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Potential Microbial Impact on Transuranic Wastes Under Conditions Expected in the Waste Isolation Pilot Plant (WIPP)

December 15, 1978—March 15, 1979

LOS ALAMOS SCIENTIFIC LABORATORY
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POTENTIAL MICROBIAL IMPACT ON TRANSURANIC WASTES UNDER CONDITIONS EXPECTED IN THE WASTE ISOLATION PILOT PLANT (WIPP)

December 15, 1978—March 15, 1979

by

Benjamin J. Barnhart, Evelyn W. Campbell, Julia M. Hardin, Eleuterio Martinez, Douglas E. Caldwell, and Richard Hallett

ABSTRACT

This progress report for the second quarter of FY 1979 relates a number of achievements in both the microbiological and chemical aspects of the project. The number of microflora in burial site soil cores to a depth of 120 cm decreased with depth. The microflora on flammable (organic) waste contaminated with Pu^{239} were enumerated and bacteria were classed as bacilli or cocci and by reaction to the gram stain. Microflora were also enumerated on a sample of LASL-simulated organic waste. Bacteria and actinomycetes isolated from burial site soil samples containing beta and gamma activity had more radioresistant isolates in the population than microorganisms from soil lacking detectable radioactivity. The alkylation of europium and thorium using methylcobalamine as a methyl donor was attempted but no organometallic product other than cobalt-cobalamine could be detected chemically or by mass spectrometry. A statistical analysis of the rates of degradation of europium tartarate and europium citrate chelates is presented.

I. INTRODUCTION

The low-level transuranic (TRU) radioactive waste that will be deposited in the WIPP will include celluloses, rubbers, plastics, and other organics; radionuclides; and actinides. This waste material may also include residual chelating chemicals from decontamination operations and chelating agents synthesized by the microorganisms as they metabolize certain organic waste constituents. The microorganisms living on waste materials will metabolize the organics and the microbial metabolites may react with TRU elements to enhance mobilities if leaching occurs.

Published information about microbial activity in radioactive wastes is scarce, and we must determine the potential for microbial interaction with radioactive elements. Our project includes investigations in several important areas of potential microbial influence on radionuclides. We are enumerating and partially characterizing the microflora in shallow burial low-level TRU waste; assessing the possibility

for elevated frequencies of radiation-resistant microorganisms in radioactive burial site soil; investigating microbial production and degradation of chelated elements, microbially caused changes in oxidation states, microbially related alkylation of TRU, and the production of gases as a result of bacterial decomposition of organic waste.

II. ASSESSMENT OF MICROFLORA IN LASL TRU BURIAL SITE SOIL AND ON TRU WASTE FROM STEEL DRUMS (LASL)

A. Background

We are using the standardized procedures described in the first quarterly report[1] to enumerate the microflora from LASL TRU shallow burial site, TA-54, Area C. These use the dilution agar-plate technique[2] to estimate colony forming units (CFU) per gram of soil or organic waste material and the most probable number (MPN) method,[3] which permits a broth culture turbidimetric estimation of population density without actual count of single cells or colonies.

B. Enumeration of Microflora at Various Depths in LASL TRU Burial Site Soil

Core samples were obtained from the dirt overburden of a LASL burial trench. A split-spoon sampler (7.5-cm diam) was hammered to 120 cm in two successive 60-cm corings. After each coring the spoon was carefully opened (Fig. 1) and each 10-cm length was packaged in zip-lock-type polyethylene bags, labeled, quick-frozen in dry ice-ethanol, and stored in a dry-ice chest in the field; then they were stored at −30°C in the laboratory for subsequent testing. Table I shows that the number of culturable microorganisms decreases with soil-sample depth. Although the numbers are larger for the MPN method, the same trend is obvious for this and the CFU technique. It was casually observed that the relative proportion of actinomycetes in the samples increased as the numbers of bacteria decreased with depth, and at 100-110 cm few fungi could be detected. Nevertheless, more than 10 000 microorganisms per gram of soil were found in the 100- to 110-cm sample, which should correspond to a depth just above the top layer of buried low-level radioactive waste.

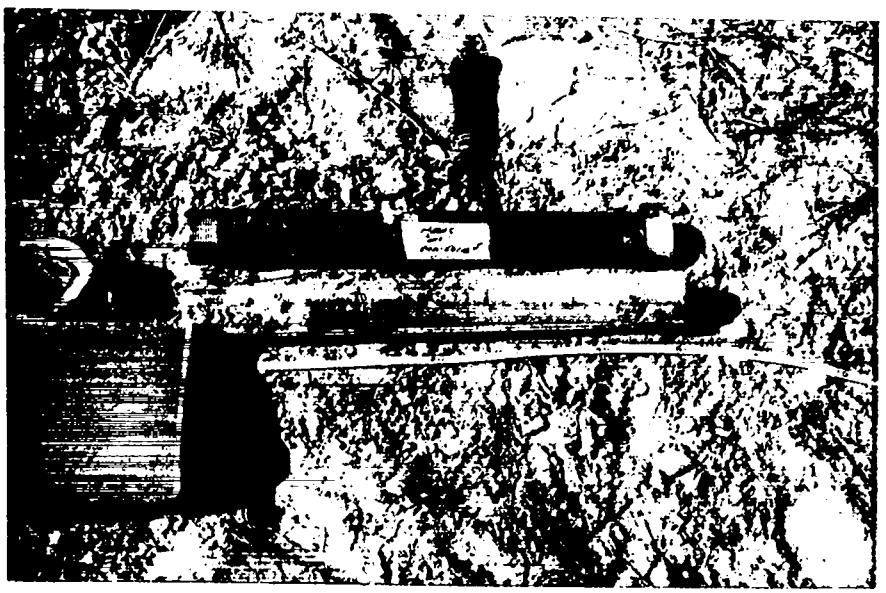

Fig. 1.
A 7.5-cm (3-in.) core of overburden soil from TRU shallow burial site.

TABLE I

ENUMERATION OF MICROFLORA IN LASL TRU SHALLOW BURIAL SITE (TA-54, AREA C) SOIL

Depth (cm)	Radioactivity[a] in Soil (γ, β)	CFU/gm Soil			MPN/gm Soil		
		Aerobic	Anaerobic	Fungi	Aerobic	Anaerobic	Fungi
5-15	ND	1.6×10^6	2.5×10^3	3.5×10^3	2.3×10^6	3.3×10^6	4.9×10^4
40-50	4×10^4, 6×10^4	1.2×10^5	1.4×10^2	5.8×10^2	4.9×10^5	1.1×10^6	1.1×10^5
100-110	2×10^3, 7×10^3	7.1×10^4	---	6.5×10^1	3.3×10^4	3.3×10^4	2.3×10^2

[a]Disintegrations per minute per gram of soil.

As shown in Table I, beta and gamma activities were greatest at a depth of 40-50 cm and lowest by an order of magnitude at 100-110 cm. The reason for this elevated radioactivity at the intermediate depth is not clear but may be due to decontamination of a piece of equipment in the field at some time in the past followed by subsequent backfilling with soil. Other reasons could be cited, such as vertical migration of radionuclides, but until additional cores are obtained and analyzed, this phenomenon will not be discussed further. The gamma emitter was tentatively identified by the radiochemistry section of LASL Group CMB-1 as Cs^{137}; the beta emitters were not identified. There was no detectable alpha activity.

Although the level of radioactivity was greatest in the 40- to 50-cm sample, the frequency of microorganisms was clearly not the lowest, so these levels of radioactivity apparently do not exert any sterilizing effect on the soil.

C. Enumeration of Microflora in Pu^{239}-Contaminated Waste From a Steel Burial Drum

Some Pu^{239}-contaminated waste materials were retrieved from a low-level steel drum in which combustibles were recently deposited by the workers in LASL Group CMB-1. The waste, put in three bags, was from work performed in gloveboxes in a plutonium work area. The bags were opened in a glovebox in that same work area, but the working surface was covered with clean nonradioactive aluminum foil. Each piece of waste material was handled with sterile forceps, pieces were cut from them with sterile scissors, and they were placed aseptically into sterile Erlenmeyer flasks containing 100 mℓ of Difco trypticase soy broth and a plastic-coated bar magnet. Each flask was stirred on a magnetic stirrer for 15 min at room temperature, after which a 5-mℓ aliquot was withdrawn and diluted 10-fold, and serially thereafter, to a 1×10^6 dilution factor in trypticase soy broth. After 14 days incubation at 28°C, the tubes were scored and the MPN was calculated.[8]

Table II shows the MPN for aerobic microorganisms and fungi. The anaerobic cultures were contaminated, which caused turbid growth for all dilutions as well as for uninoculated controls and so they were discarded. This type of assay will be repeated using a standard weight of each material sampled. Isolated microorganisms will also be tested for gas production in a glucose medium.

D. Enumeration of the Microflora in the Simulated TRU Waste Used for Gas Production and Chelate Degradation Studies at UNM

The formula for simulated TRU waste, which contains celluloses, rubbers, and plastics, can be found in Ref. 1. The eight components were cut into small pieces by hand and weighed to allow 75 to 240 mg for each component for a total of 1.0 g. We added 9.0 mℓ of Difco trypticase soy broth to the 1.0 g of this material, and the mixture was stirred on a magnetic stirrer for 15 min. Aliquots were serially diluted and incubated for 14 days at 28°C. An aliquot of the

TABLE II

MICROFLORA IN PU^{238}-CONTAMINATED WASTE FROM CMB-1

Waste Material	MPN[a] Aerobic	MPN[a] Fungi	Microflora on Solid Medium[b]
Wood chips	7.0×10^1	ND	Gram +, spore-forming rods
Kleenex box (end panel)	1.1×10^2	ND	Gram +, spore-forming rods
PVC glove (2 figures)	9.5×10^5	4.6×10^1	Gram + cocci
Tissue (1)	3.3×10^1	2×10^1	Gram +, rods
Cotton gauze (2 cm sq)	3.3×10^1	ND	No growth

[a]MPN estimate in Difco trypticase soy broth at 28°C for 14 days.

[b]An aliquot of the initial suspension (approximately a 10^1 dilution) was streaked on trypticase soy agar plates and incubated at 28°C. Isolated colonies were picked and the microflora were characterized.

TABLE III

MICROFLORAL CONTENT OF LASL SIMULATED WASTE USED FOR GAS EVOLUTION AND CHELATE DEGRADATION STUDIES AT UNM

MPN: 0.79×10^5 per gm

Characteristics: 2 different (colony morphology), gram positive, aerobic, spore-forming rods

original mixture was streaked on trypticase soy agar and on mycophil agar.

Table III shows that only 2-g-positive spore-forming bacilli were detected on the aerobically incubated petri dishes. These bacteria are the common soil and dust-borne bacteria found in most laboratories and will certainly be present in waste destined for the WIPP.

III. RADIOBIOLOGY OF LASL TRU WASTE (LASL)

Background

Radiation-resistant microorganisms have been induced by ionizing radiations in the laboratory[4] and in field conditions in which medical products were sterilized by radiation.[5] Selective enrichment of microbial populations for specific advantageous characteristics normally involves long-term radiation exposures at low dose rates that are minimally lethal to the microorganisms.

The soil microflora from the shallow burial site soil samples, which have low levels of beta and gamma emitters, provided a long-term low-dose-rate experiment. To determine whether an elevated frequency of radiation-resistant bacteria live in soil samples with detectable radioactivity, we suspended 10 g of soil from depths of 5-15 cm, 40-50 cm, and 100-110 cm in 95 mℓ of trypticase soy broth and stirred for 15 min on magnetic stirrers. Aliquots were serially diluted, plated on the surface of trypticase soy agar, and incubated for 7 days at 28°C. Isolated

TABLE IV

RELATIVE X-RAY SENSITIVITIES OF SOIL (40-50 cm, 4 to 6 x 10^4 dpm) MICROBIAL ISOLATES

Survival Key

10 — Most Resistant

9

8

7

6 — Least Resistant

Isolate No.	Relative Survival				
	0 krads	13.5 krads	27 krads	40.5 krads	54 krads
1	10	10	9	8	7
2	10	10	10	8	8-9
3	8	8	7	8	8
4	10	10	10	7	7
5	8	8	8	8	8
6	8	8	8	7	6
7	10	10	10	8	8-9
8	9	9	9	9	10
9	10	10	10	9	9-10
10	9	9	9	6	7
11	10	10	10	9	10
12	10	10	10	10	9-10
13	10	10	9	6	6-7
14	9	9	9	9	9
15	10	10	10	10	10
16	8	8	8	8	7
17	7	7	7	7	8
18	9	9	9	9	9
19	8	7	8	9	8
B. Subtilis	10	10	9	8	7-8

colonies were stabbed in the center with sterile throat swab sticks and inoculated into trypticase soy broth. A sterile wire loop was used to inoculate each of the turbid cultures onto five nutrient agar plates, and after 20 min of drying, the plates were irradiated with 250 kVP x rays at a dose rate of 900 rads per minute for total doses of 0, 13.5, 27, 40.5, and 54 krads. *Bacillus subtilis* (ATCC #6051) was included on each plate as a radiation sensitivity reference standard.

Table IV lists results of an experiment in which isolates from the 40- to 50-cm soil sample were irradiated. A survival key was formulated to permit at least a quasi-quantitative evaluation of survival of bacteria in the looped areas of each irradiated plate relative to the control unirradiated plates. An isolate was considered resistant if its survival did not decrease more than one step on the survival key. Similar evaluations were performed on the 5- to 15-cm sample, which lacked detectable radioactivity.

TABLE V

FREQUENCIES OF RADIORESISTANT MICROFLORA IN LASL TRU SHALLOW BURIAL SITE (TA-54, AREA C) SOIL

Depth (cm)	Radioactivity[a] in Soil (γ, β)	Total No. Isolates	X-ray Resistant[b] Phenotypes (No.)	(%)
5-15	ND	12	3	25
40-50	4×10^4, 6×10^4	19	13	68

[a]Disintegrations per minute per gram of soil.

[b]54 000 rads delivered at dose rate of 900 rads/min.

Table V provides a summation of relative sensitivities of isolates from 5-15 cm, and 40-50 cm. The soil sample exhibiting nanocurie levels of radioactivity has higher frequencies of radiation-resistant bacteria. Isolates with radiation-resistant phenotypes are stored at −80°C for future biochemical and genetic characterization to determine the mechanisms of radioresistance.

IV. POTENTIAL ALKYLATION OF TRU ELEMENTS (LASL)

A. Background

Microorganisms are responsible for the alkylation of many elements,[6] including the metals Hg, Sn, Pd, Pt, Au, and Tl, and the metalloids As, Se, Te, and S. When methylated, these elements become volatile and they become less polar, which increases their solubility in the lipids of biological tissues. Methylation occurs under both aerobic and anaerobic conditions.[6,7] The best studied alkylation reaction for metals is the methylation of mercury, which, like other metals, is methylated by the chemical transfer of methyl groups from methylcobalamine.[8] We methylated mercury using methylcobalamine (CH_3B12) as methyl donor.[1] This type of reaction is now being used to attempt the methylation of the lanthanide element europium, and the actinide elements thorium and plutonium.

B. Attempted Methylation of Transuranic Elements

As reported previously,[1] we could not detect a methylated form of europium using a variety of reaction conditions and detection methods. However, the question remained whether the heavy elements of the TRU series could be alkylated by the methyl donor produced by microorganisms.

We reported previously[1] that $Th(No_3)_4 \cdot 4H_2O$ appeared to be methylated as indicated by the spectral shifts indicative of the demethylation of CH_3B12. We used $ThCl_4$ instead of the nitrate salt and found a similar suggestion for methylation based on spectral shifts in the absorbance of CH_3B12. However, mass spectrometry performed by W. D. Spall at LASL determined that no methylated metal was formed even though some demethylation and degradation of CH_3B12 occurred.

Direct chemical synthesis of methylthorium or plutonium has also been elusive to other investigators because of the extreme affinity of these actinides for water molecules. These elements in the +4 valence state undergo extensive hydrolysis between pH 2.8 and 7.5 because of their high ionic charge and relatively small radius of 0.90 nm.

Our attempts at methylating these heavy metals have been thwarted to some extent by the lack of their solubility above pH 3. Below this pH the actinides are soluble but the methyl donor CH_3B12 readily degrades. When we attempted to carry out

the reactions at a higher pH (6-7) using Tris or phosphate buffers to stabilize CH_3B12, the inorganic thorium precipitated. Plutonium was not incorporated into these experiments.

Experiments are in progress using the organic buffer hexamethylenetetramine $[(CH_2)_6N_4]$ with a pK of 5.0. A 0.2 M solution of $(CH_2)_6N_4$ allows us to run the methylation reaction at pH 6.4 without apparent precipitation of thorium or breakdown of CH_3B12.

The pathways for the biologically mediated methylation of thorium or plutonium are not known. It is apparent that the methylation of actinide elements is difficult and probably requires specific physical, chemical, and biological conditions. We will continue to try to methylate these elements using microbial cultures and perhaps laboratory sod beds. Also, we may set up some transalkylation reactions to determine whether the methyl group can be transferred from other metals to the actinides in a reaction similar to that described for the methylation of tellurium in the presence of excess selenium.[9]

V. CHELATE DEGRADATION AND GAS GENERATION (UNM)

A. Background and Methods

The microbiology of chelate degradation and gas generation as well as the methods developed for studies under projected WIPP conditions were reported previously.[1,10] The IBM Statistical Analysis System (SAS) is being used to tabulate the data and to compute the amounts and rates of chelate degradation or gas generation. As data points are accumulated, graphs will also be generated by computer.

The use of gas chromatography in the analysis of CO_2 evolution was replaced with a more precise titration method by substituting 0.1 M $Ba(OH)_2$ for NaOH as the CO_2 absorbant. As CO_2 is absorbed, the $Ba(OH)_2$ provides an immediate indication of carbonate ($BaCO_3$) precipitation. The remaining $Ba(OH)_2$ was titrated using 0.1 M potassium acid phthalate. Phenolphthalin was used as the titrating indicator.

B. Results

The data for the chelate degradation studies are presented in Tables VI and VII. The data for the gas generation studies are available, but the printout program is not complete. For the statistical analysis loop of the program to operate, at least three usable data points must be supplied. Only two observed data points are available for entry into the program for the ethylenediamine tetraacetic acid (EDTA) and nitrilotriacetate (NTA) because they degrade slowly and are sampled monthly.

C. Scheduling of Project Objectives

All of the studies are on schedule. A set of 40°C chelate degradation studies have been prepared, and in addition, soil perfusion columns are being studied to determine whether the degradation rates of NTA and EDTA chelating agents can be accelerated by increasing the number of microorganisms and varying the physiochemical conditions.

The experiments that remain to be initiated at the University of New Mexico (UNM) are as follows.

(1) Bioconcentration: changes in radionuclide oxidation state due to microbial metabolism. Uranium and thorium may be studied at UNM, but plutonium will be studied at LASL. (Procedure V-D: June 1—June 30, 1978.[10])

(2) Bioconcentration: microbial degradation of radionuclide chelates. Studies of europium Desferol chelate degradation will not be performed unless positive results are obtained in the solubilization experiments. (Procedure V-H; June 1—June 30, 1978.[10]) This procedure involves the solubilization of plutonium by Desferol.

(3) Mobilization-Volatilization: alkylation reactions were negative for europium and thorium, but positive for mercury. Attempts to alkylate uranium and plutonium via the microbial pathway have yet to be made. (Procedure V-G; June 1—June 30, 1978.[10])

TABLE VI

DEGRADATION OF EUROPIUM TARTARATE CHELATE

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of Degradation (%/day)	Equation for % Degradation	R^2 Value[b]
5	25	aerobic	91% H_2O	0.0885	D = 0.0885(IP) + 32.44[a]	0.674
3	70	aerobic	91% H_2O	0.0803	D = 0.0803(IP) + 35.50	0.984
5	25	aerobic	1% H_2O	0.0117	D = 0.0117(IP) − 1.647	0.910
3	70	aerobic	1% H_2O	0.1320	D = 0.1320(IP) + 2.201	0.958
4	25	aerobic	brine	0.0455	D = 0.0455(IP) + 5.500	0.911
4	25	aerobic	nutrient	0.0368	D = 0.0368(IP) + 32.24	0.928
5	25	aerobic	91% H_2O sterile control	0.0219	D = 0.0219(IP) + 24.85	0.865
3	70	aerobic	91% H_2O sterile control	0.4016	D = 0.4016(IP) + 49.08	0.652
5	25	aerobic	brine sterile control	0.0198	D = 0.0198(IP) + 17.47	0.874
3	70	aerobic	brine sterile control	0.3783	D = 0.3783(IP) + 27.03	0.687
5	25	aerobic	nutrient sterile control	0.0323	D = 0.0323(IP) + 26.58	0.827
5	25	anaerobic	91% H_2O	0.0927	D = 0.0927(IP) + 50.39	0.664
3	70	anaerobic	91% H_2O	0.2262	D = 0.2262(IP) + 47.29	0.879
5	25	anaerobic	brine	0.0058	D = 0.0058(IP) + 3.548	0.943
5	25	anaerobic	nutrient	0.0608	D = 0.0608(IP) + 27.80	0.742
3	70	anaerobic	nutrient	0.4120	D = 0.4120(IP) + 52.37	0.637
5	25	anaerobic	91% H_2O sterile control	0.0608	D = 0.0608(IP) + 27.80	0.742
3	70	anaerobic	91% H_2O sterile control	0.4120	D = 0.4120(IP) + 52.37	0.637
5	25	anaerobic	brine sterile control	0.0163	D = 0.0163(IP) + 15.96	0.889
3	70	anaerobic	brine sterile control	0.1421	D = 0.1421(IP) + 28.33	0.951
4	25	anaerobic	nutrient sterile control	0.1117	D = 0.1117(IP) + 19.86	0.789
3	70	anaerobic	nutrient sterile control	0.4097	D = 0.4097(IP) + 58.68	0.640

[a]Percent degradation after incubation period (IP, in days).

[b]Coefficient of determination for linear regression analysis, i.e., proportion of total variation explained by regression.

TABLE VII

DEGRADATION OF EUROPIUM CITRATE CHELATE

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of Degradation (%/day)	Equation for % Degradation	R^2 Value[b]
4	25	aerobic	91% H_2O	0.2345	D = 0.2345(IP) + 33.04[a]	0.586
3	70	aerobic	91% H_2O	0.1109	D = 0.1109(IP) −0.8260	0.970
4	25	aerobic	1% H_2O	0.1816	D = 0.1816(IP) + 2.236	0.669
3	70	aerobic	1% H_2O	0.0643	D = 0.0643(IP) + 1.024	0.989
4	25	aerobic	salt brine	0.0434	D = 0.0434(IP) + 1.012	0.915
3	70	aerobic	salt brine	0.5011	D = 0.5011(IP) + 7.975	0.498
4	25	aerobic	nutrient solution	0.1920	D = 0.1920(IP) + 31.42	0.653
3	70	aerobic	nutrient solution	0.0844	D = 0.0844(IP) + 7.659	0.982
4	25	aerobic	91% H_2O sterile control	0.1514	D = 0.1514(IP) + 6.486	0.720
3	70	aerobic	91% H_2O sterile control	0.0818	D = 0.0818(IP) + 7.635	0.984
4	25	aerobic	salt brine sterile control	0.1252	D = 0.1252(IP) + 4.770	0.765
3	70	aerobic	salt brine sterile control	0.4575	D = 0.4575(IP) + 5.793	0.566
4	25	aerobic	nutrient solution sterile control	0.2081	D = 0.2081(IP) + 11.53	0.627
4	70	aerobic	nutrient solution sterile control	0.0080	D = 0.0080(IP) + 7.048	0.984
4	25	anaerobic	91% H_2O	0.2204	D = 0.2204(IP) + 35.67	0.608
4	70	anaerobic	91% H_2O	0.0030	D = 0.0030(IP) + 5.425	0.993
4	25	anaerobic	1% H_2O	0.0144	D = 0.0144(IP) + 0.830	0.971
3	70	anaerobic	1% H_2O	0.0083	D = 0.0083(IP) + 0.5490	0.999
4	25	anaerobic	salt brine	0.0414	D = 0.0414(IP) + 1.072	0.919
3	70	anaerobic	salt brine	0.5080	D = 0.5080(IP) + 6.395	0.488
4	25	anaerobic	nutrient solution	0.2708	D = 0.2708(IP) + 33.64	0.532
3	70	anaerobic	nutrient solution	0.4550	D = 0.4550(IP) + 9.672	0.570
4	25	anaerobic	91% H_2O sterile control	0.1237	D = 0.1237(IP) + 6.968	0.768
3	70	anaerobic	91% H_2O sterile control	0.1763	D = 0.1763(IP) + 10.45	0.925
4	25	anaerobic	salt brine sterile control	0.1598	D = 0.1598(IP) + 4.721	0.705
3	70	anaerobic	salt brine sterile control	0.4922	D = 0.4922(IP) + 6.125	0.512
4	25	anaerobic	nutrient solution sterile control	0.2777	D = 0.2777(IP) + 8.295	0.521
3	70	anaerobic	nutrient solution sterile control	0.0666	D = 0.0666(IP) + 6.591	0.989

[a]Percent degradation after incubation period (IP, in days).

[b]Coefficient of determination for linear regression analysis, i.e., proportion of total variation explained by regression.

VI. MILESTONES FOR THE MICROBIOLOGICAL STUDIES (WIPP R&D PROGRAM) BY QUARTERS (LASL, UNM)

	FY 78				FY 79				FY 80			
	-	-	-	4	1	2	3	4	1	2	3	4
Quarterly reports				●	●	●	○	○	○	○	○	○
Annual reports				●				○				○
Literature search and update				●	●	●	○	○	○	○	○	○
Capital equipment ordered				●	●				○			
Laboratory expendables ordered				●	●	●	○	○	○	○	○	○
Enumeration and identification of microflora in LASL TRU burial site soil (LASL)				●	●	●	○	○				
Enumeration and identification of microflora in metallic and wood LASL TRU waste containers (LASL)						●	○	○				
Identification of saltbed microflora (UNM)							○	○				
Microbial gas generation (UNM)					●	●	○	○	○	○	○	○
Abiotic reactions (LASL)												
Alkylation of heavy metals and actinides				●	●	●	○	○				
Chelation of heavy metals and actinides						●	○	○				
Microbial degradation of chelates (UNM)					●	●	○	○		○	○	
Microbial interaction with radionuclides (LASL):												
Alkylation/Volatilization						○	○	○	○	○	○	○
Chelation/Solubilization							○	○	○	○	○	○
Chelate degradation							○	○	○	○	○	○
Analysis and interpretation of data[a] (LASL, UNM)					●	●	○	○	○	○	○	○
Final conclusions and recommendations												○

● - On schedule
○ - Planned

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[a]The ongoing work may identify additional topics of research that may be included; other experiments may be deleted by mutual consent.

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