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

March 15—June 15, 1979

University of California

LOS ALAMOS SCIENTIFIC LABORATORY

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

March 15—June 15, 1979

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

March 15—June 15, 1979

by

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ABSTRACT

During the third quarter of FY 1979 we initiated several new projects and expanded others. We show, for the first time, that the chelate Desferol, which is representative of microbially produced polyhydroxamate chelates, greatly enhances the dissolution of plutonium from insoluble $^{239}PuO_2$. Radiobiological studies showed that bacterial isolates from Los Alamos Scientific Laboratory transuranic burial site soil exhibit levels of radioresistance intermediate to that of *B. subtilis* and *M. radiodurans*. Also included in this report are the first tables of data from the CO_2 gas generation studies with the corresponding modeling equations derived from the linear regression statistical analysis procedure. The data for the chelate degradation studies are updated and include data for EDTA chelate. Results of the experiment to enumerate and identify the microorganisms in the salt beds of the WIPP facility are reported as negative, as no microbial growth was detected from three salt samples.

I. INTRODUCTION

During this quarter we continued to enumerate microflora in the Los Alamos Scientific Laboratory (LASL) transuranic (TRU) burial site soil, to correlate frequencies of radioresistant bacteria with levels of radioactivity in the soil, to alkylate elements of the lanthanide and actinide series, including preparations to methylate ^{239}Pu, and to determine the production of gases caused by bacterial decomposition of organic waste. New projects are to determine the possible solubilization of plutonium by chelates of microbial origin and to quantitate the radioresistance of bacteria isolated from the TRU burial site.

II. ENUMERATION OF MICROFLORA IN LASL TRU BURIAL SITE SOIL (LASL)

A. Background

We have used standardized procedures[1] to enumerate culturable bacteria and fungi from LASL

TRU burial site soil. Assays have been repeated on the soil samples to confirm our previous results[1] and to obtain additional data for statistical calculations. The summation of these results will appear in the annual report.

A very time consuming effort was also made to obtain direct microbial counts in the soil using fluorescent microscopy.[2] The fluorescent stain fluoresceine isothiocynate (FITC) was used on soil samples immediately or after incubation to permit spore germination and the possible formation of microcolonies. The stained samples were examined under a Zeiss Epi-fluorescence microscope using an excitation wavelength range of 450-490 nm and a selective FITC filter combination.

B. Estimates of Colony Forming Units (CFU) and Most Probable Number (MPN) Estimates

These standardized procedures, and the data obtained, were described previously.[1,3] Recent data confirm our previous estimates of culturable microorganisms in core samples taken from a shallow burial site (Table I of Ref. 3).

C. Direct Enumeration of Microflora

Soil samples were prepared as previously described for CFU and MPN estimates.[1] For microscopic examination, 0.01 mℓ of 100X dilution of soil was spread over a 1.0-cm^2 well on a microscope slide. The preparations were air-dried and slightly heat-fixed. We also prepared some slides with soil amended by the addition of an aliquot of a culture of gram-positive bacilli. Fixed slides were stained using the procedure described by Bubiuk and Paul.[2] The staining solution was 10 mg FITC dissolved in 2.5 mℓ of 0.5 M $NaHCO_3$ (pH 9.6), 11 mℓ of 0.85% NaCl, and 11 mℓ of KH_2PO_4 buffer (pH 7.2).

Slides were stained for 3 min at room temperature, washed in 0.5 M $NaHCO_3$ buffer (pH 9.6) for 10 min, and then in 5% $Na_4P_2O_7$ for 2 min. The stained preparations were immediately mounted in glycerol (pH 9.6) and observed under the Zeiss Epi-fluorescence microscope.

Bacteria were easily seen on slide preparations containing amended soil and background staining was negligible, but no fluorescent microorganisms were seen in the unamended soil. This is probably because bacterial spores are impermeable to the stain, and because of the small sample size, which limited the numbers of vegetative forms.

Since spores do not stain well, 0.01 mℓ of a 100X dilution of soil was embedded in 1.5% Difco nutrient agar and incubated in a moist sterile chamber at 28°C for 2, 18, 22, 42, and 66 hours. The slides were fixed and stained as described above. As a control, 1.5% Difco nutrient agar was incubated without soil. We could not determine whether spores had germinated and formed microcolonies of vegetative forms because even the control slide fluoresced brightly. We concluded that the protein in nutrient agar stained with the FITC and could not be destained by the bicarbonate-pyrophosphate wash.

We conclude that although direct microbial enumeration by fluorescent microscopy works well for water samples, the procedure is not readily applicable to soil samples.

III. RADIOBIOLOGY OF LASL TRU BURIAL SITE SOIL (LASL)

A. Background

Soil samples from a depth of 40 to 50 cm containing 20 to 30 nCi of beta and gamma radioactivity had almost a threefold higher frequency of radioresistant bacteria than nonradioactive soil.[3] Radioresistant bacteria were isolated and their resistance levels to gamma radiation are reported in Table I. Stock cultures of 15 isolates are maintained at −80°C for future experiments designed to determine mechanisms of radioresistance.

B. Gamma-Radiation-Dose Response of Radioresistant Isolates

Ten isolates from the 40- to 50-cm soil sample containing 20 to 30 nCi of radioactivity and shown to survive 54 krads of x irradiation[3] were inoculated into broth with the ingredients of Difco Actimonycete Isolation Agar minus the agar, and incubated overnight with shaking at 28°C. The next morning the cultures were in the logarithmic phase of growth as determined by turbidometric readings

TABLE I

GAMMA-RADIATION-DOSE RESPONSE OF RADIORESISTANT SOIL ISOLATES

Radiation Dose (krads)[a]	Controls[b]		Radioresistant Isolates[c]									
	B. subtilis	*M. radiodurans*	59	63	57	60	34	54	48	56	1	6
0	187	556	412	211	96	128	57	34	109	0	78	308
25	5	493	416	237	135	167	58	20	0	91	78	121
50	0	503	235	13	66	64	4	5	7	38	6	0
75	0	381	5	0	6	2	0	0	1	2	1	0
100	0	303	0	0	0	0	0	0	0	0	0	0

[a]Gamma radiation emitted from a Co^{60} source at a calculated dose rate of 3058 rads/min.

[b]CFU of *Bacillus subtilis* (ATCC #6051) and *Micrococcus radiodurans* (ATCC #13939).

[c]CFU of bacteria isolated from a 40- to 50-cm-deep core sample taken from a LASL TRU shallow burial site and containing 20 to 40 nCi of beta and gamma activity.[3]

in a Coleman Jr. Colorimeter at a 600-nm wavelength. Each culture was diluted and pour-plated into Difco Actimonycete Isolation Agar. *Bacillus subtilis* (ATCC #6051) was grown and plated for use as a control exhibiting "normal" radiation sensitivity and *Micrococcus radiodurans* (ATCC #13939) was used as a strongly radioresistant control. All petri dishes were stored for up to 2.5 hours at 4°C and removed as needed for exposure to gamma radiation.

In our previous radiobiological experiments we used 250 kVP x-rays at a dose rate of 900 rads/min. The higher doses used in the experiments described here, however, demanded that we use another source of ionizing radiation that does not overheat the components. We irradiated 10 isolates and the two controls simultaneously in the sample chamber of a Gammacell 220 irradiation unit emitting high-intensity gamma radiation from a ^{60}Co source at a calculated dose rate of 3058 rads/min; then the dishes were incubated at 28°C for 6-7 days.

Table I shows that the radioresistance of these soil isolates was intermediate to that of the two controls. It would be desirable to determine the relative radioresistances of isolates from soil containing considerably less and considerably more radioactivity than the sample we examined.

IV. POTENTIAL ALKYLATION OF TRU ELEMENTS (LASL)

A. Background

After methylating mercury using a reaction in which methyl groups are chemically transferred from methylcobalamine, a methyl donor of microbial origin,[4,5] we used a similar protocol to try to methylate the lanthanide element europium and the actinide element thorium.[3] Neither element could be methylated even when pH, ionic strength, temperature, and molar ratios of the reactants were varied.

B. Attempt to Methylate Plutonium

Several protocols are being prepared to try to methylate ^{239}Pu using methylcobalamine (CH_3B12) as methyl donor. One of the reactions will incorporate $^{239}PuO_2$, and another, a chelated (and therefore, solubilized) form of plutonium. Specific reaction conditions and experimental results will be presented in the next quarterly report.

V. SOLUBILIZATION OF PLUTONIUM BY A CHELATE OF MICROBIAL ORIGIN (LASL-UNM)

A. Background

Hydroxamate and polyhydroxamate chelating agents are produced by microorganisms during decomposition of organic materials.[6] TRU waste contains celluloses, rubbers, and plastics which can be metabolized by bacteria and fungi and various radionuclides that may become chelated by the microbially produced agents. Solubilization by natural chelates leading to mobilization of TRU elements is a possibility that should be assessed.

Desferol is a commercially available form of the microbially produced iron chelate ferrioxamine.[7] Desferol is not complexed to iron and so is free to bind other cations. We are using this chelate to determine whether ^{239}Pu can be solubilized by a compound that is representative of naturally occurring cation-binding agents.

B. Solubilization of $^{239}PuO_2$ in Desferol

In the initial experiment ~1 mg of solid $^{239}PuO_2$ was put into a dialysis tube, which also contained 15 mℓ of deionized water, 500 mg of Desferol, and blue dextran as a color indicator to detect possible membrane leakage. The loaded tube was then suspended in a cylinder containing 200 mℓ of deionized water and a glass-covered bar magnet. The cylinder was placed on a magnetic stirrer to ensure continuous agitation of the water around the membrane. However, soon after the start of the experiment, the tubing ruptured because of constant torque exerted by the stirring magnet, and $^{239}PuO_2$ and Desferol emptied into the 200-mℓ of water.

Nevertheless, we decided to continue the experiment by sampling aliquots from near the surface of the water and determining the quantities of plutonium present by scintillation counting. Our hypothesis was that $^{239}PuO_2$ with a density of 16-17.5 g/cm^3 (Ref. 8) should settle to the bottom of the cylinder and only chelated plutonium should be detected near the surface. However, radioactivity in the samples was erratic, and it was suggested that particles of $^{239}PuO_2$ are buoyed up by surface tension. The erratic radioactivity counts were eliminated by centrifuging the aliquots for 15 min at top speed in a clinical table-top centrifuge (approximately 1500xg). A consistent, small increase in counts over a 33-day period suggested chelate-mediated solubilization of plutonium.

A subsequent experiment was set up in which $^{239}PuO_2$ and Desferol were placed outside a collodion membrane in deionized water, with only water inside the membrane. Aliquots of 100 μℓ were removed from both inside and outside the membrane daily and radioactivity counts were obtained. There was a threefold increase in counts in samples taken from outside the membrane over a 20-day period (Table II).

TABLE II

DISSOLUTION OF $^{239}PuO_2$ IN DESFEROL

Sample Time (Days)	Net[a] CPM Per 100 mℓ: Inside Membrane Control[b]	Inside Membrane Expt'l[c]	Outside Membrane Control[b]	Outside Membrane Expt'l[c]
0	2.5	31		
5	5.0	41	219	1137
11	2.4	35	124	2234
20	0	36	187	3473

[a]Total count minus background of 28 counts/min.

[b]5.5 μg (14 326 cpm) of $^{239}PuO_2$ per 100 μℓ deionized water.

[c]5.5 μg (14 326 cpm) of $^{239}PuO_2$ and 1.79 μg Desferol per 100 μℓ deionized water.

Chromatographic separation of what may be chelated plutonium from plutonium ions is being pursued using Sephadex G10 gel, which permits fractionation of molecules according to size. Elution with 0.1N HCl in 10% ethanol resulted in two distinct peaks of radioactivity. Plutonium in the first peak was shown to be associated with Desferol by adding $FeCl_3$ to an aliquot of $^{239}PuO_2$ + Desferol before chromatographic separation. The mixture (yellow due to iron-chelate complex), was visible as it moved down the column and into the first peak of eluted radioactivity. Four gel-exclusion experiments have yielded similar results , which suggests that Pu IV ions are indeed complexed with Desferol.

VI. CARBON DIOXIDE GAS GENERATION STUDIES (UNM)

Results of the gas generation studies are given in Tables III-VI. The experimental vials of the various treatments have been sampled 3-5 times over a period of 92-112 days. The linear modeling procedure used to produce gas generation equations fits the data well as shown by the high R^2 values. Low R^2 values do not necessarily mean that the data points are inaccurate, but that polynomial regression modeling may be required to more precisely fit the equation to the experimental curve.

VII. CHELATE DEGRADATION STUDIES (UNM)

Results of europium chelate degradation studies are presented in Tables VII-IX. The methods used in these experiments are described in a previous quarterly report.[8] The degradation rates were obtained using linear regression. The values summarize all chelate degradation data collected.

VIII. IDENTIFICATION AND ENUMERATION OF MAJOR GROUPS OF MICRO-ORGANISMS IN WIPP SALT BEDS (UNM)

The methods for this experiment are outlined in Ref. 9. The exact procedure consisted of dipping a piece of salt ~10-mm diam in 70% ethanol, flaming, and then grinding it to a powder in a sterile mortar. The powder was streaked on HM-2 salt agar plates using a four-quadrant-streaking method to isolate possible colonies. Two other salt samples, called "white salt deposit" and "colored salt deposit," were similarly streaked. All samples were taken from cores at a depth of 1900-2100 feet at the WIPP site. Ten replicate plates from each of the three samples were made and incubated at 32°C. No growth appeared on any of the petri dishes after an incubation period of 90 days. Colonies of halobacteria usually require 20-30 days to form visible colonies.

TABLE III

CO_2 EVOLUTION FROM SUBSTRATE

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of CO_2 Production (mg/day/g)	Equation for the Amount of CO_2 Produced[a]	R^2
5	25	A	91% H_2O	0.00948	G = 0.00948(IP) + 0.357	0.86
5	25	A	91% H_2O Sterile	0.00565	G = 0.00565(IP) − 0.150	0.96
4	70	A	91% H_2O	0.0105	G = 0.0105(IP) + 0.437	0.92
4	70	A	91% H_2O Sterile	0.00325	G = 0.00325(IP) + 0.129	0.89
5	25	A	1% H_2O	0.00315	G = 0.00315(IP) + 0.139	0.68
4	70	A	1% H_2O	0.0131	G = 0.0131(IP) + 0.570	0.97
5	25	A	Brine	0.00378	G = 0.00378(IP) + 0.256	0.95
4	25	A	Brine Sterile	0.00484	G = 0.00484(IP) − 0.088	0.97
4	70	A	Brine	0.0180	G = 0.0180(IP) + 1.15	0.94
4	70	A	Brine Sterile	0.00526	G = 0.00526(IP) − 0.131	0.98
4	25	A	Nutrient	0.00840	G = 0.00840(IP) + 0.282	0.91
5	25	A	Nutrient Sterile	0.00123	G = 0.00123(IP) − 0.0204	0.91
3	70	A	Nutrient	0.0140	G = 0.00140(IP) + 0.317	0.99
4	70	A	Nutrient Sterile	0.00325	G = 0.00325(IP) + 0.1083	0.81
4	25	An	91% H_2O	0.0139	G = 0.0139(IP) + 0.486	0.94
4	25	An	91% H_2O Sterile	0.00403	G = 0.00403(IP) + 0.0485	0.99
4	70	An	91% H_2O	0.0180	G = 0.0180(IP) + 0.369	0.94
4	70	An	91% H_2O Sterile	0.00996	G = 0.00996(IP) + 0.080	0.82
4	25	An	1% H_2O	0.00470	G = 0.00470(IP) + 0.172	0.45
4	70	An	1% H_2O	0.0159	G = 0.0159(IP) + 0.64	0.90
4	25	An	Brine	0.00799	G = 0.00799(IP) + 0.0480	0.88
4	25	An	Brine Sterile	0.00512	G = 0.00512(IP) − 0.0668	0.96
4	70	An	Brine	0.0247	G = 0.0247(IP) + 0.146	0.93
4	70	An	Brine Sterile	0.0252	G = 0.0252(IP) + 0.353	0.60
4	25	An	Nutrient	0.0120	G = 0.0120(IP) − 0.0120	0.99
4	25	An	Nutrient Sterile	0.00356	G = 0.00356(IP) − 0.0823	0.89
4	70	An	Nutrient	0.0240	G = 0.0240(IP) + 0.457	0.96
4	70	An	Nutrient Sterile	0.00697	G = 0.00697(IP) − 0.0738	0.96

[a]mg of gas evolved from 1 g waste as a function of incubation period (IP) in days.

TABLE IV

CO_2 EVOLUTION FROM ASPHALT

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of CO_2 Production (mg/day/g Substrate)	Equation for the Amount of CO_2 Produced[a]	R^2
5	25	A	91% H_2O	0.00374	G = 0.00374(IP) − 0.00258	0.99
4	25	A	91% H_2O Sterile	0.000743	G = 0.000743(IP) − 0.0147	0.82
4	70	A	91% H_2O	0.000430	G = 0.000430(IP) + 0.00548	0.73
4	70	A	91% H_2O Sterile	0.000533	G = 0.000533(IP) + 0.00704	0.86
5	25	A	Brine	0.00308	G = 0.00308(IP) + 0.0890	0.81
4	25	A	Brine Sterile	0.000742	G = 0.000742(IP) − 0.0147	0.82
4	70	A	Brine	0.00792	G = 0.00792(IP) + 1.256	0.69
4	70	A	Brine Sterile	0.000498	G = 0.000498(IP) − 0.00369	0.80
5	25	A	Nutrient	0.00558	G = 0.00558(IP) + 0.02142	0.95
4	25	A	Nutrient Sterile	0.00229	G = 0.00229(IP) − 0.0303	0.87
4	70	A	Nutrient	0.00156	G = 0.00156(IP) + 0.236	0.83
4	70	A	Nutrient Sterile	0.000734	G = 0.000734(IP) + 0.032	0.50
5	25	A	1% H_2O	0.000753	G = 0.000753(IP) + 0.064	0.82
4	70	A	1% H_2O	0.000560	G = 0.000560(IP) + 0.129	0.81
4	25	An	91% H_2O	0.000863	G = 0.000863(IP) + 0.15	0.90
4	25	An	91% H_2O Sterile	0.000321	G = 0.000321(IP) − 0.00824	0.76
4	70	An	91% H_2O	0.00862	G = 0.00862(IP) + 0.364	0.86
4	70	An	91% H_2O Sterile	0.00696	G = 0.00696(IP) + 0.0699	0.83
4	25	An	1% H_2O	0.00460	G = 0.00460(IP) + 0.0487	0.78
4	70	An	1% H_2O	0.00628	G = 0.00628(IP) + 0.343	0.84
4	25	An	Brine	0.000728	G = 0.000728(IP) + 0.170	0.77
4	25	An	Brine Sterile	0.00126	G = 0.00126(IP) − 0.0322	0.76
4	70	An	Brine	0.0179	G = 0.0179(IP) + 0.913	0.79
4	70	An	Brine Sterile	0.0165	G = 0.0165(IP) + 1.203	0.81
4	25	An	Nutrient	0.00523	G = 0.00523(IP) + 0.0958	0.87
4	25	An	Nutrient Sterile	0.00136	G = 0.00136(IP) − 0.0257	0.97
4	70	An	Nutrient	0.0104	G = 0.0104(IP) + 0.289	0.86
4	70	An	Nutrient Sterile	0.0109	G = 0.0109(IP) + 0.0641	0.83

[a]See footnote to Table III.

TABLE V

CO_2 EVALUATION ROM SAWDUST

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of CO_2 Production (mg/day/g)	Equation for the Amount of CO_2 Production[a]	R^2
4	25	An	91% H_2O	0.0215	G = 0.0215(IP) − 0.268	0.98
4	25	An	91% H_2O Sterile	0.000900	G = 0.000900(IP) + 0.0262	0.40
4	70	An	91% H_2O	0.0393	G = 0.0393(IP) + 0.444	0.96
4	70	An	91% H_2O Sterile	0.0212	G = 0.0212(IP) + 0.440	0.96
4	25	An	Brine	0.0164	G = 0.0164(IP) + 0.1307	0.99
4	25	An	Brine Sterile	0.00711	G = 0.00711(IP) − 0.126	0.99
4	70	An	Brine	0.0356	G = 0.0356(IP) + 0.557	0.97
4	70	An	Brine Sterile	0.0302	G = 0.0302(IP) + 1.152	0.92
4	25	An	Nutrient	0.0139	G = 0.0139(IP) + 0.149	0.98
4	25	An	Nutrient Sterile	0.0043	G = 0.0043(IP) − 0.0493	0.90
4	70	An	Nutrient	0.0383	G = 0.0383(IP) + 0.347	0.96
4	70	An	Nutrient Sterile	0.0284	G = 0.0284(IP) + 0.0267	0.93
4	25	An	1% H_2O	0.00975	G = 0.00975(IP) + 0.149	0.87
4	70	An	1% H_2O	0.0348	G = 0.00348(IP) + 1.038	0.93
5	25	A	91% H_2O	0.0140	G = 0.014(IP) + 0.359	0.93
4	25	A	91% H_2O Sterile	0.00278	G = 0.00278(IP) − 0.00351	0.76
4	70	A	91% H_2O	0.0139	G = 0.0139(IP) + 0.876	0.74
4	70	A	91% H_2O Sterile	0.00286	G = 0.00286(IP) + 0.173	0.96
5	25	A	1% H_2O	0.00517	G = 0.00517(IP) + 0.154	0.97
4	70	A	1% H_2O	0.00504	G = 0.00504(IP) + 0.824	0.99
5	25	A	Brine	0.00437	G = 0.00437(IP) + 0.252	0.94
5	25	A	Brine Sterile	0.00459	G = 0.00459(IP) − 0.0853	0.94
4	70	A	Brine	0.0178	G = 0.0178(IP) + 1.07	0.94
4	70	A	Brine Sterile	0.00320	G = 0.00320(IP) − 0.0668	0.89
5	25	A	Nutrient	0.013	G = 0.013(IP) + 0.144	0.69
4	25	A	Nutrient Sterile	0.0024	G = 0.0024(IP) − 0.0163	0.84
4	70	A	Nutrient	0.0164	G = 0.0164(IP) + 1.185	0.74
4	70	A	Nutrient Sterile	0.00227	G = 0.00227(IP) − 0.046	0.97

[a]See footnote to Table III.

TABLE VI

CO_2 EVOLUTION AT 40°C

Number of Observations	Aerobic or Anaerobic	Treatment	Rate of CO_2 Evolution[a] LASL Composite Waste	Asphalt	Sawdust
2	A	91% H_2O	0.0049	0	0.0087
2	A	91% H_2O Sterile	0.0006	0	0.0006
2	A	1% H_2O	0.0037	0.0008	0.0082
2	A	Brine	0.0126	0.0003	0.0128
2	A	Brine Sterile	0.0003	0.0008	0.0037
2	A	Nutrient	0.0043	0.0009	0.0022
2	A	Nutrient Sterile	0.0007	0	0
2	An	91% H_2O	0.0038	0.0017	0.0049
2	An	91% H_2O Sterile	0.0023	0	0.0012
2	An	1% H_2O	0.0085	0.0008	0.0148
2	An	Brine	0.0189	0.0046	0.0199
2	An	Brine Sterile	0.0005	0.0038	---
2	An	Nutrient	0.0033	0.0003	0.0057
2	An	Nutrient Sterile	0	0	0.0003

[a]See footnote to Table III.

TABLE VII

EDTA DEGRADATION

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of Degradation (mg/day)	Equation for Degradation[a] (%)	R^2
3	25	A	91% H_2O	0.0061	D = 0.0061(IP) − 0.0560	0.99
2	25	A	91% H_2O Sterile	0.0031	D = 0.0031(IP) − 0.0206	---
2	70	A	91% H_2O	0.0044	D = 0.0044(IP) + 0.1036	---
2	70	A	91% H_2O Sterile	0.0051	D = 0.0051(IP) + 0.1287	---
3	25	A	1% H_2O	0.0045	D = 0.0045(IP) − 0.0118	0.99
2	70	A	1% H_2O	0.0142	D = 0.0142(IP) + 0.2348	---
3	25	A	Brine Sterile	0.0024	D = 0.0024(IP) + 0.0221	0.96
2	70	A	Brine Sterile	0.0031	D = 0.0031(IP) − 0.0898	---
3	25	A	Nutrient	0.0121	D = 0.0121(IP) − 0.3144	0.99
3	25	A	Nutrient Sterile	0.0023	D = 0.0023(IP) + 0.0191	0.99
2	70	A	Nutrient	0.0048	D = 0.0048(IP) + 0.0499	---
2	70	A	Nutrient Sterile	0.0077	D = 0.0077(IP) − 0.0706	---
3	25	An	91% H_2O Sterile	0.0163	D = 0.0163(IP) − 0.7780	0.87
2	70	An	91% H_2O Sterile	0.0056	D = 0.0056(IP) + 0.2160	---
3	25	An	1% H_2O	0.0040	D = 0.0040(IP) − 0.0390	0.99
2	70	An	1% H_2O	0.0185	D = 0.0185(IP) + 0.4211	---
2	25	An	Brine	0.0010	D = 0.0010(IP) + 0.0492	---
3	25	An	Brine Sterile	0.0021	D = 0.0021(IP) + 0.0243	0.99
2	70	An	Brine	0.0020	D = 0.0020(IP) + 0.0037	---
2	70	An	Brine Sterile	0.0038	D = 0.0038(IP) − 0.1170	---
3	25	An	Nutrient	0.0225	D = 0.0225(IP) − 1.035	0.93
3	25	An	Nutrient Sterile	0.0024	D = 0.0024(IP) + 0.0176	0.98
2	70	An	Nutrient	0.0051	D = 0.0051(IP) + 0.3626	---
2	70	An	Nutrient Sterile	0.0001	D = 0.0001(IP) + 0.3744	---

[a]Degradation (D) % is given as a function of the incubation period (IP) in days.

TABLE VIII

TARTARATE DEGRADATION

Number of Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of Degradation (mg/day)	Equation for Degradation[a] (%)	R^2
6	25	A	91% H_2O	0.2064	D = 0.2064(IP) + 34.86	0.64
6	25	A	91% H_2O Sterile	0.0626	D = 0.0626(IP) + 25.18	0.88
4	70	A	91% H_2O Sterile	0.3137	D = 0.3137(IP) + 50.26	0.71
7	25	A	1% H_2O	0.1241	D = 0.1241(IP) − 2.741	0.93
4	70	A	1% H_2O	0.1656	D = 0.1656(IP) + 1.558	0.95
5	25	A	Brine	0.0832	D = 0.0832(IP) + 5.850	0.93
6	25	A	Brine Sterile	0.0987	D = 0.0987(IP) + 18.15	0.89
3	70	A	Brine	0.3280	D = 0.3280(IP) + 19.66	0.86
4	70	A	Brine Sterile	0.4445	D = 0.4445(IP) + 26.21	0.83
5	25	A	Nutrient	0.3732	D = 0.3732(IP) + 41.19	0.80
6	25	A	Nutrient Sterile	0.0860	D = 0.0860(IP) + 27.28	0.82
3	70	A	Nutrient Sterile	0.1266	D = 0.1266(IP) + 51.46	0.99
7	25	An	91% H_2O	0.2521	D = 0.2521(IP) + 53.33	0.68
6	25	An	91% H_2O Sterile	0.1849	D = 0.1849(IP) + 29.37	0.75
4	70	An	91% H_2O	0.4771	D = 0.4771(IP) + 48.66	0.90
4	70	An	91% H_2O Sterile	0.3521	D = 0.3521(IP) + 53.58	0.70
6	25	An	Brine	0.1106	D = 0.1106(IP) + 3.893	0.96
6	25	An	Brine Sterile	0.0831	D = 0.0831(IP) + 16.56	0.89
4	70	An	Brine Sterile	0.1566	D = 0.1566(IP) + 25.33	0.69
6	25	An	Nutrient	0.1849	D = 0.1849(IP) + 29.37	0.75
5	25	An	Nutrient Sterile	0.0682	D = 0.0682(IP) + 20.11	0.84
4	70	An	Nutrient	0.3521	D = 0.3521(IP) + 53.58	0.70
4	70	An	Nutrient Sterile	0.3890	D = 0.3890(IP) + 60.33	0.69

[a]Degradation (D) % is given as a function of the incubation period (IP) in days.

TABLE IX

CITRATE DEGRADATION

Number Observations	Temp (°C)	Aerobic or Anaerobic	Treatment	Rate of Degradation (mg/day)	Equation for Degradation[a] (%)	R^2
4	25	A	91% H_2O	0.2149	D = (T x 0.2149) + 33.04	0.58
5	25	A	91% H_2O Sterile	0.0105	D = (T x 0.0105) + 6.713	0.67
4	70	A	91% H_2O Sterile	0.1623	D = (T x 0.1623) + 9.580	0.92
4	25	A	1% H_2O	0.1177	D = (T x 0.1177) + 2.236	0.66
4	70	A	1% H_2O	0.0735	D = (T x 0.0735) + 1.587	0.96
5	25	A	Brine	0.0192	D = (T x 0.0192) + 1.379	0.85
5	25	A	Brine Sterile	0.0095	D = (T x 0.0095) + 4.940	0.77
4	70	A	Brine Sterile	0.0691	D = (T x 0.0691) + 5.664	0.84
5	25	A	Nutrient	0.1249	D = (T x 0.1249) + 34.25	0.61
5	25	A	Nutrient Sterile	0.0183	D = (T x 0.0183) + 11.94	0.61
4	70	A	Nutrient	0.1565	D = (T x 0.1565) + 9.858	0.90
5	70	A	Nutrient Sterile	0.2860	D = (T x 0.2860) + 11.76	0.85
5	25	An	91% H_2O	0.1538	D = (T x 0.1538) + 39.97	0.52
5	25	An	91% H_2O Sterile	0.0140	D = (T x 0.0140) + 7.275	0.70
5	70	An	91% H_2O	0.1652	D = (T x 0.1652) + 7.770	0.89
4	70	An	91% H_2O Sterile	0.1763	D = (T x 0.1763) + 11.70	0.94
5	25	An	1% H_2O	0.0570	D = (T x 0.0570) + 0.4777	0.97
4	70	An	1 % H_2O	0.0579	D = (T x 0.0579) + 1.056	0.96
5	25	An	Brine	0.0096	D = (T x 0.0096) + 1.146	0.95
5	25	An	Brine Sterile	0.0128	D = (T x 0.0128) + 5.055	0.64
4	70	An	Brine	0.0698	D = (T x 0.0698) + 6.277	0.79
4	70	An	Brine Sterile	0.0908	D = (T x 0.0908) + 5.760	0.83
5	25	An	Nutrient	0.1084	D = (T x 0.1084) + 36.79	0.45
5	25	An	Nutrient Sterile	0.0080	D = (T x 0.0080) + 8.410	0.62
4	70	An	Nutrient Sterile	0.1933	D = (T x 0.1933) + 8.912	0.92

[a]Degradation (D) % is given as a function of the incubation period (IP) in days.

IX. 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

[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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