Waste Isolation Pilot Plant

Compliance Certification Application

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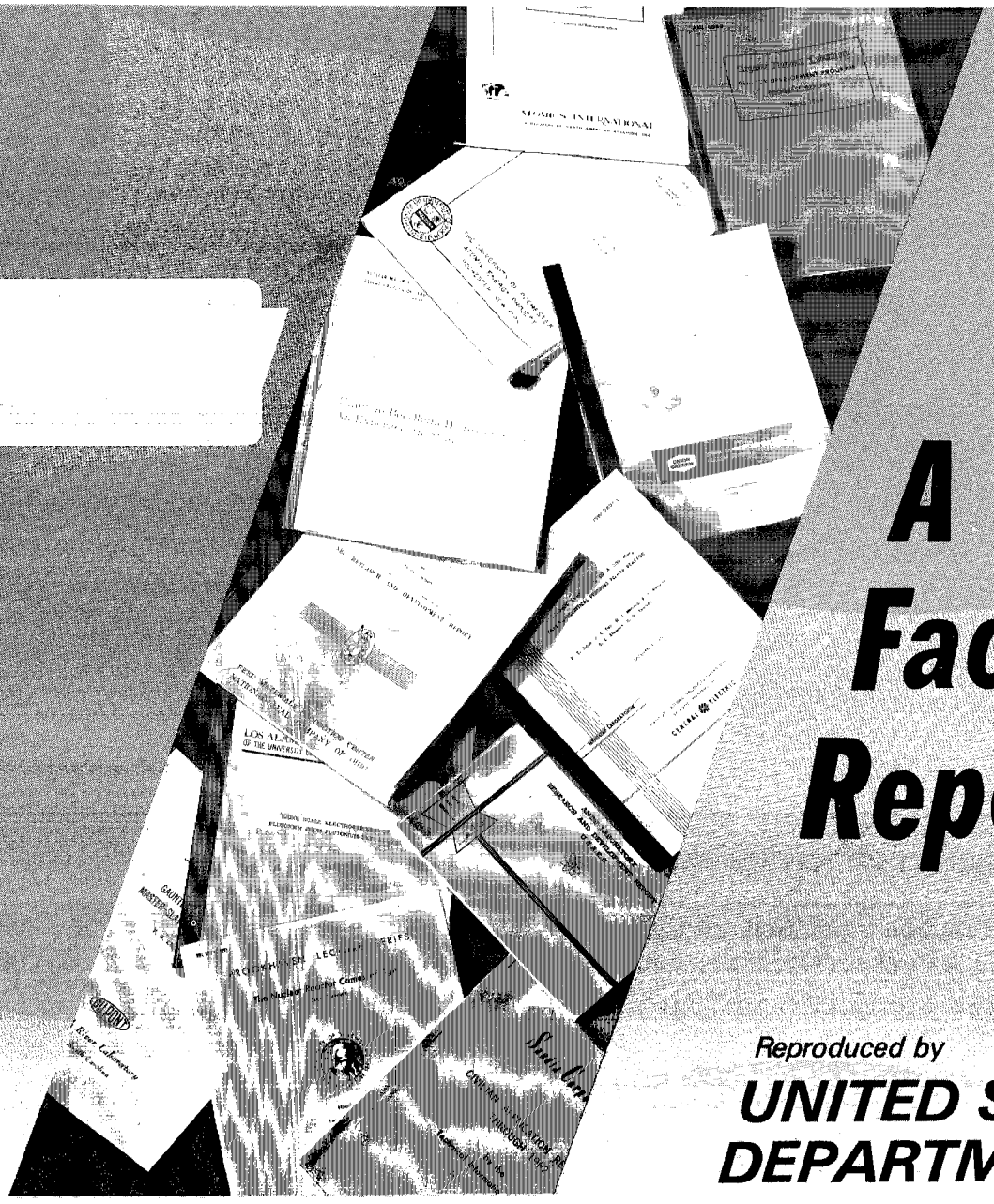

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Potential Microbial Impact on Transuranic Wastes Under Conditions Expected in the Waste Isolation Pilot Plant (WIPP)

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October 1, 1978—September 30, 1979

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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