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

Annual Report

October 1, 1978—September 30, 1979

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

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by

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

ABSTRACT

We confirmed our previous results showing elevated frequencies of radiation-resistant bacteria in microorganisms isolated from shallow transuranic (TRU) burial soil that exhibits nanocurie levels of beta and gamma radioactivity. Research to determine whether plutonium could be methylated by the microbially produced methyl donor, methylcobalamine, was terminated when literature and consulting radiochemists confirmed that other alkylated transuranic elements are extremely short-lived in the presence of oxygen. We placed greater emphasis on investigation of the dissolution of plutonium dioxide by complex formation between plutonium and a polyhydroxamate chelate similar to that produced by microorganisms. New chromatographic and spectrophotometric evidence supports our previous results showing enhanced dissolution of alpha radioactivity when ^{239}Pu dioxide was mixed with the chelate Desferol. Microbial degradation studies of citrate, ethylenediamine tetraacetate (EDTA), and nitrilo triacetate (NTA) chelates of europium are in progress. Current results are summarized. All of the chelates were found to degrade. The average half-life for citrate, NTA, and EDTA was 3.2, 8.0, and 28 years, respectively.

Microbial CO_2 generation is also in progress in 72 tests on several waste matrices under potential WIPP isolation conditions. The mean rate of gas generation was 5.97 μg CO_2/g waste/day. Increasing temperature increased rates of microbial gas generation across treatments of brine, varying water content, nutrient additions, and anaerobic conditions. No microbial growth was detected in experiments to enumerate and identify the microorganisms in rocksalt cores from the proposed WIPP site. This report contains the year's research results and recommendations derived for the design of safe storage of TRU wastes under geologic repository conditions. This was the final quarter for the LASL Life Sciences Division effort. Future LASL research for this project will be conducted by LASL's CMB Division in collaboration with the Biology Department, University of New Mexico.

I. INTRODUCTION

The WIPP is primarily intended for the terminal isolation of defense-related transuranic (TRU) radioactive waste. This material may include celluloses, rubbers, plastics, etc.; radionuclides including actinides; and, quite possibly, residual chelating chemicals from decontamination operations and microorganisms. The bacteria and fungi in the wastes are derived from humans who work with the materials, laboratory animals, air, and soil. Microorganisms can metabolize organic materials in radioactive waste and, as a consequence, generate significant quantities of gas. Microbial metabolites may also react with radionuclides to enhance their mobilities in solution if leaching occurs.

To quantify both the potential for microbial interactions with TRU-contaminated waste materials and the effects of such interactions on the WIPP, a research program was initiated in June 1978. This study involves the research of Douglas E. Caldwell at the University of New Mexico (UNM) Biology Department, with experimental support and additional studies at the Los Alamos Scientific Laboratory (LASL) under the direction of Benjamin J. Barnhart, Life Sciences Division. LASL participation began in August 1978 and ended September 30, 1979. These studies are important to the Sandia Laboratories' WIPP TRU waste characterization program.[1]

Although microbial activity in radioactive wastes has not been a prominent area of research, there are some relevant reports. Colombo et al.[2] have enumerated and classified as aerobic or anaerobic bacteria from trench water in low-level radioactive waste disposal sites at Maxey Flats, Kentucky, and West Valley, New York. Au[3] and Au and Beckert[4] have analyzed the microbial population in soil of the Nevada Test Site. However, the LASL-UNM project is the first to report on the numbers and partial characterizations of microorganisms extant in radioactive waste and to assess the potential microbial alteration of the chemical and physical states of transuranic elements. We have also identified the existence of radioresistant bacteria in microbial populations found in LASL low-level shallow trench burial sites.

Microbial interaction with radionuclides may cause enhanced volatilization via alkylation reactions, or in solubilization and concentration by chelation and degradation of chelates, respectively. Microbial production and degradation of chelated radionuclides, changes in radionuclide oxidation states, and alkylation reactions are also being investigated.

Microorganisms will cause production and transformation of gases within the WIPP disposal site. Carbon dioxide is the major gas expected as a result of bacterial decomposition of organic waste. The rates of CO_2 production under WIPP conditions are under continuing study. In addition, other gases that may be produced and their rates of production are being identified.

This report summarizes all the pertinent data and results obtained on these microbial interaction topics.

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

A. Background

We have established standard operating procedures for enumerating microflora from LASL TRU shallow burial site TA-54, Area C, using the dilution agar-plate technique[5] to estimate colony forming units (CFU)/g of soil and the most probable number (MPN) method,[6] which permits estimation of population density without an actual count of single cells or colonies.

It was necessary to develop these procedures using soil not contaminated with radioactivity.[7] The characterized soil was collected from just outside the fenced TRU burial site along the southern perimeter. The microbiological enumerations are reproducible and serve as a reference to compare with TRU-contaminated soil samples collected in the TA-54 area.[8] These experiments have shown that it is possible to collect samples in plastic bags, freeze them in dry ice and ethanol at the collection site, and store them in a laboratory freezer without a loss in microorganisms until it is convenient to set up the cultures.

A very time-consuming effort was also made to obtain direct microbial counts in the soil using fluorescent microscopy.[9] The fluorescent stain fluorescene isothiocynate (FITC) was used on soil samples immediately or after incubation to permit spore germination and formation of microcolonies. The

stained samples were examined under a Zeiss Epifluorescence microscope using an excitation wavelength range of 450-490 nm and a selective FITC filter combination.

B. Estimation of CFU by Dilution Agar-Plate Technique

All manipulations were performed in a Bioquest Biological laminar flow cabinet. A 10-g aliquot of moist soil sample was transferred to an Erlenmeyer flask containing 95 mℓ of sterile 3X distilled water or 1% peptone broth and a sterile stirring rod. The flask was placed on a magnetic stirrer for 15 min to disperse soil particles. Immediately, 10 mℓ from the suspension was diluted serially to 10^9. A 1-mℓ portion of each dilution was transferred to each of three petri dishes, and about 12 mℓ of molten agar, cooled to 42°C, was poured into each inoculated dish. After the dishes were incubated at 28°C for 7 days, colonies were counted except where noted. Dishes from the dilution at which 30 to 300 colonies had developed were considered satisfactory to count.

Table I shows the results of three experiments with 10-g aliquots of the same soil sample and growth medium used in each case.

These experiments resulted in an average of 1.94×10^6 CFU/g of soil (incubated at 28°C for 7 days under aerobic conditions). The excellent agreement among experiments shows that our technique is reproducible. Either 3X distilled H_2O or 1% peptone can be used as a diluent for microorganisms, and numbers and types of colonies are essentially the same using 1:10 or 1:100 trypticase soy agar (TSA).

The anaerobic cultures resulted in an average of 2.66×10^9 CFU/g of soil with good agreement between experiments. Anaerobic agar (Difco) was the medium of choice in later experiments. Except for *Clostridium*, colonies appearing were facultative anaerobes.

TABLE I

CFU PER GRAM OF SOIL

Sample	Bacteria		Fungi
	Aerobic	Anaerobic	
Soil collected outside fenced area TA-54, Area C C 2940 on 10/20/78 plated immediately on TSA 1:10	1.33×10^6	1.99×10^8	Mycosel 3.6×10^3 at 3 days
Aliquot of same sample stored 11 days in freezer, plated on 10/31/78 on TSA 1:10	1.61×10^6	3.03×10^3	
TSA 1:100	1.59×10^6	Anaerobic agar (Difco)	Mycosel 3.5×10^3 at 3 days
Aliquot of same sample using 1% peptone as diluent, plated on 12/4/78 on TSA 1:10	2.19×10^6	2.97×10^3	Mycosel
TSA 1:100	2.98×10^6	Anaerobic agar (Difco)	3.2×10^4 at 7 days

Mycosel (fungus selection agar) in the first two experiments had to be counted in 3 days before overgrowth made them impossible to count. In the third experiment, no colonies formed at 3 days, indicating that the agar was too hot when the plates were poured, so the result (counted at 7 days) cannot be compared with the other experiments.

C. Estimation of Microbial Population Density by MPN

One-mℓ portions of each soil diluation were used to inoculate a series of five tubes, each containing trypticase soy broth (TSB) or thioglycollate medium. The tubes were examined microscopically for evidence of microbial growth after 7 days at 28°C and recorded as positive or negative. The negative dilution tubes were incubated an additional 7 days. The first experiment (October 20, 1978) showed growth in all tubes, indicating that additional dilutions must be made to find the weakest dilution at which growth can be obtained. Results of the October 31, 1978 experiment (tubes incubated for 14 days at 28°C) are shown in Table II.

The MPN of organisms in the original sample was calculated using a factor from the table of MPN for use with 10-fold dilutions and 5 tubes per dilution.[6] The factor was multiplied by the appropriate dilution factor to obtain the MPN of the original sample.

TSB 1:10 or 1:100 gives the same result of 2.3×10^6 MPN microorganisms/g of soil under aerobic conditions. The growth in the tubes containing Sabouraud with antibiotics may include some bacteria since the antibiotics (added to prevent bacteria from growing) are unstable with heat and on standing.

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

Core samples were obtained from the dirt overburden of a LASL low-level TRU waste burial trench. A split-spoon sampler (7.5-cm diam) was hammered to a depth of 120 cm in two successive 60-cm corings.[8] After each coring the spoon was carefully opened 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 III 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 the CFU technique. We 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/g 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.

As shown in Table III, 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

TABLE II

MPN MICROORGANISMS PER GRAM OF SOIL

Medium	Bacteria		Fungi
	Aerobic	Anaerobic	
TSB			
1:10	2.3×10^6		
1:100	2.3×10^6		
Thioglycollate medium		3.3×10^6	
Sabouraud broth with antibiotics			4.9×10^4

TABLE III

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

Depth (cm)	Radioactivity[a] in soil (γ,β)	CFU/g Soil[b]			MPN/g Soil[c]		
		Aerobic	Anaerobic	Fungi	Aerobic	Anaerobic	Fungi
5-15	ND	$1.5 \pm 0.2 \times 10^6$	$2.8 \pm 0.4 \times 10^3$	3.5×10^3	2.3×10^6 (0.7–7.6)	3.3×10^6 (1.0–10.9)	4.9×10^4 (1.49–16.2)
40-50	4×10^4, 6×10^4	$1.3 \pm 0.1 \times 10^5$	$1.4 \pm 0.8 \times 10^2$	5.8×10^2	4.9×10^5 (1.49–16.2)	1.1×10^6 (0.33–3.6)	1.1×10^5 (0.33–3.6)
100-110	2×10^3, 7×10^3	$4.5 \pm 1.7 \times 10^4$		6.5×10^1	3.3×10^4 (1.0–10.9)	3.3×10^4 (1.0–10.9)	2.3×10^2 (0.70–7.6)

[a]dpm/g soil.
[b]Mean and standard deviation of duplicate or triplicate plate counts.
[c]Numbers in parentheses are 95% confidence limits.[6]

is not clear but may be due to decontamination of a piece of equipment in the field followed by backfilling with soil. Other possibilities could be cited, but until additional samples are analyzed, this phenomenon will not be discussed further. The gamma emitter was tentatively identified by the radiochemistry section of LASL Group CMB-1 as ^{137}Cs; the beta emitters were not identified; and 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 sterilize the soil.

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

Some ^{239}Pu-contaminated waste materials were retrieved from a steel drum in which combustibles had been deposited recently by LASL employees after work performed in gloveboxes in a plutonium work area. We opened the bags in a glovebox in the same work area, but we covered the working surface with clean nonradioactive aluminum foil. Each piece of waste material was handled with sterile forceps, pieces were cut from them with sterile scissors, and each piece was placed aseptically into a sterile Erlenmeyer flask containing 100 mℓ of Difco TSB and a sterile 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 diluted to a 1×10^5 dilution factor in TSB. After 14 days incubation at 28°C, the tubes were scored and the MPN was calculated.[6] Table IV shows the MPN for aerobic microorganisms and fungi. The anaerobic cultures were contaminated, so they were discarded.

F. Enumeration of Microflora in 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. 7. We cut the eight components by hand into small pieces and weighed them to allow 75 to 240 mg of each component for a total of 1.0 g. We added 9.0 mℓ of Difco TSB to the 1.0-g portions and stirred the mixture on a magnetic stirrer for 15 min. Aliquots were serially diluted and incubated for 14 days at 28°C. An aliquot of the original mixture was streaked on TSA and on mycophil agar.

Table V shows that only two gram-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.

G. Direct Enumeration of Microflora

Soil samples were prepared as described for CFU and MPN estimates.[7] For microscopic examination, 0.01 mℓ of 100X dilution of soil was spread over a

TABLE IV

MICROFLORA IN ^{239}Pu-CONTAMINATED WASTE

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 fingers)	9.5×10^3	4.6×10^1	Gram +, cocci
Tissue (1)	3.3×10^1	ND	Gram +, rods
Cotton gauze (2 cm^2)	3.3×10^1	ND	No growth

[a]MPN estimate in Difco TSB at 28°C for 14 days.
[b]An aliquot of the initial suspension (~10^1 dilution) was streaked on TSA plates and incubated at 28°C. Isolated colonies were picked and the microflora were characterized.

TABLE V

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

MPN: 0.79×10^3/g

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

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 [see Can. J. Microbiol. **16**, 57-62 (1970)].

Slides were stained for 3 min at room temperature, and washed in 0.5 *M* $NaHCO_3$-5% $Na_4P_2O_7$ buffer. The stained preparations were immediately mounted in glycerol 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 the small sample size limited the number 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 WASTE (LASL)

A. Background

Radiation-resistant microorganisms have been induced by ionizing radiations in the laboratory[10] and in field conditions in which medical products were sterilized by radiation.[11] 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 microorganism.

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 TSB and stirred for 15 min on magnetic stirrers. Aliquots were serially diluted, plated on the surface of TSA, and incubated for 7 days at 28°C. Isolated colonies were stabbed in the center with sterile sticks and inoculated into TSB. Each turbid culture was looped 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 min 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. 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.[8] Similar evaluations were performed on the 5- to 15-cm sample, which lacked detectable radioactivity.

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, which survived 54 krads of x-irradiation,[8] were inoculated into broth with the ingredients of Difco Actinomycete 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 in a Coleman Jr. Colorimeter at a 600-nm wavelength. Each culture was diluted and pour-plated into Difco Actinomycete 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 inoculated petri dishes were stored for up to 2.5 hours at 4°C and removed as needed for exposure to gamma radiation.

In 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.[9] 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 VI 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

Microorganisms are responsible for the alkylation of many elements, including the metals mercury, tin, palladium, platinum, gold, and thallium, and the metalloids arsenic, selenium, tellurium, and sulfur.[12] 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.[12,13] 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[14] [(CH_3B12), an analog of vitamin B12 and an intermediate product of bacterial metabolism]. This methyl donor was used to methylate mercury and in attempts to methylate the lanthanide element europium, and the actinide elements thorium and plutonium.

TABLE VI

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 ^{60}Co 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. Heading numbers represent number of isolate.

As reported in Ref. 7, we could not detect a methylated form of europium using a variety of reaction conditions and detection methods. However, the question remained as to whether the heavy elements of the TRU series could be alkylated by the methyl donor produced by microorganisms.

We reported that $Th(NO_3)_4 \cdot 4H_2O$ appeared to be methylated, as indicated by the spectral shifts accompanying the demethylation of CH_3B12.[7] 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 determined that no methylated metal was formed, even though some demethylation and degradation of CH_3B12 occurred.

Direct chemical synthesis of methylthorium or methylplutonium has also been elusive 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 to methylate these heavy metals were 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. Experiments were then designed to incorporate the organic buffer hexamethylenetetramine $[(CH_2)_6N_4]$ with a pH of 5.0. A 0.2 *M* solution of $(CH_2)_6N_4$ buffered 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. Methylation of actinide elements is difficult and probably requires specific physical, chemical, and biological conditions. An obvious extension of these experiments would be to try to methylate these elements using microbial cultures, and perhaps laboratory sod beds. It would also be interesting to 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.[15]

B. Attempted Methylation of Plutonium

A soluble form of plutonium is needed as a reactant in aqueous methyl transfer reactions. To satisfy this need quickly, we dissolved metallic ^{239}Pu (weapon grade) in 4*M* HCl. We made two unsuccessful attempts to convert the resulting mixture of plutonium ions (predominantly III and IV) to the IV oxidation state. Although Pu(III) is reactive, the IV

state is more reactive in general, and III-complexes are less stable.[16]

In the first experiment, we diluted the stock solution of plutonium in deionized water to a final acid concentration of 0.1 *M* HCl. Then, to reduce the plutonium to the III oxidation state, we added 1 *M* NH_2OH-HCl until the solution turned green.[16] We added $NaNO_2$ to oxidize the III ions to the IV oxidation state and the solution became almost colorless. The solution was diluted in 0.1 *M* HCl and the absorption spectrum between 400 and 625 nm was determined on a Carey recording spectrophotometer. This scan showed that the plutonium had not been converted to the IV state, but displayed the spectrum of Pu(III).[16]

All conditions were the same in the second experiment, except that the reduction and oxidation steps were performed on the concentrated stock of plutonium (85.3 m*M* in 4 *M* HCl) before diluting the acid to 0.1 *M*. Again, a spectral scan showed no indication of the IV state, but indicated that the plutonium had polymerized.[17]

The 0.1 *M* HCl was adopted as plausible since higher concentrations of mineral acids are not compatible with most living microorganisms that can produce methylcobalamine. We also felt that higher acid concentrations would probably affect the organic molecule of the methyl donor, methylcobalamine.

In any event, we stopped the experiments when we learned that it was highly unlikely that we would detect any plutonium in a methylated form due to the short half-life of alkylated actinides.[18-20]

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

A. Background

Chelates of microbial origin may be found where heterotrophic microorganisms exist that require iron or other metals.[21] Hydroxamates and interchelins are strong metal complexing molecules produced when bacteria grow in an iron-deficient environment.[22] These chelates enhance the dissolution of the metals with which they complex, and therefore, have the potential of increasing their mobilities. The mobilization of TRU elements in radioactive-waste disposal sites by both naturally occurring and man-made chelates such as those used in decontamination procedures was considered to be a real possibility. We initiated this area of experimentation using the commercially available polyhydroxamate Desferol. Unfortunately, the lack of continued funding will not permit the LASL Life Sciences Division to continue these experiments and to include experiments designed to demonstrate whether microbial cultures would mobilize plutonium via chelation. However, these experiments may be continued by UNM in collaboration with LASL Group CMB-1.

B. Dissolution of Plutonium by a Polyhydroxamate Chelate

The experimental design was to place insoluble PuO_2 and the chelating agent together in water. The dioxide of weapon-grade ^{239}Pu and Desferol, a modified ferrioxamine,[21] were used in the following experiments. The pH ranged from 5.5 to 6.0 due to variations in pH of the deionized water.

In the first experiment, PuO_2, Desferol, and blue dextran in 10 mℓ of water were placed in a collodian dialysis membrane and immersed in a cylinder containing 200 mℓ of water. The high-molecular-weight dye was added to help detect breaks in the membrane. Concentrations of each component and other specifics were reported in Ref. 9. Even though the membrane ruptured after 10 days, aliquots were taken from the 200-mℓ mixture, centrifuged to remove PuO_2 polymers, and counted to determine solubilized radioactivity. A consistent increase in counts over 20 days suggested chelate-mediated solubilization of plutonium (Table VII). Chromatographic separation on Sephadex G10 resulted in two peaks of radioactivity, as shown in Fig. 1. In a similar chromatographic run in which $FeCl_3$ was added, brown Desferol-Fe (III) appeared in the first peak. These results suggested that the plutonium in this peak was complexed with Desferol.

In the next experiment, $^{239}PuO_2$ and Desferol were placed in 140 mℓ of water and a collodian membrane containing 5 mℓ of water was immersed in it. Aliquots were removed from inside and outside the membrane daily for 35 days, centrifuged, and the supernatants counted. The data reported in Ref. 10 showed more than a threefold increase in solubilized counts over a 20-day period, whereas the control with only PuO_2 showed no increase.

TABLE VII

DISSOLUTION OF $^{239}PuO_2$ IN DESFEROL

Sample Time (Days)	Net[a] CPM per 100 $\mu\ell$			
	Inside Membrane		Outside Membrane	
	Control	Expt[b]	Control	Expt 1[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 $\mu\ell$ deionized water.
[c]5.5 μg (14 326 cpm) of $^{239}PuO_2$ and 1.79 μg Desferol per 100 $\mu\ell$ deionized water.

Chromatographic separation of aliquots was obtained again on Sephadex G10 as described in Ref. 9. Two peaks of radioactivity resulted, as in the previous experiment; a Ferric ion-Desferol complex eluted with the first peak, again suggesting a plutonium-Desferol complex.

In an attempt to determine the nature of the material in the first peak (Fig. 1), which might contain chelated Pu(IV) ions, a sample was taken from the first experimental cylinder (described above), centrifuged, chromatographed on Sephadex G10, and the fractions under the first peak were pooled. Based on total count the peak contained 3×10^4 μmoles of plutonium. An absorption spectrum of this solution was compared with spectra of solutions of Pu(IV) ions with and without Desferol and with Desferol only (Fig. 2). The pooled experimental fractions had an absorption maximum at A_{304} nm, Pu(IV) ions plus Desferol at a broad peak from 325 to 400 nm, the plutonium ions exhibited no maximum, and Desferol had virtually no absorption. Similar absorption profiles have been observed for hydroxamate-Fe(III) complexes.[21]

To further characterize the plutonium in both peaks eluted from the G10 column, the fractions under each peak were pooled and an aliquot was extracted with a mixture of phenol:chloroform (1:1). Mixtures of Pu(IV) and Desferol prepared at specific concentrations and in various ratios were also extracted with this solvent. Chelated metal ions are soluble in phenol:chloroform while the unchelated metals are soluble in an aqueous phase.[23]

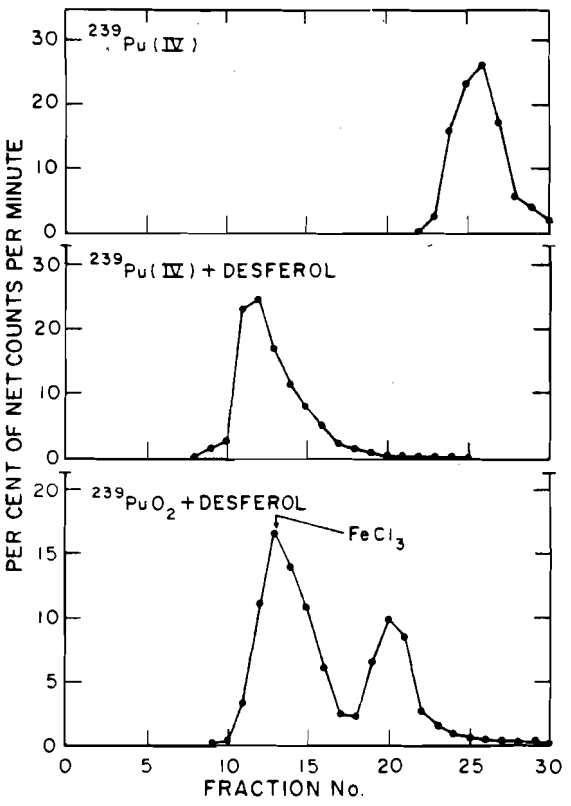

Fig. 1.
Chromatographic separation of plutonium ions and plutonium-Desferol complex. The material was eluted from a Sephadex G-10 column (1 × 20 cm) with 0.1 M HCl in 10% ethanol. The Desferol chelate of Fe Cl_3 eluted simultaneously with the more rapidly eluting radioactivity believed to be a plutonium-Desferol chelate.

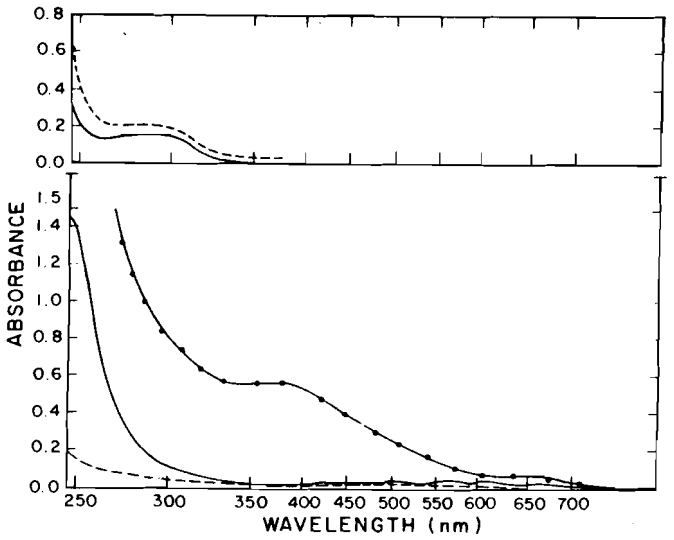

Fig. 2.
Upper panel, ultraviolet absorption spectra of rapidly eluting material from a Sephadex G-10 column. Lower panel, ultraviolet absorption spectra of $^{239}Pu(IV)$-Desferol complex (·−·), $^{239}Pu(IV)$ ions (—) and Desferol (− − −).

Extraction of peak I resulted in 69% of the radioactivity partitioned in the organic phase. We extracted 26% of the radioactivity from a mixture of 2.2×10^{-2} *M* Pu(IV) and 4.6×10^{-2} *M* Desferol, pH 1.0. On the other hand, radioactivity from a control solution of Pu(IV) ions partitioned into the aqueous phase. All of the detectable radioactivity in the second Sephadex G10 peak was also extracted into the aqueous rather than the organic phase. These results support further the possibility that under appropriate conditions, plutonium can be chelated by at least one microbially produced chelate, Desferol.

VI. CARBON DIOXIDE GAS GENERATION STUDIES (UNM)

A. Background

The generation of CO_2, H_2S, CH_4, O_2, NO, N_2O, and SO_2 by simulated organic TRU waste (LASL composite waste, 34% cellulose, 24% polyethylene, 12% polyvinyl chloride, 7.5% each of neoprene, latex, hypalon, and butyl rubber), CM cellulose, bovine serum albumen, and asphalt were studied in FY 78.[7] These preliminary studies showed that CO_2 was the predominant gas formed aerobically unless proteinaceous organic matter was present, in which case, small quantities of H_2S were also produced. Also, the simulations did not adequately model anaerobic conditions since no methane was produced. As a result, studies of gas generation in FY 79 focused on CO_2 evolution.[8,9] Simulations of anaerobic gas generation are planned for FY 80. The following treatments were used: aerobic, N_2-purged (anaerobic), 25°C, 40°C, 70°C, 1% H_2O, 91% H_2O, 91% brine, and nutrient solution.[24] The present study was to determine the effects of brine, temperature, nutrient addition, and waste composition (alone and in various combinations) on gas generation. Sawdust, asphalt, and LASL composite waste were used as substrates. There were no replicates due to the large number of treatments. However, replicates are planned for FY 80 to provide confidence intervals on gas production rates for key treatments.

B. Rates of Carbon Dioxide Generation

The mean rate of carbon dioxide generation for the 72 simulations (with sterile controls) was 5.97 μg/day/g with a standard deviation of 3.33. The results from each simulation are given in Tables VIII-X for simulated organic matrix TRU waste (LASL composite waste), asphalt, and sawdust, respectively. As seen in Table XI, despite the effects of oxygen, water, brine, and nutrient addition, temperature shows a positive correlation with gas generation rates for all three substrates. Varying the oxygen tension, concentration of water, nutrient level, and the addition of brine had no significant effect on rates of degradation at the levels tested (across treatments).

Gas chromatography consistently gave lower results than the alkaline absorption method for measuring rates of CO_2 evolution because of the loss of CO_2 during incubations over periods of months (CO_2 diffused from the test ampoules). Thus the alkaline absorption technique is more accurate because it traps CO_2 as it forms, preventing the pressurization of the test ampoules and consequent loss of product.

Both 1% H_2O and saturated salt brine provided enough water for biological degradation to occur. The rates of CO_2 generation under extreme conditions (high temperature, high salt, and anaerobiosis) had a much higher standard deviation than the rates observed under moderate conditions. This suggests

TABLE VIII

MICROBIAL PRODUCTION OF CO_2 FROM SIMULATED ORGANIC MATRIX TRU WASTE (LASL COMPOSITE WASTE)[a]

Rate (μg/day/g)[b]	Rate (μg/day/g)[c]	Treatment
Aerobic		
		25°C
2.1	3.8	91% H_2O
0.3	none detected	1% H_2O
---	none detected	brine
---	7.2	nutrient
		40°C
---	4.3	91% H_2O
---	3.1	1% H_2O
---	12.3	brine
---	3.6	nutrient
		70°C
0.3	7.2	91% H_2O
0.026	9.8	1% H_2O
---	12.7	brine
---	10.7	nutrient
Anaerobic		
		25°C
2.8	9.9	91% H_2O
0.053	5.5	1% H_2O
---	7.4	brine
---	11.6	nutrient
		40°C
---	1.5	91% H_2O
---	6.2	1% H_2O
---	18.4	brine
---	3.3	nutrient
		70°C
none detected	8.0	91% H_2O
none detected	11.9	1% H_2O
---	none detected	brine
---	17.0	nutrient

[a]Net rates after subtraction of sterile controls.
[b]Determined by gas chromatography (μg CO_2 generated/g waste/day).
[c]Determined by titration of $Ba(OH)_2$ trap for carbon dioxide.

TABLE IX

MICROBIAL PRODUCTION OF CO_2 FROM ASPHALT[a]

Rate (μg/day/g)[b]	Rate (μg/day/g)[c]	Treatment
Aerobic		
		25°C
1.0	3.0	91% H_2O
none detected	---	1% H_2O
---	2.3	brine
---	3.3	nutrient
		40°C
---	---	91% H_2O
---	0.8	1% H_2O
---	---	brine
---	0.9	nutrient
		70°C
none detected	---	91% H_2O
none detected	---	1% H_2O
---	7.4	brine
---	8.3	nutrient
Anaerobic		
		25°C
0.26	0.5	91% H_2O
none detected	4.3	1% H_2O
---	---	brine
---	3.9	nutrient
		40°C
1.7	---	91% H_2O
0.8	---	1% H_2O
0.8	---	brine
0.3	---	nutrient
		70°C
none detected	1.7	91% H_2O
none detected	6.0	1% H_2O
---	1.4	brine
---	---	nutrient

[a]Net rates after subtraction of sterile controls.
[b]Determined by gas chromatography (μg CO_2 generated/g waste/day).
[c]Determined by tritration of $Ba(OH)_2$ trap for carbon dioxide.

TABLE X

MICROBIAL PRODUCTION OF CO_2 FROM SAWDUST

Rate (μg/day/g)[a]	Treatment
Aerobic	
	25°C
11.2	91% H_2O
2.4	1% H_2O
0.0	brine
13.0	nutrient
	40°C
8.1	91% H_2O
5.1	1% H_2O
9.1	brine
2.2	nutrient
	70°C
2.8	91% H_2O
5.0	1% H_2O
14.6	brine
14.1	nutrient
Anaerobic	
	25°C
20.6	91% H_2O
8.9	1% H_2O
9.3	brine
9.6	nutrient
	40°C
3.7	91% H_2O
13.6	1% H_2O
18.7	brine
5.4	nutrient
	70°C
18.1	91% H_2O
13.6	1% H_2O
5.4	brine
9.9	nutrient

[a]Determined by titration of $Ba(OH)_2$ trap for carbon dioxide.

that a lag period is required for microbial adaptation to occur. During this period, organisms (enriched) that can survive under adverse conditions may be selected. Long-term enrichments under these conditions would yield organisms capable of more rapid degradation rates. Thus, the *in situ* rates of gas generation in the WIPP facility, after an initial adaptation period, will probably be higher than in short (1- to 2-yr) simulations. Due to the variability in the concentration of salt, humidity, substrates present, microflora present, temperature, and inorganic nutrient availability, it is not possible to accurately forecast the rate of CO_2 evolution unless these conditions are defined. However, the conditions most likely to produce biological gas generation are the addition of brine to wastes and the storage of wastes under humid conditions. The present results confirm this. As a result, the simulated organic matrix TRU waste (LASL composite waste) will be incubated in brine and at 100% relative humidity with 10 replicates and 3 sterile controls (total of 23 simulations). The large number of replicates will allow an analysis of variation in the rates of gas generation. In addition, a duplicate set of flasks will be purged with nitrogen and amended with iron sulfide to lower the redox potential sufficiently to obtain rates of methanogenesis.[25] Preliminary results show that this treatment provides strictly anaerobic conditions despite the presence of oxygen, which is continuously scavenged by the ferrous sulfide reductant.

The limit of detection for the $Ba(OH)_2$ titration procedure was 0.01 mg CO_2/g of waste. Simulations were sampled every 30-60 days. The total amount of waste in the simulations planned for FY 80 has been increased from 1 to 25 g to increase the sensitivity of the procedure to 0.40 μg/g. In addition, NaOH will replace $Ba(OH)_2$ because the $BaCO_3$ precipitate that formed as the CO_2 was trapped reduced the efficiency of trapping. An equal volume of an equimolar solution of $BaCl_2$ is added to the NaOH after incubation, before titration.

All the simulations involved only 1 g of waste material. When these results are scaled up to kg quantities, the time course and magnitude of degradation may vary from that found in the small simulations. In addition, highly biodegradable materials (insects, rodents, etc.) that may occur *in situ* could greatly increase the rate of gas generation from cellulosics and other more refractory organics.

TABLE XI

AVERAGE RATES OF CO_2 GENERATION[a] (ACROSS TREATMENTS) IN 72 CO_2 SIMULATIONS (WITH STERILE BACKGROUND CONTROLS)

Conditions	LASL Composite	Sawdust	Asphalt
25°C	5.1 (3.9)[b]	9.4 (6.3)	2.3 (1.5)
40°C	6.5 (5.8)	8.2 (5.5)	0.88 (0.45)
70°C	8.6 (5.5)	10.4 (5.5)	4.9 (3.2)
aerobic	4.8 (4.6)	7.3 (5.1)	3.4 (2.9)
anaerobic	7.4 (5.8)	11.4 (5.6)	2.0 (1.9)
91% H_2O	4.45 (3.5)	10.8 (7.4)	1.0 (1.0)
1% H_2O	3.8 (4.7)	8.1 (4.7)	2.0 (2.5)
91% brine	2.9 (3.0)	11.4 (5.2)	2.9 (3.0)
nutrient	8.9 (5.3)	9.0 (4.5)	3.4 (3.2)

[a]Standard deviation given in parentheses.
[b]Rate of CO_2 generation given as μg/day/g.

The initial linear rates of CO_2 evolution given here do not necessarily reflect long-term degradation rates. Rates should decline over a period of years due to the depletion of labile organics, leaving more refractory compounds to degrade at a slower rate. If there is pressurization due to gas evolution, the rate may decline further.

VII. CHELATE DEGRADATION STUDIES (UNM)

A. Background

The chelate degradation studies and methodology described in Ref. 7 were preliminary studies of chelate degradation using europium as an analog of plutonium. Studies of plutonium chelates planned for FY 80 at LASL have been canceled.

B. Results

Results of europium chelate degradation, given in Tables XII-XIV, show that citrate, EDTA, and NTA, with europium chloride present in 10-fold molar excess, were biodegraded under simulated WIPP conditions. The average half-life (across treatments) for NTA was 7.9 years (std dev, 4.0 years); for EDTA, 27.9 years (std dev, 21.5 years); and for citrate, 3.2 years (std dev, 2.0 years). The temperatures studied (70 and 25°C) were boundary conditions expected for the WIPP.[26] However, intermediate temperatures near 40°C, the optimum for most mesophilic microorganisms, would probably yield more rapid degradation rates. Rates of tartarate degradation are not given because the autoclave procedure for the sterilization of controls resulted in the decomposition of tartarate to CO_2. As a result, the sterile controls showed more CO_2 generation than the biological treatments.

Although each chelate degraded, the rates were low. This suggests that lanthanide coordination complexes are refractory, having half-lives of several years in WIPP simulations. The minimum sensitivity of the assay was 0.074% degradation. This represents a total of 2.3×10^{-5} μCi (50 dpm of the radioactive europium tracer) degraded to CO_2, trapped, and detected by scintillation counting. A minimum of 3.1×10^{-2} μCi (68 000 dpm) of europium, thorium, and sodium chelates in soil are being run to determine whether the cause of low degradation rates is the lanthanides, actinides, or conditions in the WIPP simulations. All simulations involved 1 g of waste material. If these are scaled up to kg quantities, the time course and magnitude of degradation may vary from that found in the simulations. In addition, the presence of highly

biodegradable materials (insects, rodents, etc.), which may occur in waste packages sent to WIPP, could greatly increase chelate degradation due to cometabolism.

VIII. IDENTIFICATION AND ENUMERATION OF MAJOR GROUPS OF MICROORGANISMS IN WIPP SALT BEDS (UNM)

No halophiles or other bacteria were found in surface sterilized crystals taken from the salt formations at the WIPP site.[9]

IX. CONCLUSIONS (LASL-UNM)

The numbers of culturable bacteria found in radioactive LASL TRU burial site soil and flammable waste contaminated with ^{239}Pu show that populations of microorganisms coexist quite well with radionuclides, including weapon-grade plutonium. These are viable, metabolically active microbes whose potential effects on long-term storage of radioactive wastes must not be overlooked. These effects include possible mobilization by microbially produced chelates that, as we have shown, can mediate dissolution of essentially insoluble plutonium dioxide, and by possible gas pressurization of radioactive waste storage vessels or enclosures as the result of microbially produced carbon dioxide. An analysis of the experimental results over the course of this project shows that microorganisms produce far more gas than that produced by physical or chemical processes.[26]

We investigated the relative radiation sensitivities of bacteria isolated from radioactive shallow burial site soil. As many as 68% of the bacteria from soil exhibiting gamma and beta radioactivity were more resistant than the common soil bacterium *Bacillus subtilis* to ionizing radiation, i.e., x and gamma radiation. Approximately 25% of the isolates from nonradioactive soil were more resistant than *B. subtilis*. Although the radioresistant bacteria were less resistant than *Micrococcus radiodurans*, our results showed that microbes not only flourish in soil containing nanocurie levels of alpha and beta activity, but that this selective pressure results in elevated frequencies of radiation-resistant bacteria.

These results indicate that the potential effects of microbial interactions with radioactive waste are not limited to laboratory situations but must be considered in designing long-term radwaste storage capabilities. Reduction or elimination of organic carbon sources in radionuclide-contaminated waste would reduce the numbers of heterotrophic microorganisms. The effects of chemosynthetic bacteria on the integrity of inorganic waste and waste containers has not been considered.[27]

X. PROPOSED RESEARCH FOR FY 80

Gas generation studies will continue through FY 80 at UNM. The current simulations will be continued until the rates of generation decrease to zero. New simulations will also be initiated to provide a confidence interval on gas generation rates and to determine CH_4 production rates under strict anaerobic conditions (in addition to CO_2 production). The simulated organic matrix TRU waste (LASL composite waste) will be incubated in brine at 100% relative humidity with 10 replicates and 3 sterile controls to allow an accurate statistical analysis of gas generation rates. A duplicate set of flasks will be purged with nitrogen and amended with iron sulfide to lower the redox potential enough to obtain rates of methanogenesis. Preliminary results show that this treatment provides strict anaerobic conditions despite the presence of oxygen, which is continuously scavenged by the ferrous sulfide reductant.

The limit of detection for the $Ba(OH)_2$ titration procedure was 0.01 mg CO_2/g of waste. Simulations were sampled every 30-60 days. The total amount of waste in simulations planned for FY 80 has been increased from 1 to 25 g to increase the assay sensitivity to 0.4 μg/g. In addition, NaOH will replace $Ba(OH)_2$ in the traps since the $BaCO_3$ precipitate that forms as the CO_2 is trapped reduces the efficiency of CO_2 detection.

Studies (conducted in collaboration with LASL's CMB Division) of the effect of plutonium on microbial gas generation and microbial viability have begun and will be described in another report.

TABLE XII

EUROPIUM-NTA CHELATE DEGRADATION (NET RATES AFTER SUBTRACTION OF STERILE CONTROLS)

Rate (% degraded/day)	Treatment
Aerobic	
	25°C
0.14	nutrient supplement
1.02	91% H_2O
0.0	1% H_2O
0.001	brine
	70°C
0.0	nutrient supplement
0.0	91% H_2O
0.11	1% H_2O
0.08	brine
Anaerobic	
	25°C
0.3	nutrient supplement
0.0	91% H_2O
0.0	1% H_2O
0.005	brine
	70°C
0.01	nutrient supplement
0.0	91% H_2O
0.0	1% H_2O
0.0	brine

TABLE XIII

EUROPIUM-EDTA CHELATE DEGRADATION (NET RATES AFTER SUBTRACTION OF STERILE CONTROLS)

Rate (% degraded/day)	Treatment
Aerobic	
	25°C
0.01	nutrient supplement
0.003	91% H_2O
0.001	1% H_2O
---	brine
	70°C
0.0	nutrient supplement
0.0	91% H_2O
0.01	1% H_2O
---	brine
Anaerobic	
	25°C
0.2	nutrient supplement
---	91% H_2O
0.0	1% H_2O
0.0	brine
	70°C
0.005	nutrient supplement
---	91% H_2O
0.01	1% H_2O
0.0	brine

TABLE XIV

EUROPIUM-CITRATE CHELATE DEGRADATION (NET RATES AFTER SUBTRACTION OF STERILE CONTROLS)

Rate (% degraded/day)	Treatment
Aerobic	
	25°C
0.11	nutrient supplement
0.2	91% H_2O
0.0	1% H_2O
0.01	brine
	70°C
0.0	nutrient supplement
---	91% H_2O
0.0	1% H_2O
---	brine
Anaerobic	
	25°C
0.14	nutrient supplement
0.14	91% H_2O
0.0	1% H_2O
0.0	brine
	70°C
---	nutrient supplement
0.0	91% H_2O
0.0	1% H_2O
0.0	brine

XI. 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			•		•	•	•		o	o	o	o
Annual reports								•				o
Literature search and update			•		•	•	•	•	o	o	o	o
Capital equipment ordered			•		•	•	•	•	o	o	o	o
Laboratory expendables ordered			•		•	•	•	•	o	o	o	o
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)						•	•	o				
Identification of saltbed microflora							•	•				
Microbial gas generation (UNM)					•	•	•	•	o	o	o	o
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-UNM)					•	•	•	•	o	o		
Alkylation/Volatilization						o	o	o				
Chelation Solubilization							o	o				
Chelate degradation												
Plate counts								•	o	o	o	o
Gas generation								•				
Effect of PuO_2 on microbial								•	o	o	o	o
activity (LASL, UNM) gas								•	o	o	o	o
generation Gas viability of								•	o	o	o	o
microoganisms												
Effect of microbial activity on the oxidation state of plutonium												
Analysis and interpretation of data[a] (LASL, UNM)					•	•	•	•				
Conclusions and recommendations								•				o

•On schedule
oPlanned
[a]The ongoing work may identify additional topics of research that may be included, and other experiments may be deleted by mutual consent of primary investigator and WIPP technical program managers.

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