supported only a modest increase in growth of these bacteria. The results of this study indicate that bacteria are active in the trenches, and the radioactivity and organic compounds present in the trenches are not toxic to these bacteria.

I. INTRODUCTION

Most of the low-level radioactive wastes buried in the commercially operated shallow land disposal sites contain natural and man-made organic materials. Microorganisms play an active role in the decomposition of organic materials under anaerobic conditions and produce CO_2, CH_4, H_2, H_2S, and other organic compounds. Gas samples collected from the trenches at Maxey Flats, Kentucky, contained carbon-14 and tritium activity (Colombo, Weiss, and Francis, unpublished results, 1977). Microorganisms can act upon existing organo-radionuclide complexes to change the forms of radionuclides. Alternatively, the microbial metabolites may react with radionuclides to form complexes and influence their mobility in the environment.

Microbial activity in radioactive wastes has received little attention due to the common belief that the radiation levels may be lethal to microorganisms and generally will not support any microbial growth. To date no information is available regarding the microbial activities that may affect the long term storage and disposal of radioactive wastes and the possible release of radioactivity in the biosphere.

A preliminary study was conducted to determine the population distribution and growth of microorganisms present in the trench water samples at the commercial low-level radioactive waste disposal sites. Water samples accumulated in the trenches were collected from Maxey Flats, Kentucky, and West Valley, New York, and were analyzed for the presence of bacteria and the ability of these bacteria to grow in the trench water under anaerobic conditions. The results of this study are discussed in this quarterly progress report.

II. MICROBIOLOGY

A. Sample Collection:

Water samples from trenches at Maxey Flats, Kentucky, and West Valley, New York, were collected under anoxic conditions as described by Colombo *et al.*[1] The schematic of the water sample collection system is shown in Figure 1. Thoroughly cleansed sample bottles were autoclaved and filled with nitrogen prior to collection of water samples. Approximately five gallons of trench water was flushed through the collection system before collecting samples for microbiological, inorganic, organic, and radiochemical analyses.

For microbiological analyses, about 100 ml of the trench water sample was collected anoxically into sterile nitrogen filled 150 ml glass serum bottles fitted with Teflon lined serum stoppers (Figure 1). The serum bottles were packed in ice during the collection of water samples. Samples thus collected were immediately shipped to BNL and stored at $4^{o}C$. The bacterial populations were enumerated within 24 hours of sample collection. To determine the effect of time on bacterial populations, microbilogical analysis was also performed on seven day old water samples.

B. Sample Filtration:

For microbial growth studies, trench water samples were filter sterilized using 0.45 μ millipore membrane filters. The filtration unit used in this study as shown in Figure 2 was autoclaved prior to sample filtration. The filtrate was collected in a clean, sterile, amber glass bottle and stored at $4^{o}C$.

C. Inorganic, Organic, and Radiochemical Analyses:

A partial characterization of inorganic, organic, and radiochemical constituents of the trench water samples from Maxey Flats and West Valley are presented in Tables 1 and 2, respectively. Detailed analyses of the trench waters will be furnished in a subsequent report. The Eh and pH of the samples were measured in-line during collection in the field. The methods used for the analysis of inorganic, organic, and radiochemical constituents of the trench water samples are described in detail by Colombo *et al.*[2,3]

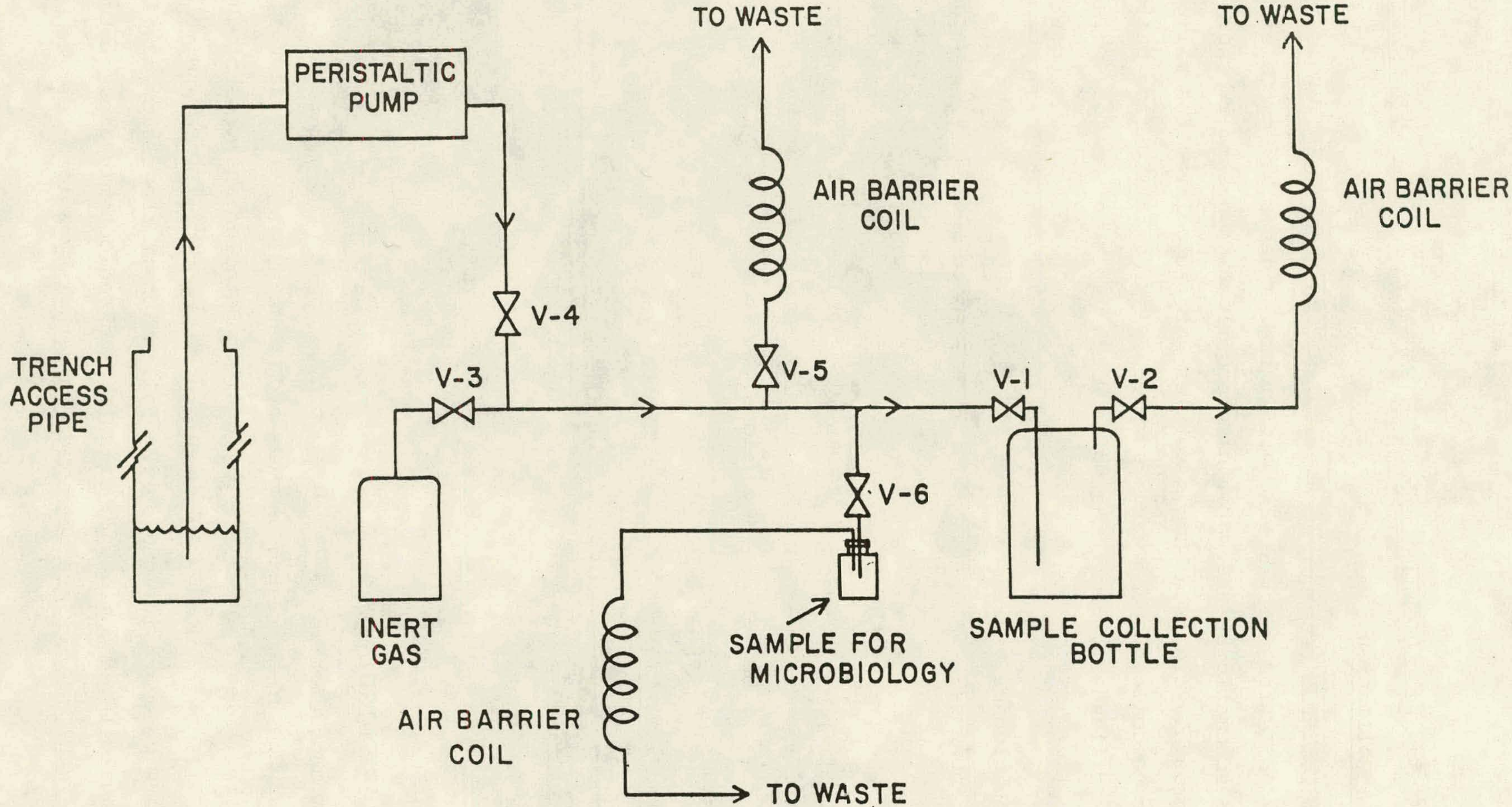

Figure 1. Schematic Water Sample Collection System.

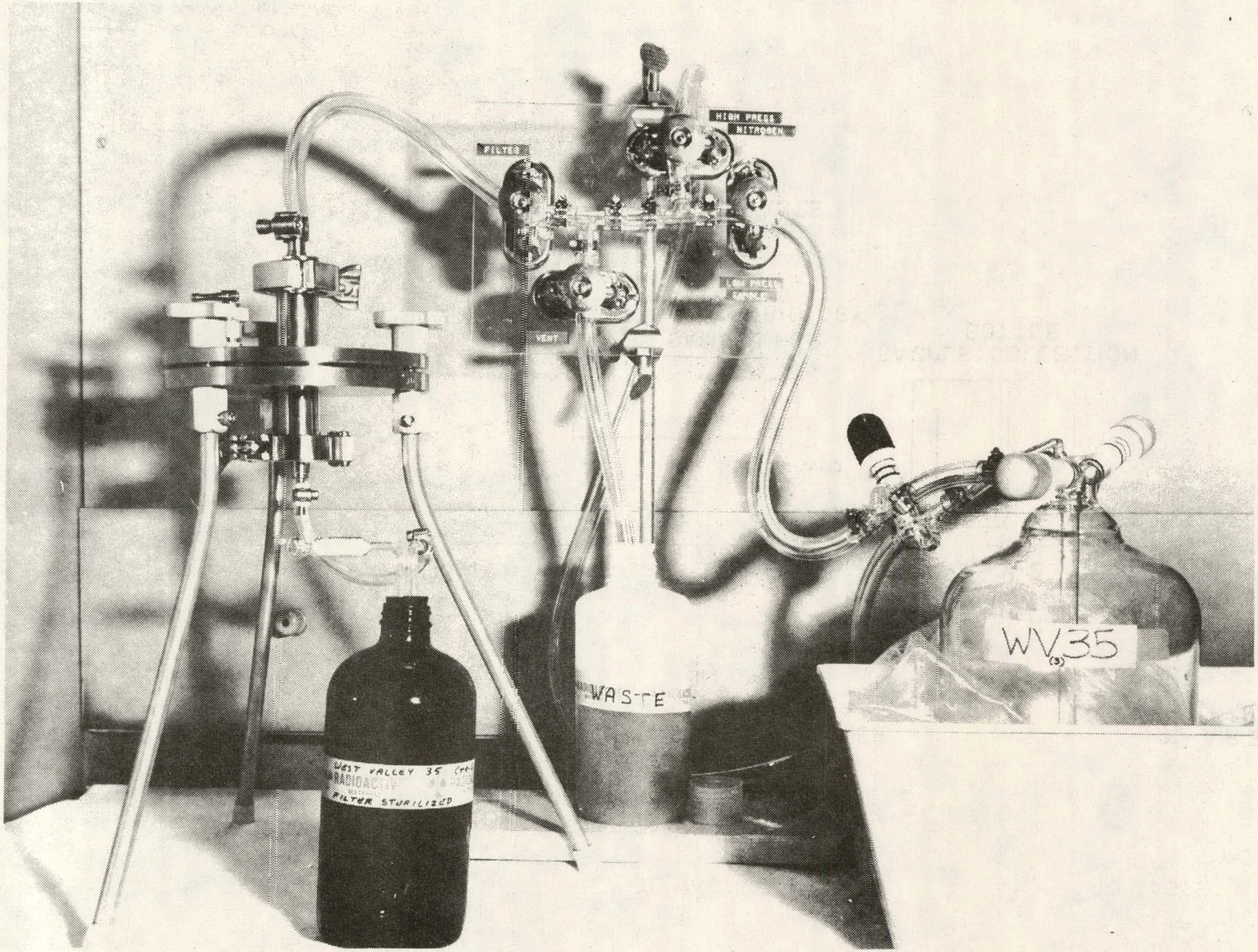

Figure 2. Filtration of Trench Water Sample.

Table 1

Partial Characterization of Organic, Inorganic and Radiochemical Constituents of Maxey Flats Trench Water Samples (July 1977)

	Trench 2	Trench 26	Trench 32
Dissolved Organic Carbon (mg/ℓ)	90	770	990
Inorganic Carbon (mg/ℓ)	330	100	380
pH	7.4	7.3	7.9
Eh (mv)	- 111	- 75	- 200
Ammonia-N (mg/ℓ)	36	115	117
Nitrite+Nitrate-N (mg/ℓ)	0.08	0.04	0.04
Gross Alpha (pCi/ℓ)	$6.73x10^3$	$2.71x10^4$	$3.07x10^4$
Gross Beta (pCi/ℓ)	$2.38x10^4$	$1.18x10^5$	$1.79x10^6$
Tritium (pCi/ℓ)	$2.09x10^7$	$1.28x10^8$	$2.27x10^9$
Americium-241 (pCi/ℓ)	$2.69x10^3$	N.D.	N.D.
Cesium-137 (pCi/ℓ)	N.D.	$5.10x10^3$	$4.58x10^3$
Cobalt-60 (pCi/ℓ)	$9.36x10^3$	$1.44x10^3$	$3.35x10^4$
Strontium-90 (pCi/ℓ)	$4.03x10^3$	$3.08x10^4$	$5.63x10^5$
Plutonium-239,240 (pCi/ℓ)	$2.75x10^2$	$3.51x10^3$	$2.92x10^3$
Plutonium-238 (pCi/ℓ)	$9.38x10^3$	$1.26x10^5$	$1.14x10^5$

N.D. - Not Detected.

Table 2

Partial Characterization of Organic, Inorganic and Radiochemical Constituents of West Valley Trench Water Samples (November 1977)

	Trench 3	Trench 5	Trench 8
Dissolved Organic Carbon (mg/ℓ)	1600	3600	2700
Inorganic Carbon (mg/ℓ)	90	50	100
pH	7.5	7.1	6.7
Eh (mV)	+ 25	+ 25	- 120
Ammonia-N (mg/ℓ)	230	16	120
Nitrite+Nitrate-N (mg/ℓ)	N.D.	3.0	< 0.1
Gross Alpha (pCi/ℓ)	N.D.	6.26×10^{2}	8.74×10^{3}
Gross Beta (pCi/ℓ)	2.39×10^{6}	2.57×10^{6}	7.23×10^{5}
Tritium (pCi/ℓ)	4.38×10^{8}	1.31×10^{9}	3.90×10^{9}
Americium-241 (pCi/ℓ)	N.D.	N.D.	N.D.
Cesium-137 (pCi/ℓ)	2.92×10^{5}	2.69×10^{5}	1.36×10^{5}
Cobalt-60 (pCi/ℓ)	2.49×10^{4}	1.98×10^{3}	3.3×10^{2}
Plutonium-239,240 (pCi/ℓ)	8.9×10^{1}	3.2×10^{1}	3.0×10^{2}
Plutonium-238 (pCi/ℓ)	6.4×10^{1}	3.81×10^{2}	1.41×10^{5}

N.D. - Not Detected

D. Enumeration of Bacteria in Trench Waters:

Water samples from trenches 2, 26, and 32 at Maxey Flats, Kentucky, (July 1977) and from trenches 3, 5, and 8 at West Valley, New York, (November 1977) were collected and the bacterial populations were enumerated. Trenches were selected that contained various concentration levels of radionuclides and organics based on previous analyses. The standard dilution agar plate method, and the most probable number (MPN) technique were used to enumerate the distribution of aerobic and anaerobic organisms. Compositions of the various culture media used in this study are shown in Table 3.

E. Dilution Agar Plate Method:

Ten-fold serial dilutions of the trench water sample were prepared using 90 ml sterile distilled water blanks. One milliliter of a suitable dilution of the trench water sample was transferred into a sterile petri dish and mixed with trypticase soy agar which was cooled to 42^{o}C. Six plates were prepared for each dilution and triplicate plates were incubated under aerobic and anaerobic conditions at 28^{o}C. Anaerobic incubation was achieved by using anaerobic jars with disposable gas generators and anaerobic indicators (BBL Gas-Pak 150, anaerobic system, Bioquest, Cockeyville, Maryland).

F. Most-Probable-Number (MPN) Technique:

The MPN of aerobic and anaerobic bacterial populations of the trench water samples was determined according to the method described by Alexander.[4] The MPN method permits estimation of population density without an actual count of single cells or colonies. Ten-fold serial dilutions of the trench water samples from Maxey Flats were prepared. One milliliter aliquots from appropriate serial dilutions were added to each of five tubes containing trypticase soy broth and to five tubes containing fluid thioglycollate medium to enumerate the aerobic and anaerobic organisms, respectively. All tubes were incubated for three weeks at 28^{o}C. At the end of the incubation period each dilution set of tubes was examined macroscopically for evidence of microbial growth. Tubes were considered positive if there was any visible turbidity.

Table 3

Composition of Culture Media

Nutrient Agar

Beef Extract	3 g
Peptone	5 g
Agar	15 g
Distilled Water	1000 ml
pH	6.9

Trypticase Soy Broth

Trypticase Peptone	17.0 g
Phytone Peptone	3.0 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g
Dextrose	2.5 g
Distilled Water	1000 ml
pH	7.3

Trypticase Soy Agar

Trypticase Peptone	15.0 g
Phytone Peptone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Distilled Water	1000 ml
pH	7.3

Table 3 (Cont'd)

Fluid Thioglycollate Medium

Trypticase	15.0 g
L-Cystine	0.5 g
Dextrose	5.0 g
Yeast Extract	5.0 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 g
Resazurin	0.001 g
Agar	0.75 g
Distilled Water	1000 ml
pH	7.1

Mineral Salts Medium

$(NH_4)_2SO_4$	0.45 g
NaCl	0.9 g
$MgSO_4 \cdot 7H_2O$	0.18 g
$CaCl_2 \cdot 2H_2O$	0.1 g
NH_4Cl	0.5 g
KH_2PO_4	1.5 g
K_2HPO_4	2.2 g
Distilled Water	1000 ml
pH	7.2

The bacterial populations of the Maxey Flats and West Valley trench water samples are shown in Tables 4 and 5. Based on colony morphology, only a few variety of bacteria were found in the dilution agar plates. Some actinomycetes colonies were also found in the plates. There were no marked differences between the total number of aerobic and anaerobic bacteria enumerated from trench water samples which were analyzed at 24 hours and at 7 days after collection.

Several individual colonies of bacteria growing in aerobic and anaerobic plates were isolated on the basis of colony morphology, and the purity of these cultures were checked. The colonies were maintained on nutrient agar slants or thioglycollate medium. Several of the pure cultures were tested for their ability to grow in the presence or absence of oxygen and were characterized as strict aerobes, strict anaerobes, and facultative anaerobes. The results are presented in Table 6. Most of the isolates are facultative anaerobes, and further tests are underway to identify these organisms.

G. Growth of Bacteria in Trench Water:

Although aerobic, anaerobic, and facultative organisms are present in the trench waters, it is not known which of these organisms are actively growing in the trenches. To distinguish and isolate those organisms that are capable of growth in the trench water from those organisms that are dormant or not actively growing, 1 ml of the raw trench water sample from Maxey Flats trenches 26 and 32, and West Valley trenches 3 and 5 were added to 50 ml of the respective filter sterilized trench waters. The inoculated trench water samples were incubated anaerobically (BBL Gas-Pak anaerobic system) at 28°C. At periodic intervals, 1 ml aliquots were removed from the enrichment cultures and transferred to their respective filter sterilized trench waters. After a few days, the inoculated trench water samples turned cloudy indicating bacterial growth. Several days after incubation, most of the trench water turned black indicating the possible formation of metal sulfides. Microbiological examination of the trench water samples revealed the presence of a mixed bacterial population in all of the trench water samples.

Table 4

Bacterial Analyses of Maxey Flats Trench Water Samples

Sample*	Colony Forming Units (CFU)/mℓ		Most-Probable-Number (MPN)/mℓ	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Trench 2 A	1.2×10^3	1.9×10^2	4.9×10^3	1.7×10^4
B	6.2×10^3	3.4×10^2	1.1×10^4	7.9×10^3
Trench 26 A	4.7×10^2	4.1×10^2	3.3×10^3	2.3×10^4
B	2.0×10^3	1.5×10^4	4.9×10^3	4.9×10^4
Trench 32 B	4.8×10^4	1.2×10^4	7.9×10^5	4.9×10^5

* A - Samples were analyzed within 24 hours of collection.

B - Samples were analyzed at day 7 after collection.

Table 5

Bacterial Analyses of West Valley Trench Water Samples

	Colony Forming Units (CFU)/mℓ	
Sample*	Aerobic	Anaerobic
Trench 3	$2.4x10^{4}$	$1.5x10^{4}$
Trench 5	$2.7x10^{3}$	$4.5x10^{3}$
Trench 8	$3.9x10^{2}$	$9.0x10^{1}$

* Samples were analyzed within 24 hours of collection.

Table 6

Physiological Characterization of Bacteria Isolated from Trench Waters: Relation to Free Oxygen

Sample	Source of Isolates	No. of Isolates Tested	Number of Isolates: Strict Aerobes	Strict Anaerobes	Facultative Anaerobes
Maxey Flats, Kentucky					
Trench 2	Aerobic	11	0	0	11
	Anaerobic	9	0	1	8
Trench 26	Aerobic	8	0	0	8
	Anaerobic	9	0	1	8
Trench 32	Aerobic	5	0	0	5
	Anaerobic	9	0	2	7
West Valley, New York					
Trench 3	Aerobic	6	4	0	2
	Anaerobic	4	0	3	1
Trench 5	Aerobic	5	0	0	5
	Anaerobic	4	0	1	3
Trench 8	Aerobic	3	0	0	3
	Anaerobic	3	0	3	0

A preliminary study was conducted with mixed cultures isolated from Maxey Flats trenches 26 and 32 to determine the ability of the bacteria to grow in trench water (a) diluted with sterile distilled water (1:1), (b) amended with glucose (0.5%), (c) amended with mineral salts, and (d) amended with glucose (0.5%) and mineral salts. One tenth of one milliliter of 2 week old cultures from trenches 26 and 32 were used to inoculate filter sterilized undiluted and diluted trench waters with and without amendments. Inoculated and uninoculated controls were incubated for 96 hours under anaerobic conditions at 28°C. Growth was determined turbidimetrically by measuring the absorbance at 470 nm with a Spectronic-20 Colorimeter and the results are shown in Tables 7 and 8. Trench water samples amended with glucose and mineral salts showed an increase in turbidity.

A detailed study was undertaken to determine the growth of bacteria in undiluted filter sterilized trench water and trench water supplemented with mineral salts. For this purpose, 0.5 ml of a 7 day old mixed culture from Maxey Flats trenches 26 and 32, and West Valley trenches 3 and 5 were used to inoculate 100 ml of the respective filter sterilized trench water with and without mineral salts. The composition of the mineral salts are given in Table 3. The inoculated cultures were incubated at 28°C in anaerobic jars. At periodic intervals 1 ml aliquots were removed from each culture flask and the bacterial populations were enumerated by the serial dilution agar pour plate method. Triplicate plates of trypticase soy agar medium were used for each appropriate dilution series, and the plates were incubated anaerobically at 28°C for 3 days. The bacterial populations were enumerated after 3 days, and the results are expressed as colony forming units/ml (Figures 3 to 6). Addition of mineral salts to the trench water had some influence on the growth of bacteria, but not to the extent that it is a growth limiting factor in the trench water. The bacteria capable of growth in trench waters from Maxey Flats and West Valley were found to be a mixed culture. On the basis of colony morphology, there appears to be three to five types of colonies, however, there may be more types of bacteria present that were not distinguishable by colony morphology alone. Several of these colonies have been isolated for future studies.

In conclusion, a preliminary microbiological investigation at the low-level radioactive waste disposal sites has revealed that (i) microorganisms are present in the trench waters, (ii) they are able to grow under anoxic conditions using the nutrients present in the trench water, (iii) the organic compounds present in the trench water are not toxic to these bacteria, and (iv) the radionuclides present in the trench water are not lethal to these bacteria.

The results of this study suggest that a more comprehensive investigation should be made of the microbial activities in the trenches and trench waters at the low-level radioactive waste disposal sites relevant to their effect on the long term storage, solubility, mobility, and migration of radionuclides from the sites.

Table 7

Growth of Bacteria Isolated from

Maxey Flats - Trench 26

Additions to Trench Water	Optical Density (OD) at 470 nm Trench Water Undiluted	Diluted
No additions	0.05	0.02
Glucose (0.5%)	0.19	0.18
Mineral Salts	0.12	0.05
Glucose (0.5%) + Mineral Salts	0.90	0.76

Table 8

Growth of Bacteria Isolated from

Maxey Flats - Trench 32

Additions to Trench Water	Optical Density (OD) at 470 nm Trench Water Undiluted	Diluted
No additions	0.49	0.29
Glucose (0.5%)	0.58	0.28
Mineral Salts	0.31	0.35
Glucose (0.5%) + Mineral Salts	0.78	1.05

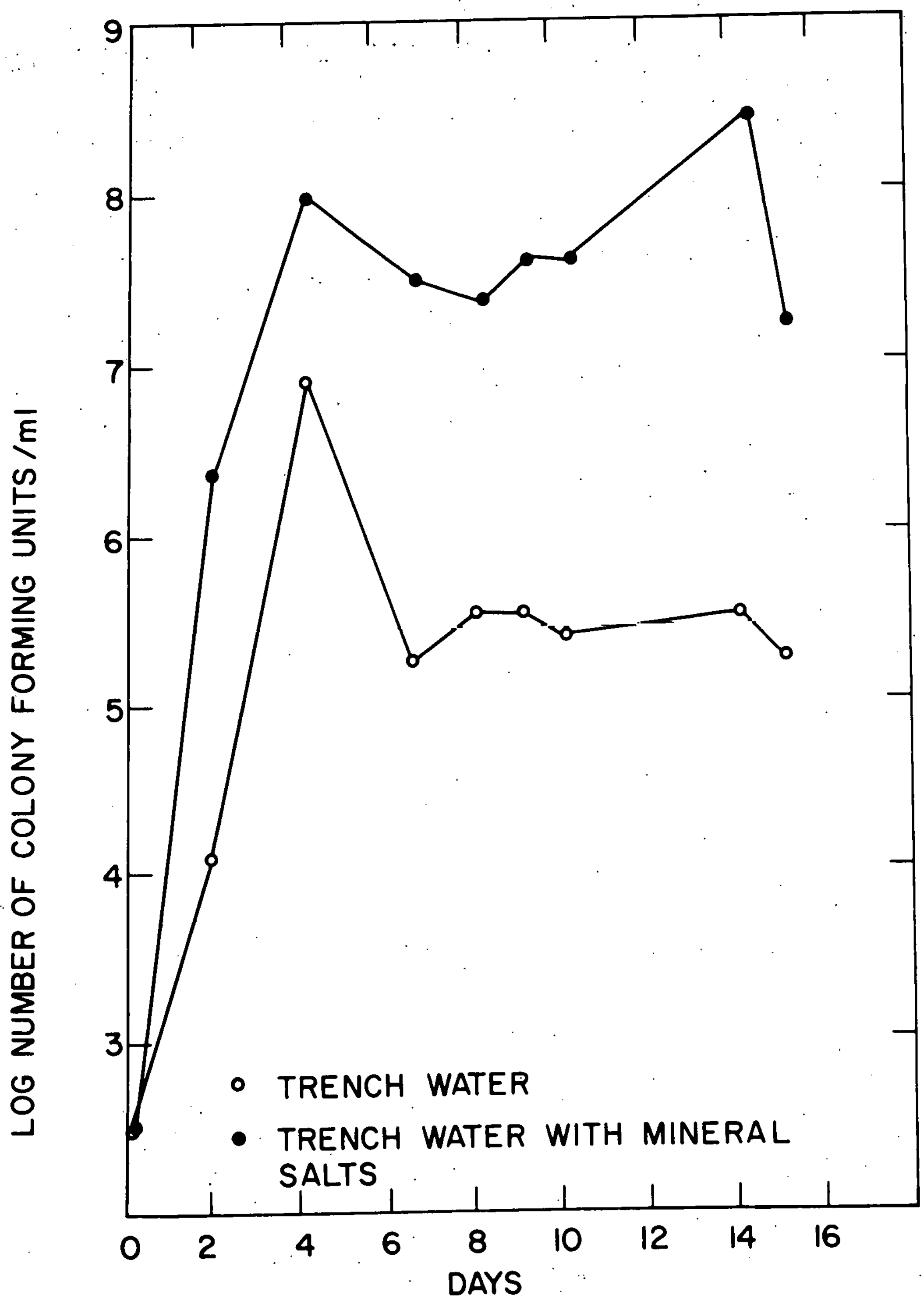

Figure 3. Anaerobic Growth of Mixed Culture Bacteria in Maxey Flats Trench 26 Water Sample.

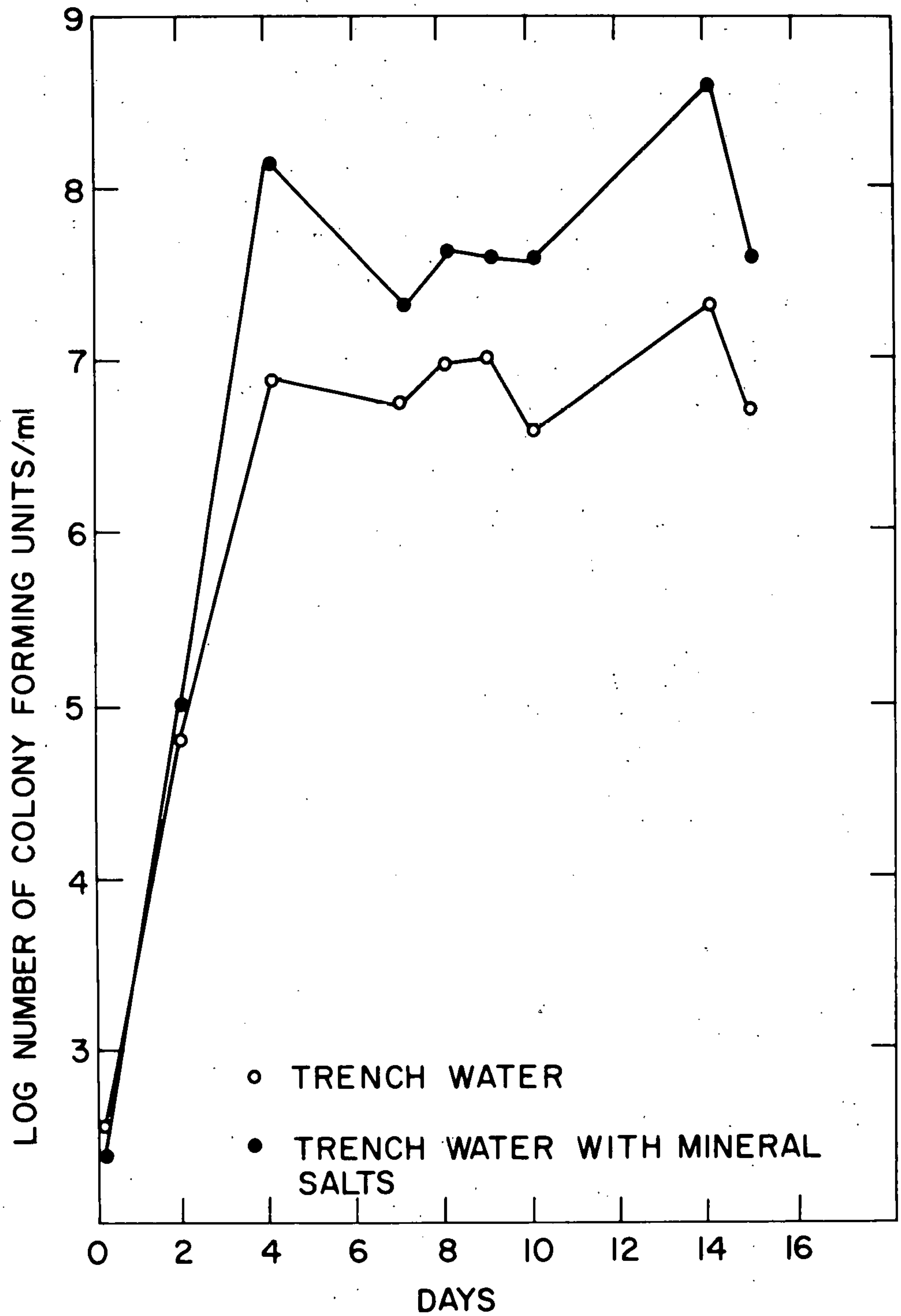

Figure 4. Anaerobic Growth of Mixed Culture Bacteria in Maxey Flats Trench 32 Water Sample.

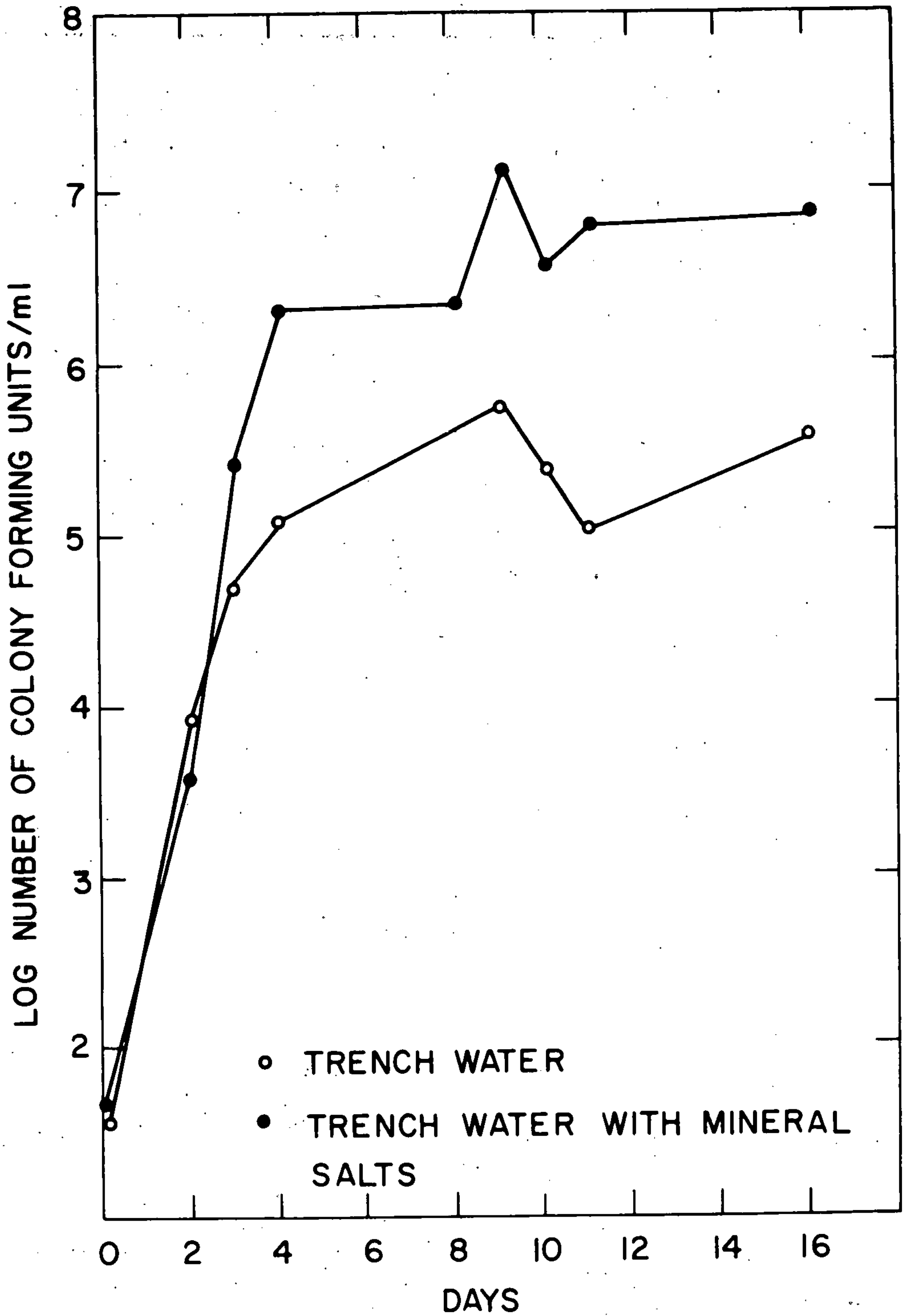

Figure 5. Anaerobic Growth of Mixed Culture Bacteria in West Valley Trench 3 Water Sample.

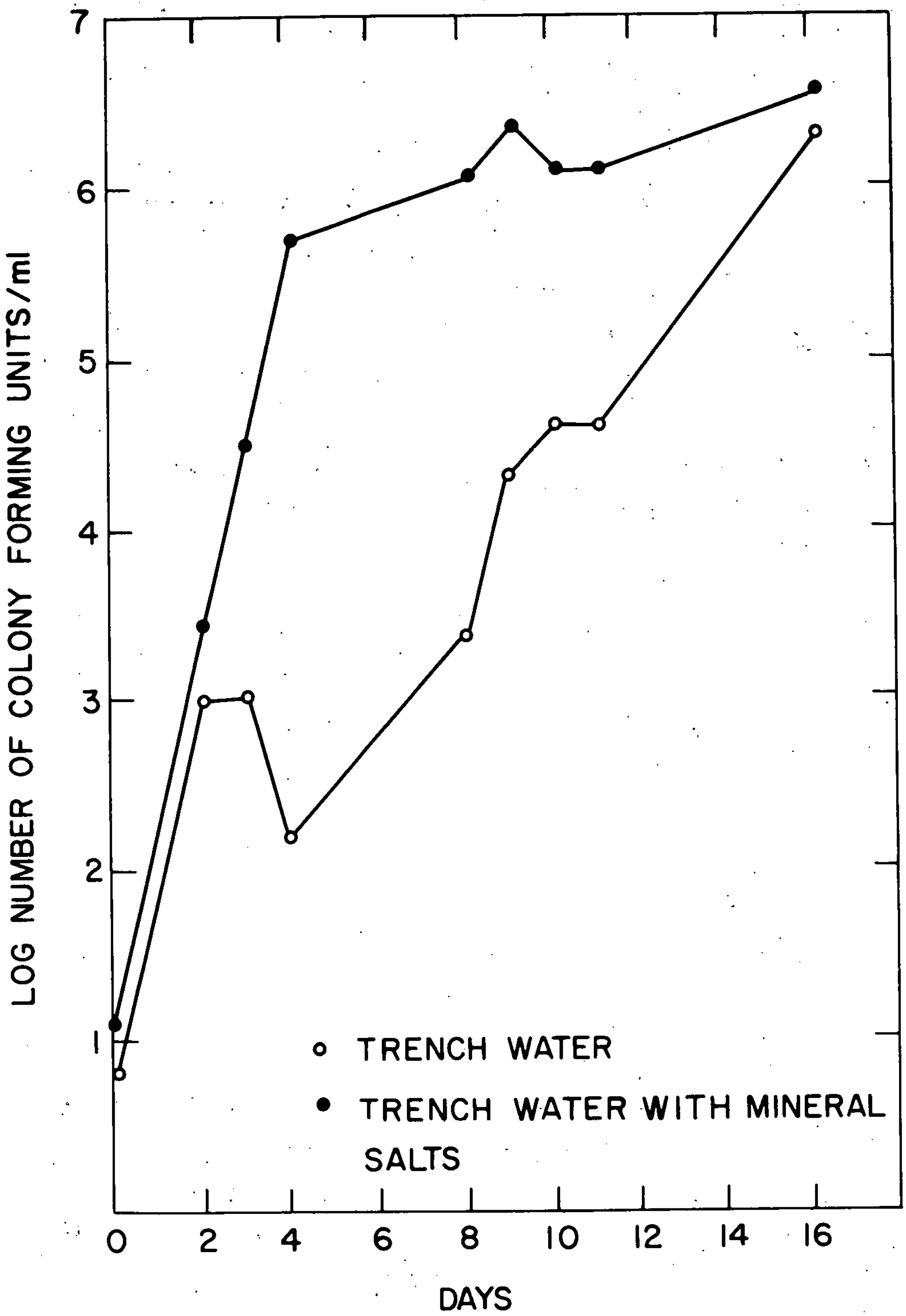

Figure 6. Anaerobic Growth of Mixed Culture Bacteria in West Valley Trench 5 Water Sample.

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1 Philip M. Garrett, Atomic Industrial Forum, Inc., 7101 Wisconsin Avenue, Washington, D.C. 20014

1 Stephen Lamanna, Babcock & Wilcox, P.O. Box 1260, Lynchburg, Virginia 24505

1 Danny R. Jackson, Ecology & Ecosystems Analysis Section, Battelle Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201

1 A.M. Platt, Battelle Pacific Northwest Laboratories, Richland, Washington 99352

1 Lloyd J. Andrews, Chem-Nuclear Systems, Inc., P.O. Box 1866, Bellevue, Washington 98009

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1 John C. Rodgers, Health Division, Los Alamos Scientific Laboratory, P.O. Box 1663, Mail Stop 690, Los Alamos, New Mexico 87545

8 Gerald Parker, Assistant to the Commissioner of Radiological Health, Massachusetts Department of Health, 80 Bryston Street (Room 835), Boston, Massachusetts 02116

1 John Pomeroy, National Academy of Sciences, Committee of Radioactive Waste Management, National Research Council, 2101 Constitution Avenue, Washington, D.C. 20418

1 Terry R. Lash, Natural Resources Defense Council Inc., 2345 Yale Street, Palo Alto, California 94306

1 John May, Newport News Industrial Corporation, 230 41st Street, Newport News, Virginia 23607

1 N. Richard Werthamer, New York State Energy Research and Development Authority, Room 2425, 230 Park Avenue, New York, New York 10017

1 J. Pferd, New York State Geological Survey, Education Building Annex, Room 973, Albany, New York 12224

1 Charles F. Eason, Director of Governmental Affairs, Nuclear Engineering Company, Inc., P.O. Box 7246, Louisville, Kentucky 40207

1 J.P. Duckworth, Nuclear Fuel Services, Inc., P.O. Box 124, West Valley, New York 14171

1 C.R. Moore, Nuclear Fuel Services, Inc., Suite 600, 600 Executive Boulevard, Rockville, Maryland 20852

1 W. Rodgers, Nuclear Safety Associates, 5101 River Road, Bethesda, Maryland 20016

1 T.A. Russell, NUS Corporation, 4 Research Place, Rockville, Maryland 20850

1 R. Blanco, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37830

1 Tsuneo Tamura, Environmental Sciences Division, Oak Ridge National Laboratory, Building 3504, P.O. Box X, Oak Ridge, Tennessee 37830

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2 D. Smith, Director, Technology Assessment Division (AW-459), Office of Radiation Programs, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C. 20460

1 Frederick Au, U.S. Environmental Protection Agency, Las Vegas, Nevada 89104

1 Richard Blanchard, U.S. Environmental Protection Agency, Eastern Environmental Radiation Facility, P.O. Box 3009, Montgomery, Alabama 36109

1 T. Fields, Hazardous Waste Management Division (AW-465), U.S. Environmental Protection Agency, 401 M Street S.W., Washington, D.C. 20460

1 W. Holcomb, Technology Assessment Division (AW-459), Office of Radiation Programs, U.S. Environmental Protection Agency, 401 M Street S.W., Washington, D.C. 20460

1 Bruce Mann, Radiation Program, U.S. Environmental Protection Agency, P.O. Box 15027, Las Vegas, Nevada 89114

1 G.L. Meyer, Office of Radiation Programs, U.S. Environmental Protection Agency, 401 M Street S.W., Washington, D.C. 20460

1 Charles Phillips, Eastern Environmental Radiation Facility, U.S. Environmental Protection Agency, P.O. Box 3009, Montgomery, Alabama 36109

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