NUREG/CR-6188
INEL-95/0153
Vol. 2

Microbial Degradation of Low-Level Radioactive Waste

Annual Report for FY 1994

Prepared by
R. D. Rogers, M. A. Hamilton, R. H. Veeh, J. W. McConnell, Jr.

Idaho National Engineering Laboratory
Lockheed Idaho Technologies Company

Prepared for
U.S. Nuclear Regulatory Commission

AVAILABILITY NOTICE

Availability of Reference Materials Cited in NRC Publications

Most documents cited in NRC publications will be available from one of the following sources:

1. The NRC Public Document Room, 2120 L Street, NW., Lower Level, Washington, DC 20555-0001

2. The Superintendent of Documents, U.S. Government Printing Office, P. O. Box 37082, Washington, DC 20402-9328

3. The National Technical Information Service, Springfield, VA 22161-0002

Although the listing that follows represents the majority of documents cited in NRC publications, it is not intended to be exhaustive.

Referenced documents available for inspection and copying for a fee from the NRC Public Document Room include NRC correspondence and internal NRC memoranda; NRC bulletins, circulars, information notices, inspection and investigation notices; licensee event reports; vendor reports and correspondence; Commission papers; and applicant and licensee documents and correspondence.

The following documents in the NUREG series are available for purchase from the Government Printing Office: formal NRC staff and contractor reports, NRC-sponsored conference proceedings, international agreement reports, grantee reports, and NRC booklets and brochures. Also available are regulatory guides, NRC regulations in the *Code of Federal Regulations*, and *Nuclear Regulatory Commission Issuances*.

Documents available from the National Technical Information Service include NUREG-series reports and technical reports prepared by other Federal agencies and reports prepared by the Atomic Energy Commission, forerunner agency to the Nuclear Regulatory Commission.

Documents available from public and special technical libraries include all open literature items, such as books, journal articles, and transactions. *Federal Register* notices, Federal and State legislation, and congressional reports can usually be obtained from these libraries.

Documents such as theses, dissertations, foreign reports and translations, and non-NRC conference proceedings are available for purchase from the organization sponsoring the publication cited.

Single copies of NRC draft reports are available free, to the extent of supply, upon written request to the Office of Administration, Distribution and Mail Services Section, U.S. Nuclear Regulatory Commission, Washington, DC 20555-0001.

Copies of industry codes and standards used in a substantive manner in the NRC regulatory process are maintained at the NRC Library, Two White Flint North, 11545 Rockville Pike, Rockville, MD 20852-2738, for use by the public. Codes and standards are usually copyrighted and may be purchased from the originating organization or, if they are American National Standards, from the American National Standards Institute, 1430 Broadway, New York, NY 10018-3308.

NUREG/CR-6188
INEL-95/0153
Vol. 2

Microbial Degradation of Low-Level Radioactive Waste

Annual Report for FY 1994

Manuscript Completed: August 1995
Date Published: August 1995

Prepared by
R. D. Rogers, M. A. Hamilton, R. H. Veeh, J. W. McConnell, Jr.

Idaho National Engineering Laboratory
Lockheed Idaho Technologies Company
Idaho Falls, ID 83415

Prepared for
Division of Regulatory Applications
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission
Washington, DC 20555-0001
NRC Job Code L1807

DISCLAIMER

NUREG/CR-6188 is not a substitute for NRC regulations and compliance is not required. The approaches and/or methods described in this NUREG/CR are provided for information only. Publication of this report does not necessarily constitute NRC approval or agreement with the information contained herein.

ABSTRACT

The Nuclear Regulatory Commission stipulates in 10 CFR 61 that disposed low-level radioactive waste (LLW) be stabilized. To provide guidance to disposal vendors and nuclear station waste generators for implementing those requirements, the NRC developed the Technical Position on Waste Form, Revision 1. That document details a specified set of recommended testing procedures and criteria, including several tests for determining the biodegradation properties of waste forms. Cement has been widely used to solidify LLW; however, the resulting waste forms are sometimes susceptible to failure due to the actions of waste constituents, stress, and environment. The purpose of this research program is to develop modified microbial degradation test procedures that will be more appropriate than the existing procedures for evaluating the effects of microbiologically influenced chemical attack on cement-solidified LLW. Groups of microorganisms indigenous to LLW disposal sites are being employed that can metabolically convert organic and inorganic substrates into organic and mineral acids. Such acids aggressively react with cement and can ultimately lead to structural failure. Results over the past year on the application of mechanisms inherent in microbially influenced degradation of cement-based material are the focus of this annual report. Data-validated evidence of the potential for microbially influenced deterioration of cement-solidified LLW and subsequent release of radionuclides has been developed during this study.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Microbial Degradation of Low-Level Radioactive Waste Annual Report for FY 1994

INTRODUCTION

Regulatory Background

Microorganisms have been isolated in samples taken from low-level radioactive waste (LLW) environments (Francis et al. 1980). This realization, combined with scientific data that demonstrate the existence of microorganisms under hostile conditions once thought to exclude them, has raised concerns that microbial activity at LLW disposal sites could affect the long-term stability of the disposed waste. Due to apprehension over possible microbial effects, the United States Nuclear Regulatory Commission (NRC) requires microbial activity be addressed as one of the requirements for determining the stability of Class B and C LLW [10 CFR 61.56(b)(1)]. This methodology is needed to ensure that these radioactive wastes can be disposed of safely for 300 to 500 years. The NRC developed the Technical Position on Waste Form, Revision 1 (NRC 1991), to provide guidance for disposal vendors and nuclear station waste generators to implement the stability requirements of 10 CFR 61 (NRC 1987). That document details a specified set of required testing procedures and criteria, including several tests for determining the biodegradation properties of waste forms.

Concerns were voiced over the appropriateness of the Technical Position tests for microbial degradation of cement containing LLW at the NRC-sponsored "Proceedings of the Workshop on Cement Stabilization of Low-Level Radioactive Waste" (Tokar 1989). It became apparent during the deliberations that improved tests would be required if meaningful information was to be obtained from biodegradation studies on cement wastes. A major difficulty identified was that current testing procedures did not adequately determine the bioeffects on cementitious materials because the microorganisms used for the accelerated testing were not associated with cement degradation. Further, it was pointed out that there were literature references that identified specific microorganisms as the causative agents of cement degradation. The conclusions reached by the workshop were that "there appeared to be a clear need to specify more appropriate microbes for such [Technical Position] tests" and that "this [need] might require a substantial research effort" (Tokar 1989).

Researchers familiar with the required Technical Position biodegradation tests, American Society for Testing and Materials (ASTM) G21 and G22 (ASTM 1981a; 1981b), have not found them to be applicable for use with cement-solidified LLW. Doubts raised concerning the tests included (a) the lack of demonstrable evidence that specific microorganisms, required by the ASTM standard tests, could promote degradation of cement waste forms under any test conditions, (b) the lack of flexibility in determining the duration of time required for test completion, and (c) the lack of flexibility in determining test specimen size and preparation (Rogers and McConnell 1988).

The NRC's Advisory Committee on Nuclear Waste has also raised concerns about biodegradation testing of LLW. In a letter to the NRC dated September 6, 1990, the Committee indicated that biodegradation testing should be specified, for cementitious waste matrices, using bacteria that are likely to affect cement and any organic component exposed during the decomposition process.

Based on the NRC needs, the objectives of the program have been to (a) develop biodegradation test procedures to determine cement waste stability, (b) test the developed procedures on simulated nonradioactive waste and, if successful, continue with actual solidified LLW, (c) recommend modified test procedures for consideration as revisions to the Technical Position, based on the best procedures developed, (d) investigate the environment at LLW sites to determine if microorganisms capable of degrading waste-form

materials are present, and (e) determine if microbial action can promote the release of radionuclides from LLW and solidified LLW.

It is anticipated that the research conducted for this program will provide data, information, and recommendations, which the NRC can use as a basis for (a) the implementation of 10 CFR 61 requirements that deal with the stability of cementitious LLW specimens when challenged by known cement-degrading microorganisms under optimal conditions for cement degradation, (b) modifications to the Technical Position testing procedures to more realistically determine the microbial effects on cement-solidified LLW, (c) guidance to licensees concerning a determination of the susceptibility of cement-stabilized LLW to biodegradation, and (d) the determination of microorganism-related radionuclide releases. This work will provide a basis for including microbial-degradation potential in predicting overall safety and performance of new LLW disposal facilities.

The following tasks were identified as requirements to reach the objectives stated above. The status of each task is also given.

Task 1: Literature Review—A literature review was conducted to provide information on test sophistication, methods, and materials. Information was gathered on the impact that biological organisms have on cement. This included information on conditions that promote deterioration and what organisms are responsible. Information on the occurrence and extent of microbial populations at LLW disposal sites together with the prevalent type of microorganism was investigated. This task resulted in the preparation of a NUREG/CR report. This task is complete.

Task 2: Develop Program Plan—A program plan was written that contains detailed program requirements, including a test plan, testing procedures, required equipment and facilities, waste form composition (cement and type of LLW to be used), identification of microbial species, and a bibliography. Input for the plan came from past experience, the literature review, and involvement with recognized peers. This task resulted in a letter report. This task is complete.

Task 3: Develop Microbial Culture Collection—A collection of microbial cultures was initiated. The intent of this activity is to obtain organisms that are expected to interact with the selected cement-solidified waste material(s). Microorganisms were obtained from other culture collections (commercial and private) as well as from environments where there is evidence of natural degradation of solidification materials. Pure cultures of promising microorganisms were developed and are being maintained. This task resulted in a letter report. This task is complete.

Task 4: Determine Microorganisms Capable of Cement Degradation—Work was conducted to determine which microorganisms from the culture collection of Task 3 are capable of degrading cement. This task required an in-depth study of the biodegradation potential of the individual microbial species. Growth of the test microorganisms was monitored, and analysis was conducted to detect the presence of microbially produced metabolites, which could affect the test material. Different methodologies that integrate exposure time and microbial activity were evaluated. Microbial isolates were classified that were determined by the screening procedures to be potential candidates for degradation studies. A knowledge of the types of microorganisms that promote degradation was useful for comparison with microorganisms endemic to existing and proposed LLW disposal sites. Cement-degrading microorganisms were determined and reported in a letter report. This task is complete.

Task 5: Develop Procedures for Growing Candidate Microorganisms—Media necessary for the promotion of active growth of the candidate microorganisms from Task 4 were obtained or developed. This involved determining the appropriate energy and carbon source, mineral nutrients, pH range, and temperature range. Methods were developed for incubation and preservation of the candidate microorganisms. Procedures such as freeze drying, low-temperature freezing of liquid cultures, or storage on solid media were evaluated. Procedures for growing

candidate microbes were developed and are documented in a letter report. This task is complete.

Task 6: Develop Initial Test Methodology for Assessing Biodegradation—It has been suggested by researchers at the INEL and Brookhaven National Laboratory that standard biodegradation tests (such as ASTM G21 and G22) are not applicable for use with some materials used to solidify LLW. Concerns raised over these tests included specimen size and preparation, the lack of demonstrable evidence that the microorganisms required by the standard tests stated above could promote degradation of the materials in question, and lack of flexibility in determining the duration of time required for test completion. Cement testing methodology was developed to answer the above concerns. Such methods included exposure of cement to a wide range of microbial species (especially to those known to affect cement); determination of the appropriate size for test specimens; use of alternative energy sources (carbon and non-carbon) to provide opportunity for assessing the effects of co-metabolism or biologically produced chemicals on specimen structure; and adequate time for a reasonable interaction between the microbial component and the test specimen. Testing of vendor-supplied simulated waste forms exposed to *N. europea* is completed. Analysis of samples of test lixiviant for radioactive content has been completed. Compilation of those data is being conducted. This task will result in a letter report.

Task 7: Develop Criteria for Evaluation of Test Effects—It will be necessary to designate some occurrence as a measurable endpoint for the cement specimen testing. Both biological and physical parameters were investigated. For example, effects on cement could be determined directly by measuring metabolic activity such as by-product production (CO_2, acid, etc.) or use of consumables (oxygen, carbon, inorganic, etc.). Some physical parameters such as specimen weight loss or visual deterioration will also be used. Work is being conducted on evaluation of methods that will determine to what extent biofilms develop on the surface of the waste forms. It is thought that the detection of biofilm development can be used as an indicator of the potential onset of cement degradation. Direct observation of biofilm development is continuing with scanning electron microscopy and light microscopy with selective straining methodology. This task resulted in a preliminary test evaluation method, which is to be reported by letter. This task is in progress.

Task 8: Apply Biodegradation Test to Actual LLW—The testing method developed in Task 6 was evaluated on actual cement solidification LLW samples containing different types of waste, including decontamination ion-exchange resins, evaporator concentrates, and filter sludges. Testing *Thiobacillus thiooxidans* using sections of actual LLW waste forms (Peach Bottom and Nine Mile Point) for 8 weeks has been completed. Visual observation indicate that these waste forms performed similarly to the simulated waste forms in prototype testing, that is, they were in various stages of disintegration. Photographs of the operation were taken on day 30 and again on day 60. Radiochemical analysis of lixiviant recovered from the test has been completed. Effects were reported by letter. This task is complete.

Task 9: Apply Current Technical Position (Rev. 1) to LLW Containing Decontamination Radwaste—The effect of decontamination solutions on the integrity of LLW containing them was to be evaluated. Under current regulations, these wastes need to be tested for biodegradation. Selected cement-solidified wastes and decontamination LLW corresponding to those tested in Task 8 were to be examined using the current Technical Position method of ASTM G21 and G22. The results of that testing were to be compared to those obtained from the newly developed tests (Task 8). These comparisons could then be used by the NRC to judge the effectiveness of the new test protocol. This task was cancelled as being unnecessary.

Task 10: Determine Radionuclide Releases from LLW by Microbial Action—Studies are being performed to determine the releases of radionuclides from actual stabilized cement LLW containing ion-exchange resins, and activated LLW materials by microorganisms. The

investigations involve Class B low-level radioactive waste obtained from operating nuclear power stations (Peach Bottom and Nine Mile Point). The ion-exchange resins used in this task were those typically used in LLW applications at the nuclear stations and focused on decontamination waste containing chelating agents. Analytical results from Task 8 tests are being studied in detail. This task is in progress.

Technical Background

Microbially influenced degradation (MID) of concrete has been reviewed elsewhere (Rogers et al. 1993). MID is thought to occur when microorganisms present in the environment produce mineral or organic acids that dissolve or disintegrate the cement matrix. The rate of degradation cannot be determined by measuring the generation of metabolically produced gases because the microorganisms are not metabolizing the cement matrix. The particular mechanisms of biological acid attack are consistent with those that have been associated with chemical attack.

A knowledge of the types of microorganisms that promote cement degradation is useful for selecting appropriate candidate organisms. Preliminary data (Rogers et al. 1993, 1994) suggest that the activity of three different genera of bacteria must be understood to provide a comprehensive evaluation of microbial degradation of selected cement-solidified waste materials. These include organic acid-producing heterotrophic bacteria, nitrifying bacteria, and sulfur-oxidizing bacteria. For the purposes of the work, suitable sources, from which these microorganisms have been isolated, include samples from sites with natural microbial activity, actual or proposed LLW locations, and use of inocula from existing culture collections.

While the literature suggests that the above microorganisms are ubiquitous in the environment, it was important in this study to demonstrate their presence in soils representative of present and proposed commercial LLW sites. The sources of microorganisms, with steps for confirming their viability in selective environments, have been discussed previously (Rogers et al. 1994). However, by way of review, data on the isolation as well as the activity and significance of MID bacteria have been provided.

Because of the number of individual bacterial isolates and the complexity of the testing procedures, it was necessary to use only representatives of the three main species of microorganisms in the actual testing procedure. It was assumed that the action of a representative species would be indicative of the activity of others in the group. This premise was shown to apply for the mineral acid-producing bacteria (nitrifying and thiobacilli species), but not for the heterotrophs. This was because organic acid production is not limited to a single genera of heterotrophs. Therefore, for this study, a known, aggressive heterotrophic bacterium (*Pseudomonas cepacia*) was selected. Strength of acid production was determined by the pH change of the solution in which the organism was being cultivated.

Heterotrophic Bacteria. A diverse group of microorganisms that degrade concrete are included with the heterotrophic bacteria. Heterotrophic microorganisms, capable of producing organic acids through the assimilation of organic carbon compounds, can be found everywhere. Organic acids such as lactic, citric, gluconic, malic, and many others are by-products of their metabolism. Several organic acids are produced on an industrial scale through the metabolic activity of these microbes. In addition, several types of these organisms, collected from a wide range of environments, are known to use an organic acid mechanism for the active extraction of phosphate from phosphate ores (Rogers and Wolfram 1992). They are known to promote acidic conditions that can be less than pH 3.

In work conducted to determine the microbiological parameters associated with the English nuclear waste disposal effort (Dunk 1991), wide diversities of heterotrophic microorganisms were isolated from simulated, cement-solidified, plutonium-contaminated materials. Many cultured microbes could grow in alkaline conditions (pH 11) common on the surface of concrete. Unfortunately, the studies were not carried out

over a sufficient time to determine if growth of the organisms influenced the integrity of the concrete. Studies on the effects of heterotrophic degradation of cement-solidified LLW have also been conducted by the French (N. Langomazino, personal communication 1990, Libert et al. 1993). They used a microbial growth medium containing concrete powder and common soil (the initial source of microbes). Several species of microbes were isolated by this method and maintained good growth in an alkaline medium (pH 9). Also, these microbes grew well in the presence of cement and produced organic acids. A fungus and the bacterium *P. cepacia* were selected for the concrete degradation studies. Their data showed that by 7 months, porosity had increased 11% in the samples, and at the end of 11 months, there had been a 50 to 85% loss of $Ca(OH)_2$ in the treated concrete samples, resulting in an 80% loss of compressive strength. Further results, from a 2-year study, demonstrated that the organic acid-producing fungi were responsible for a significant loss of calcium from a defined leach layer of 0.2 cm.

Though several heterotrophic bacterial species were isolated from the collected soil samples, it was possible to select only one as the candidate heterotrophic bacterium for use in the testing protocol. The selection was made after several isolates were screened to determine which could produce organic acids. This was accomplished using a bioassay that could detect bacteria capable of dissolving calcium compounds. Only one or two of the isolates exhibited the ability for extreme dissolution of calcium compounds. Of these, *P. cepacia* was selected for application in the waste form work.

Nitrifying Bacteria. The second group of microorganisms known to promote concrete degradation are the nitrifying bacteria. These bacteria (e.g., *Nitrosomonas* and *Nitrobacter*) are chemoautotrophs. They obtain energy through the oxidation of inorganic nitrogen compounds and have been isolated from a variety of soils. Growth of nitrifying organisms on concrete is not sequential as observed for the sulfur-oxidizing bacteria. However, in controlled experiments (using a simulation chamber), it was found that mixed cultures of *Nitrosomonas* and *Nitrobacter* inoculated on concrete blocks produced about 14 mL of 65% nitric acid per block per year (Mansch and Bock 1992). This was sufficient acid to dissolve the concrete and produce the breakdown product calcium nitrate. Thus, the relationship between ammonia-oxidizing and nitrite-oxidizing bacteria is mutualistic rather than antagonistic.

There was evidence from the soil sampling study that representative soil samples from all locations contained a nitrifying bacterial population. These data show that both ammonia oxidizers and nitrite oxidizers were present in soils from all eight of the sampling sites (Rogers et al. 1994). It was significant that nitrifying bacteria were found in all of the surface soils and most of the subsurface samples. Populations of ammonia oxidizers were confirmed in at least one soil sample from each geographic location. The number of ammonia oxidizers ranged from 10^1 to 10^3 per gram of soil (wet weight) in the 17 soil samples that hosted them. The presence of nitrite oxidizers was confirmed at all depths for all but one soil location. The number of these microbes ranged from 10^1 to 10^4 per gram of soil (wet weight) in the 21 soil samples in which they were found.

A representative of *Nitrosomonas* was used as a culture for testing the effects of nitrifying bacteria. Although species of the nitrite-oxidizing genus *Nitrobacter* convert nitrite to nitrate as the second step in nitrification, this is merely an oxidation step. The acidification step of ammonia oxidation to nitrite by *Nitrosomonas* and other ammonia-oxidizing genera is thought to constitute the step resulting in cement-based material solubilization.

Sulfur-Oxidizing Bacteria. Sulfur-oxidizing bacteria (genus *Thiobacillus*) are the microorganisms most often associated with the biological degradation of concrete structures. These organisms were responsible for the catastrophic, biogenic sulfuric-acid attack of the Hamburg sewer system (Sand and Bock 1988) and are now recognized as the causative factor in degradation of concrete sewer pipe elsewhere (Islander et al.

1991; Mori et al. 1992). Thiobacilli are chemoautotrophs that obtain energy by oxidizing reduced, inorganic sulfur sources such as elemental sulfur, thiosulfate, and polythionates, while assimilating CO_2 as their sole carbon source.

Literature data suggest that, regardless of the initial populations of the various species of thiobacilli in soils, once the process of sulfur oxidation begins, *T. thicoxidans* or *T. ferrooxidans* will eventually become the dominate organisms (Rogers et al. 1993). Although dominance depends on the environmental conditions, both species appear to be appropriate choices for use in MID evaluations. Our previous work (Rogers et al. 1994) showed that soil from all LLW sampling locations had the presence of sulfur oxidizers in at least one of the soil profiles sampled. It appeared that these bacteria numbered approximately 10^2 to 10^3 per gram of soil.

As has been discussed, sulfur oxidation under conditions conducive for cement degradation (production of sulfuric acid) suggested that *T. thiooxidans* and *T. ferrooxidans* were the sulfur-oxidizing organisms of choice for use in developing part of a standardized test. Accordingly, an effort was made to selectively isolate these particular sulfur-oxidizing organisms from the collected environmental samples. Isolates from the various environmental samples were cultured under conditions conducive to long-term maintenance of pure cultures.

MATERIALS AND METHODS

Prior work on this program (Rogers et al. 1994) provides information on the materials and methods that were used to develop a microbial culture collection from disposal sites, develop procedures for growing candidate microorganisms, determine the microorganisms capable of cement degradation, and develop testing methodology and criteria for evaluation of test effects. Therefore, these details are not provided in this document. Further work has been ongoing on the latter tests with a focus on waste form failure criteria.

It was necessary to designate some occurrence or change as failure criteria for the cement specimens. Physical, chemical, and biological parameters are being investigated. Before specimens were tested, their dry weight and physical dimensions were determined. In addition, a total chemical analysis was performed on INEL-fabricated and vendor-supplied simulated waste forms. Total quantities of Ca, Al, and Si were obtained so that the percentages of these elements leached from the waste forms during the evaluation could be determined. Also, the physical effects (color change, swelling, spalling, crumbling) were documented with photographs.

EXPERIMENTAL RESULTS

Development of Testing Methodology

It has been determined that standard biodegradation tests (such as ASTM G21 and G22) are not applicable for use with some materials used to solidify LLW (Rogers et al. 1994). Therefore, testing methods were devised and evaluated using a selected microorganism from each of the three different genera known to degrade concrete. The evaluations were designed using the optimum conditions of growth, type exposure to the test specimen, and reaction time to promote degradation of simulated, cement-solidified LLW. This approach was used to provide the necessary flexibility to be able to conduct an evaluation of microbial activity, exposure methodology, specimen size, and test duration. It was intended that the newly developed biodegradation tests be very conservative. That is, they were designed to determine if microbial activity or the products of this activity affect the integrity of cement-solidified waste forms in a short time frame, quantifiable by physical or chemical observations.

A conservative method was developed that provided candidate organisms with optimum growth conditions while ensuring that the waste forms were provided a maximum opportunity for exposure to microorganisms and their metabolic products. This was accomplished by using a microbial propagation system coupled with a specimen exposure chamber (Figure 1). The candidate microorganisms were intended to be introduced to the test specimens in several ways, including continual immersion in which specimens were completely immersed in a continual flow of solution, as well as intermittent immersion and intermittent misting. The evaluation of these methods was stated previously, and some test results have been reported (Rogers et al. 1994). Results from the remaining tests are provided in this report.

Preliminary work with each test method was conducted using simulated waste-form specimens that contained a similar mixture of cement and waste as that of actual waste forms, but without the radioactive contamination. This provided the opportunity to determine, under the conditions of the testing methodology, how candidate organisms might react to actual solidified LLW. Representative formulations for the INEL-fabricated simulated LLW waste-form specimens and the control (cement mortar waste-form specimens)

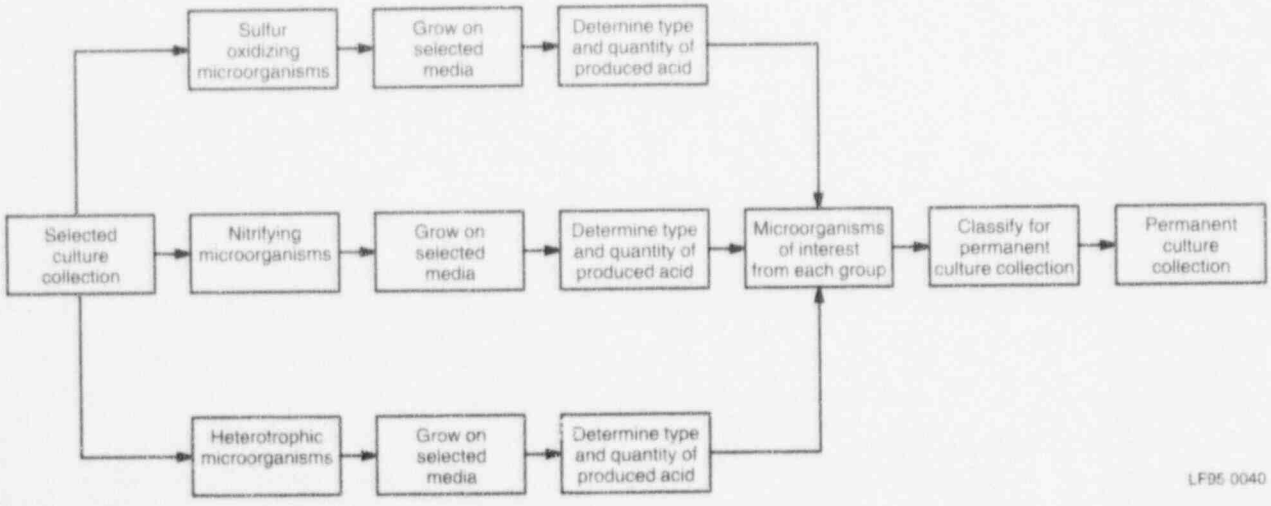


Figure 1. Schematic of proposed methods for testing the effects of MID on cement waste forms.

are found in Figure 2. They were similar to those listed by Colombo and Neilson (1979). The formulation of the control cement mortar was as specified in the ASTM "Standard Test Methods of Compressive Strength of Hydraulic Cement Mortar" (ASTM 1990) and included Portland type II cement, quartz sand, and water mixed at a 1:2.75:0.5 ratio. For the waste containing salts, the formulation includes sodium sulfate, ammonium sulfate, and sodium chloride in water mixed at a ratio of 1:1 Portland type II cement to waste. The simulated ion-exchange resin waste contained ion-exchange resin and water mixed at a 1:1.5 ratio Portland type II cement to waste. All of the simulated waste form specimens (including the ASTM formulation) manufactured at INEL were molded as cylinders 1.8 cm in diameter by 2.2 cm long.

Vendor-supplied, simulated waste forms were also used. They included both ion-exchange resin and boric acid salts formulations. The vendor's process and formulations for waste form production are proprietary; however, it is known that the formulation includes a pozzolanic cement mixture. The vendor used its standard manufacturing methods to produce simulated, solidified ion-exchange resin and evaporator bottoms waste forms molded into cylinders 5 cm by 10.2 cm. Before use, these waste forms were cut into pie-shaped specimens 3 cm wide, 2.5 cm long, and 1 cm thick. These simulated waste-form specimens were then tested in a manner similar to the INEL-supplied simulated waste-form specimens using the testing matrix shown in Figure 3. The two types of simulated waste-form specimens and the control cement mortar specimens were exposed to selected MID species from the three genera of microorganisms. Control solutions of sterile nutrient feed were also used for each set of experiments. The exposure time had to be determined for each testing scenario because the time requirement for observable effects was not known.

In a final test, radioactive waste-form specimens from two actual commercial nuclear power plant waste forms were evaluated. Those waste forms, 5 cm in diameter by 10 cm high, were obtained by INEL personnel during solidification of the plant wastes. The first waste form was composed of decontamination ion-exchange resin in a proprietary formulation of pozzolanic cement from the Peach Bottom Atomic Power Station Unit 3. The second consisted of filter sludge in a proprietary, pozzolanic cement mixture from the Nine Mile Point Nuclear Plant Unit 1.

Total Immersion.

Heterotrophic Bacteria. Preliminary work has been conducted with a strain of *Pseudomonas cepacia*. As mentioned earlier, it was selected as representative of the organic acid-producing heterotrophs. Results from this work suggested that during the test (60 days), the buffering capacity of the cement formulations was sufficient to resist chemical attack from the organic acids produced by *Pseudomonas* (Rogers et al. 1994). Therefore, no further testing was conducted with the heterotrophic species.

Nitrifying Bacteria. Although nitrifying bacteria produce acidity through their metabolic activity, most of these bacteria grow optimally in buffeted systems in which the pH is maintained between 7.5 and 8.5 (Schmidt and Belser 1982). Unlike thiobacilli whose metabolic activity results in overwhelming the buffering capacity of cement-derived calcium hydroxide, nitric acid formation by nitrifying bacteria is continuously being neutralized by the loss of the calcium constituent.

The continuous flow reactor system was evaluated (as described in a previous section) for application to the nitrifying bacteria using mixed cultures of *Nitrosomonas* and *Nitrobacter* (Rogers et al. 1994). Data from this work showed that the continuous flow reactor system was not suitable for optimum growth of these bacteria. Also, results showed that it was not necessary to use mixed cultures of the ammonia-oxidizing and nitrite-oxidizing bacteria to achieve optimum mineral acid production. In addition, the growth of these bacteria under continuous flow bioreactor conditions required the addition of a buffer to maintain slightly alkaline growth conditions.

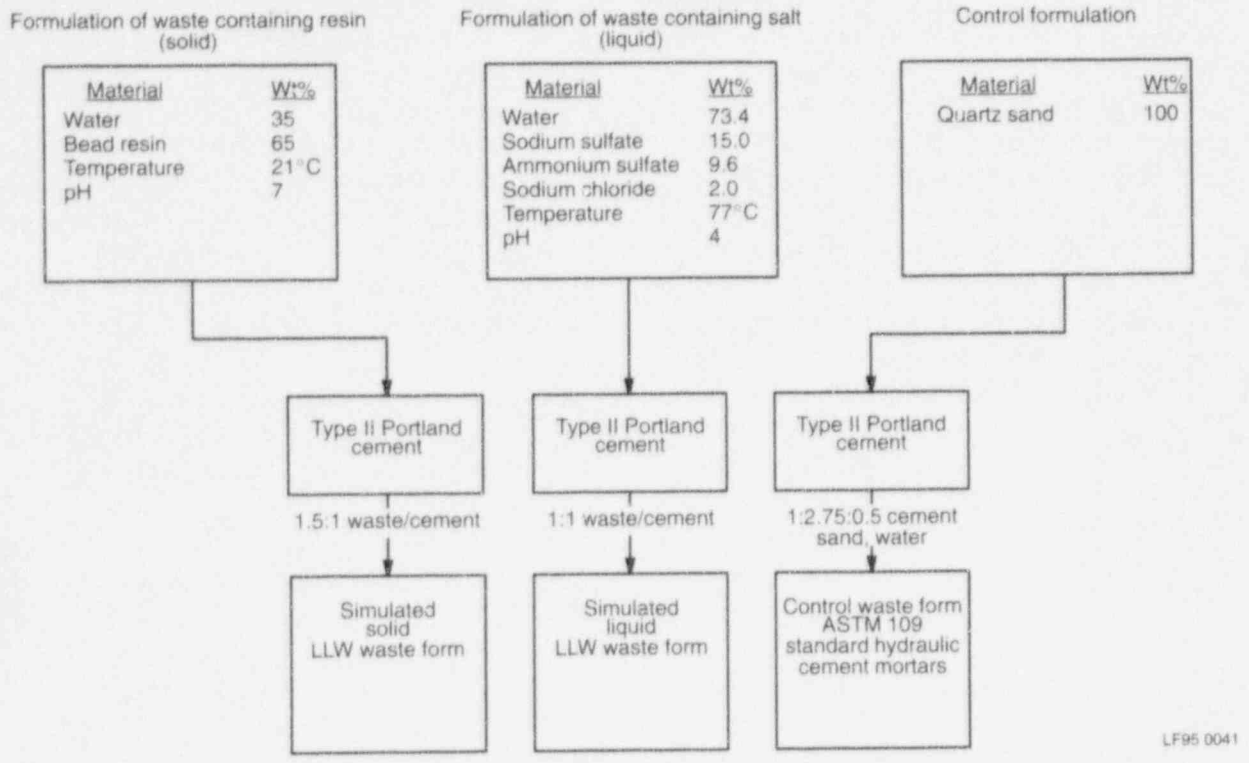


Figure 2. Examples of possible composition of simulated low-level waste forms.

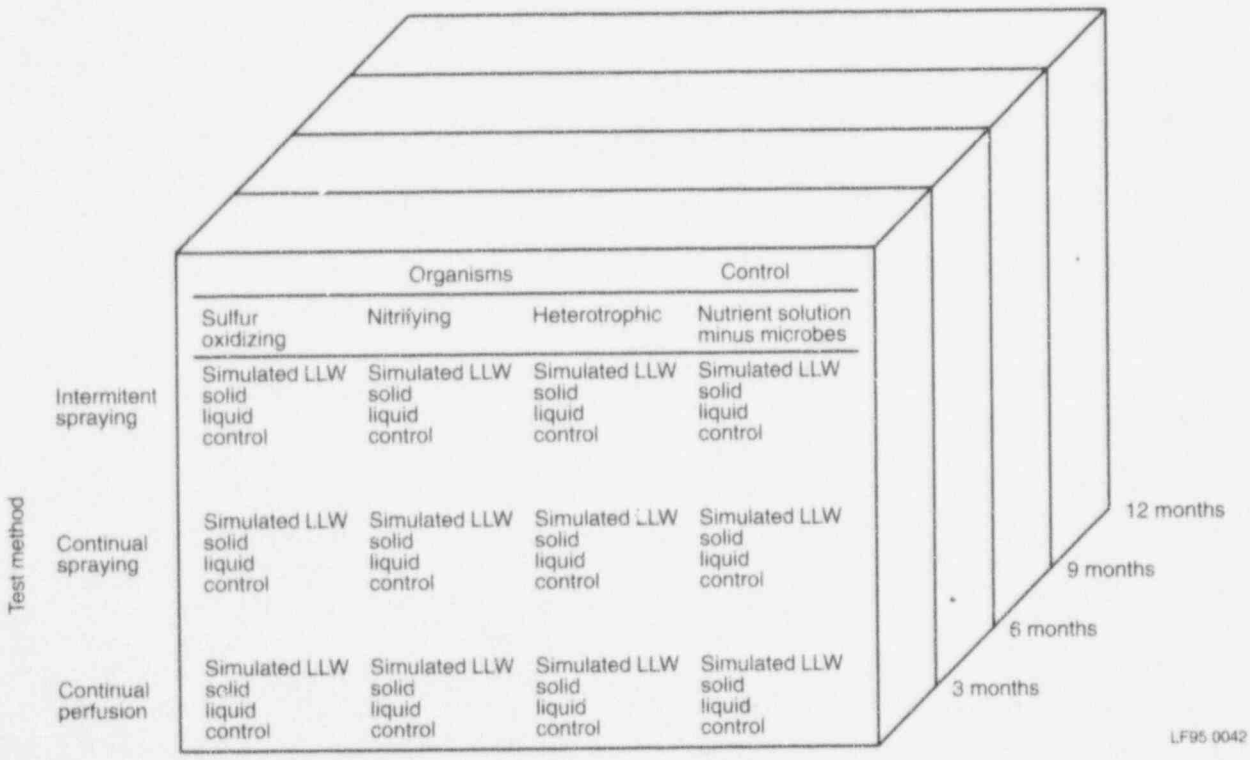


Figure 3. Matrix for testing simulated low-level waste forms.

Therefore, the use of the continuous flow design for waste form exposure was discontinued for these microorganisms and another design was evaluated.

A bioreactor design used successfully by other researchers to grow nitrifying bacteria was based on a fed-batch design. Fed-batch reactor systems are particularly suited to the growth and monitoring of slow-growing microorganisms (Grady and Lim 1980; Bailey and Ollis 1986). They combine the desirable aspects of both continuous-flow and batch reactor systems. This year, an evaluation of the fed-batch system was initiated to determine if it satisfied program needs. A pure culture of the ammonia-oxidizing bacteria *Nitrosomonas europea* was inoculated into nutrient solutions (see Table 1 for content). Preliminary work with the method was encouraging. It was possible to monitor the solution concentrations of ammonia and nitrite, thus providing a quantitative assessment of acid production. Also, the concentration of Ca in exposed media was monitored to determine the quantity of cement-based Ca that was solubilized. The results of the evaluation were sufficiently encouraging to warrant further studies.

Waste forms used in the initial fed-batch study were the INEL-supplied simulated resin and salts and the ASTM control. The study was designed so that one each of the simulated waste forms was exposed to both the biotreatment and a sterile medium control. To accomplish this, sterilized waste forms (ethylene oxide treatment) were placed individually into six 250-mL flasks. Sterile medium (~100 mL) inoculated with *N. europea* (with a final concentration of 1×10^7 cells/mL) was added to flasks containing representative samples of either the two waste forms or the ASTM control (treatments). Sterile medium without inocula was added to the remaining three flasks (sterile controls). All the flasks were sealed with sterile rubber stoppers modified to accept influent and effluent tubes for the exchange of medium. Prepared flasks were then placed into a covered, insulated water bath and incubated at 28°C in the dark. Every 48 hours, the liquid in all flasks was removed and replaced with fresh sterile medium (this medium was automatically inoculated in those flasks containing the microbial treatment). The pH of the collected effluent was recorded, followed by quantitative determination of the nitrite concentration. A decrease in solution pH with a concomitant increase in the concentration of nitrite were used as an indication of metabolic activity by the bacteria. Confirmation of bacterial growth was obtained by direct counts of planktonic cells (unattached) using light microscopy.

Table 1. Results of most probable number enumeration of pellet surfaces.

	Number of cells (cm^{2-1})					
Sampling day	ASTM inoculated	Vendor inoculated	PWR inoculated	ASTM control	Vendor control	PWR control
Day 26	8.5×10^4	$>1.2 \times 10^6$	8.0×10^5	<12	<12	<12
Day 61	2.2×10^5	$>1.2 \times 10^6$	8.0×10^5	<12	<12	<12
Day 113	4.8×10^4	9.0×10^4	3.2×10^4	<12	<12	<12

PWR = INEL-supplied specimens.

Results from the first week of an initial test indicated that the bacteria were not active. There had been no increase in nitrite content; solution pH had not decreased from an initial high of between 11 and 12; and direct counts showed that cell numbers were decreasing. The apparent reason for the lack of microbial viability was an alkaline solution pH (>9). *Nitrosomonas* are known to prefer a pH range between 7.5 and 8.5 (Bock, personal communication, 1993). However, data from the literature (Diercks et al. 1991; Mansch and Bock 1992) indicate that these bacteria do grow on the surface of concrete under natural and laboratory conditions. The question of how to initiate growth on fresh concrete was answered by Bock. He pointed out that before growth occurs on fresh concrete test specimens, it is necessary to condition the exposed concrete surfaces to duplicate the natural process of carbonization (carbonate formation) that results in a decrease of surface pH. The conditioning process has been carried out by Bock in test chambers by allowing medium to percolate over a specimen for several weeks before attempting inoculation. We elected to accelerate conditioning by immersing specimens in sterile water fortified with weak carbonic acid generated by bubbling with carbon dioxide. It was found that a 1 to 2-week treatment (depending on waste formulation) in the resulting solution was sufficient to duplicate the natural process that reduces surface pH to near 8.5. Conditioned specimens were then used in subsequent studies.

Results 48 hours after initiation of the second study using conditioned simulated waste-form specimens indicated that the *N. europea* bacteria were able to tolerate the presence of the test specimens. By day 12, the pH of the inoculated solutions had decreased to 6.0, 8.5, and 7.1, respectively, for the ASTM control, resin, and salts waste forms. In addition, nitrite concentrations had increased to 6, 5, and 6 mM, respectively. Results at the end of the 66-day study clearly demonstrated a relationship between pH and nitrite production. It was seen that as pH decreased, the concentration of nitrite increased (an inverse relationship). The pH during the study fluctuated, but the average remained close to the day-12 values. A total of 5.5, 1.8, and 4.9 mM of nitrite was produced in the ASTM, resin, and salts waste-form specimen treatments, respectively. This corresponded to 0.34, 0.11, and 0.31 mL of concentrated nitric acid. The continued production of relatively stable amounts of nitrite showed that bacterial activity was maintained each time after medium replacement.

It was noted in this study that after 2 weeks of incubation, the treated waste-form specimens had become contaminated with an additional microbial species. Biochemical tests identified the contaminant as *Pseudomonas fluorescence*. This is a heterotrophic bacteria that is capable of growth using only minute quantities of organic carbon. Initially, there was concern that these microbes could affect the activity of the *N. europea*. However, the contamination of the simulated waste-form specimens in the sterile medium (after 45 days) showed that the presence of *P. fluorescence* had no effect on solution pH or on the production of nitrite. Sterile cotton swabs were used to obtain surface samples of all the waste-form specimens to determine if colonization by with either species of bacteria had occurred. Results from spreading on a heterotrophic medium and incubation of inoculated sterile nitrifier medium showed that both species of bacteria had successfully colonized the surface of the specimens.

At the end of 66 days, the waste-form specimens were retrieved and prepared for analysis. Their physical appearance can be seen in Figures 4 through 9. The ASTM controls and salts formulations of both treatment and controls remained intact while the resin waste-form specimens disintegrated. Those specimens exposed to the microbial treatment showed some color change as well. A small portion of each specimen was taken for image analysis using environmental scanning electron microscopy (ESEM). Using a sterile spatula, surface scrapings were taken from these portions for further study using appropriate staining techniques. The remainder of each portion was rinsed in sterile water to remove planktonic cells. This was followed by sonication of the solids in sterile phosphate buffer to remove attached biofilm and/or individual bacterial cells.

94-102-1-18

Figure 4. ASTM control waste-form specimens after being immersed for 66 days in sterile nitrifier medium.

94-102-1-26

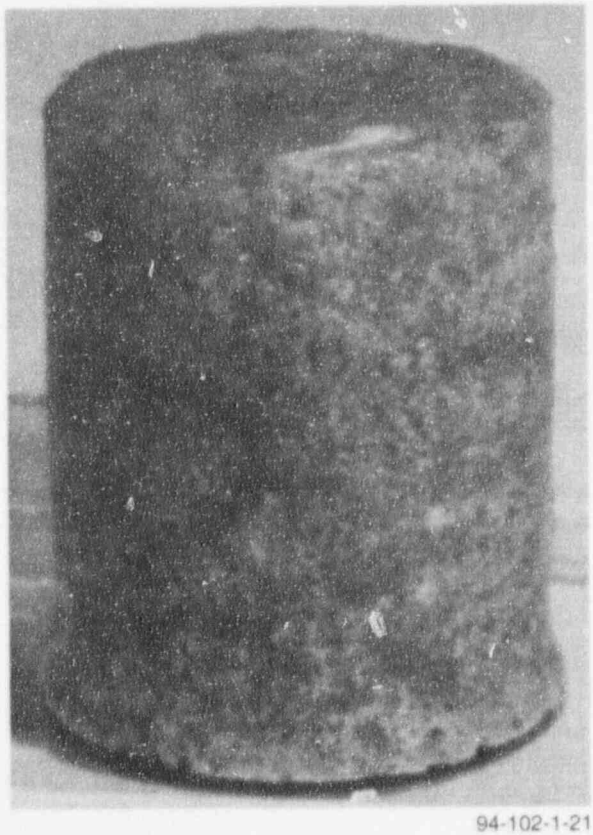
Figure 5. INEL-supplied simulated evaporator bottoms waste-form specimen after being immersed for 66 days in sterile nitrifier medium.

Aliquots of those solutions were incubated in the nitrifier medium to confirm the presence of nitrifying bacteria adhering to the waste form surface. The surface scrapings were subjected to a combined staining procedure designed to detect both the presences of biofilm exopolysaccheride (EPS) and bacterial cells. Alcian blue stain was used to highlight the presence of biofilm EPS, while acridine orange stain was used to show the presence of viable bacteria imbedded in the biofilm. Examination of the stained material was conducted with both light and fluorescence microscopy. In addition, the surface pH of each waste-form specimen was determined.

Examination with the ESEM showed the presence of an amorphous material on the surface of all waste-form specimens (both treatment and sterile control) (Figures 10 through 14). Experts familiar with biofilm formation identified this material as an organic-mineral matrix consistent with biofilm formation. The presence of some individual bacterial cells was also detected, but it was assumed that most of them were immersed in the biofilm. Confirmation of these observations was obtained from the staining procedure (Figure 15). Note that the presence of the background blue color confirms the presence of biofilm EPS material (Figure 15a) while the bright, fluorescing orange oval shapes indicate the presence of viable cells associated with the biofilm matrix (Figure 15b). Results from plating of the surface scrapings confirmed the earlier findings obtained from swabbing the surfaces of the waste-form specimens. *P. fluorescence* was present on the surface of all waste form specimens, and in addition, *N. europea* was identified by most-probable-number analysis (Schmidt and Belser 1982) as a co-colonizer of the treated waste-form specimens. All of the data clearly demonstrate that bacteria were capable of colonizing the

94-102-1-22

Figure 6. INEL-supplied simulated ion-exchange resin waste-form specimen after being immersed for 66 days in sterile nitrifier medium.



94-102-1-21

Figure 7. ASTM control waste-form specimen after being immersed for 66 days in medium containing nitrifying bacteria.

94-102-1-29

Figure 8. INEL-supplied simulated evaporator bottoms waste-form specimen after being immersed for 66 days in medium containing nitrifying bacteria.

94-102-1-25

Figure 9. INEL-supplied simulated ion-exchange resin waste-form specimen after being immersed for 66 days in medium containing nitrifying bacteria.

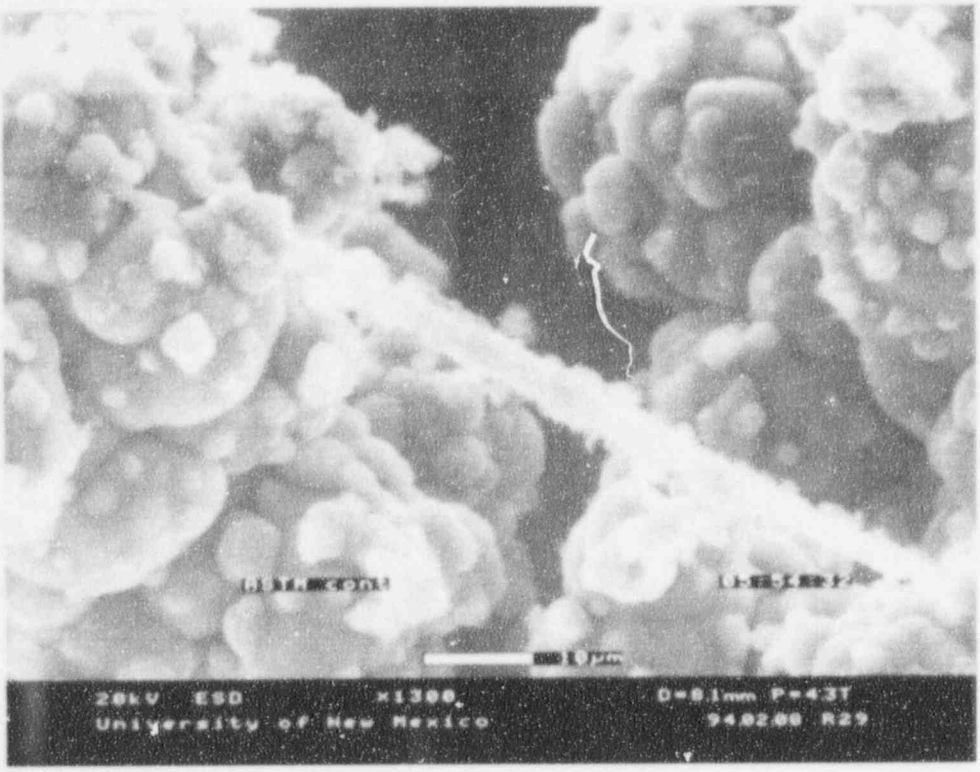


Figure 10. ESEM photograph of ASTM control waste-form specimen after exposure to sterile medium.

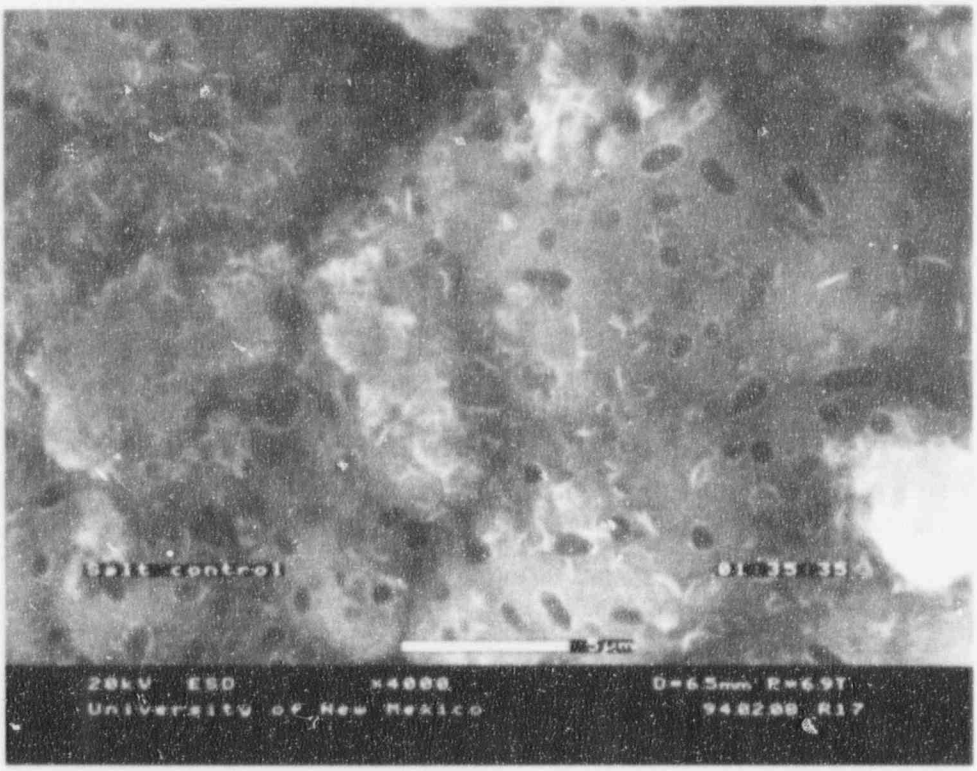


Figure 11. ESEM photograph of evaporator bottoms INEL-supplied simulated waste-form specimen after exposure to sterile medium.

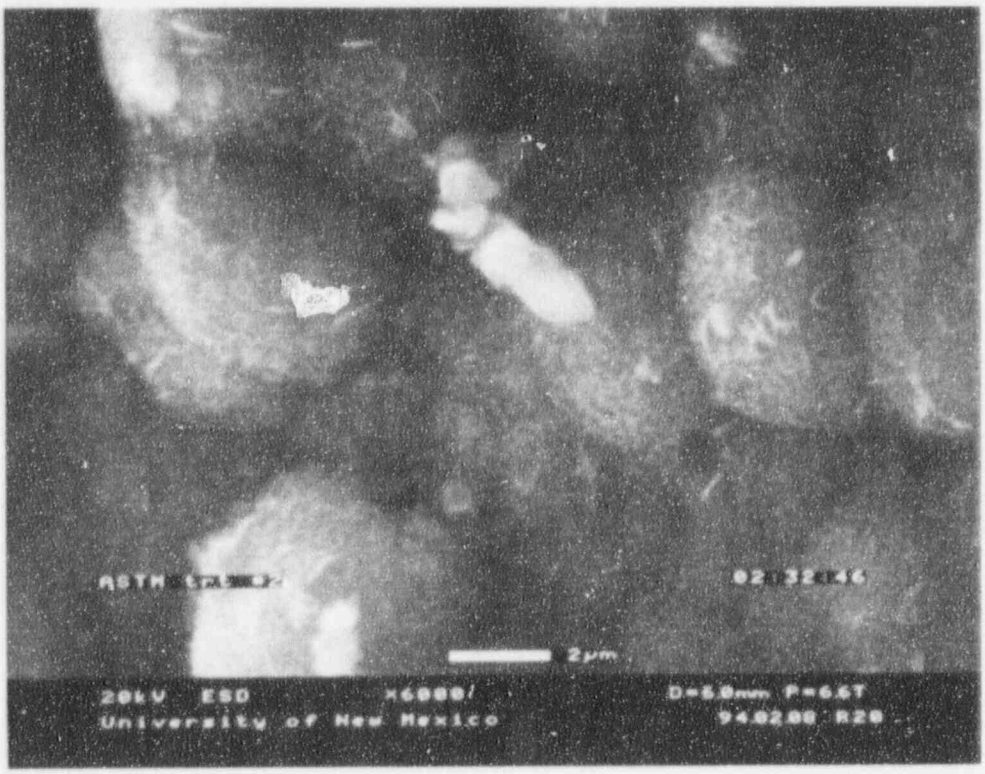


Figure 12. ESEM photograph of ASTM control waste-form specimen after exposure to nitrifying organisms.

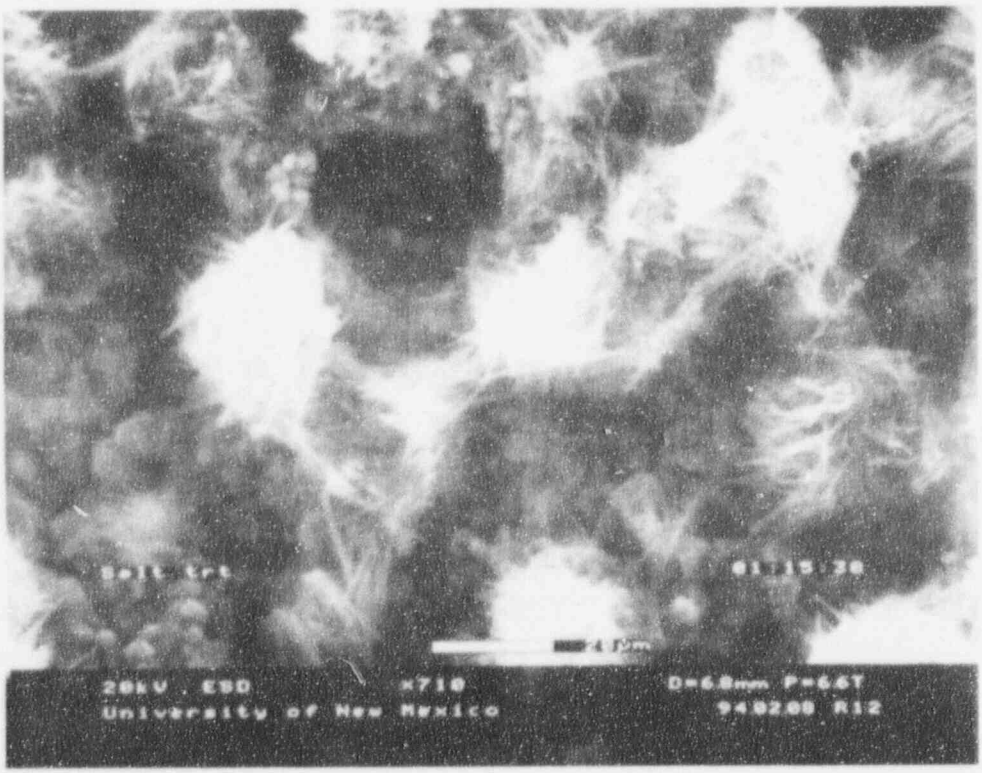


Figure 13. ESEM photograph of evaporator bottoms INEL-supplied simulated waste-form specimen after exposure to nitrifying organisms.

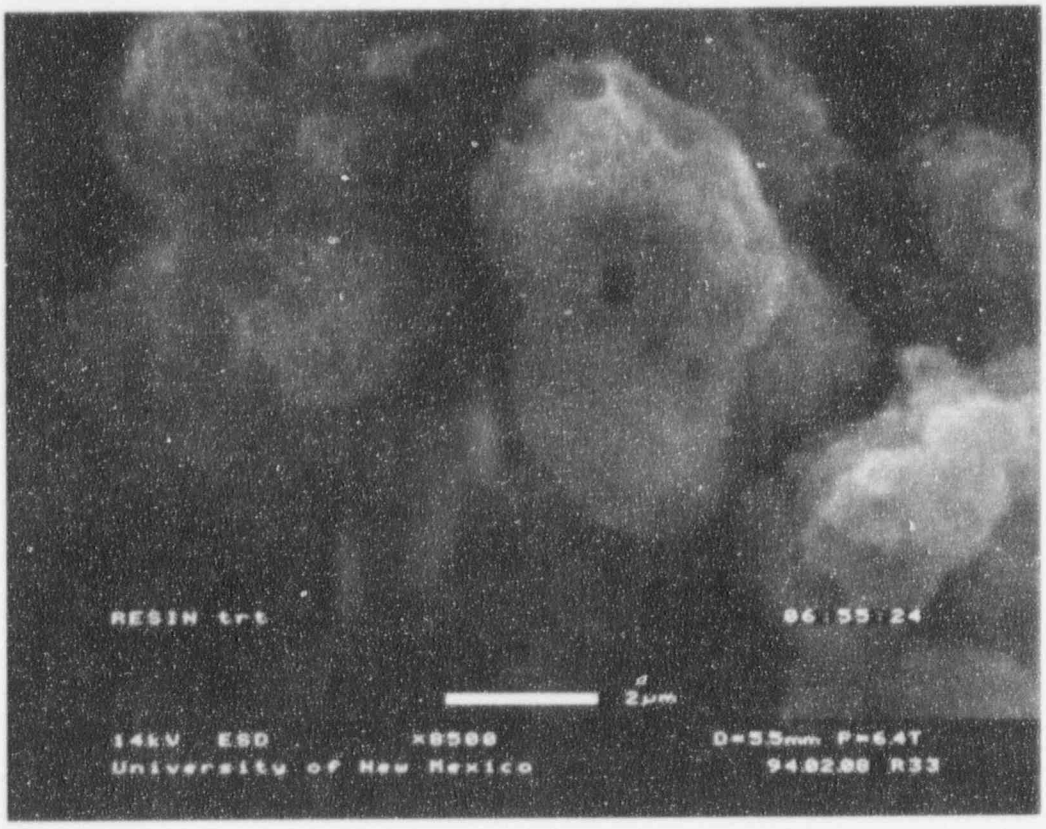
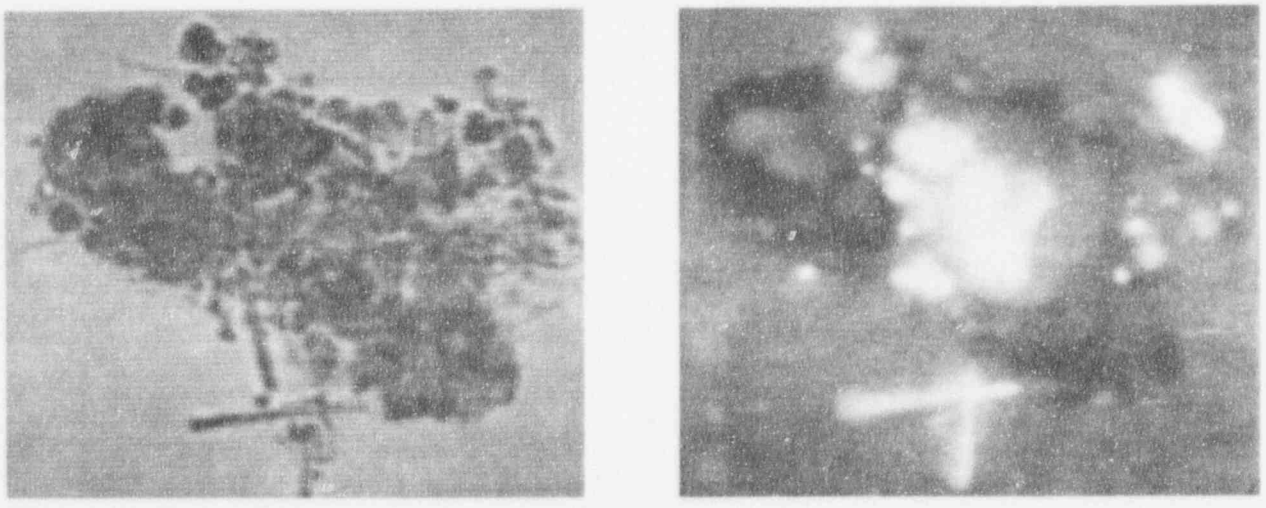


Figure 14. ESEM photograph of ion-exchange resin INEL-supplied simulated waste-form specimen after exposure to nitrifying organisms.



(a) (b)

Figure 15. Biofilm staining of INEL-supplied simulated waste-form specimen after exposure to nitrifying organisms (a) stained blue to demonstrate presence of biofilm EPS material and (b) fluorescent orange stain demonstrating presence of viable cells.

surface of the simulated cement waste-form specimens.

The colonization by the *N. europea* resulted in the beginning of an MID attack. This is confirmed by the increased loss of Ca in the treated samples. The percent loss of Ca from the INEL-supplied simulated waste-form specimens exposed to *N. europea* is summarized in Figure 16. The ASTM standard and the evaporator bottoms waste-form specimen each had a greater loss of Ca due to the nitrifying microbe (~12 and 6%, respectively) than they did from the sterile medium (~3%). The ion-exchange resin waste-form specimens exposed to the biotreatment and the sterile medium both lost comparable quantities of Ca (~17%). The increased loss of Ca demonstrates that the activity of the nitrifying bacteria was beginning to affect the chemical integrity of two of the test specimens. It is apparent, however, that a more thorough evaluation of the effects of *N. europea* will require a test period longer than 60 days.

In the next study, the vendor-supplied simulated ion-exchange resin and the evaporator bottoms waste-form formulations were used. In addition, the ASTM formulation was included as the control waste form. Before use, the simulated waste forms and control were sliced into pellets approximately 0.5 cm thick. For the vendor-supplied waste forms, this resulted in pie-shaped pellets approximately 0.5 cm thick with 0.9 cm × 0.9 cm × 1.3 cm side dimensions, with a bisecting groove cut nearly to the depth of pellet thickness. Before the pellets were used, they were exposed to the carbonization process (as described for the previous study) and then sterilized with ethylene oxide. Sterilized pellets were then transferred aseptically into 250-mL Erlenmeyer flasks (five each), which were subsequently immersed in the 28°C water bath, and the cover was replaced.

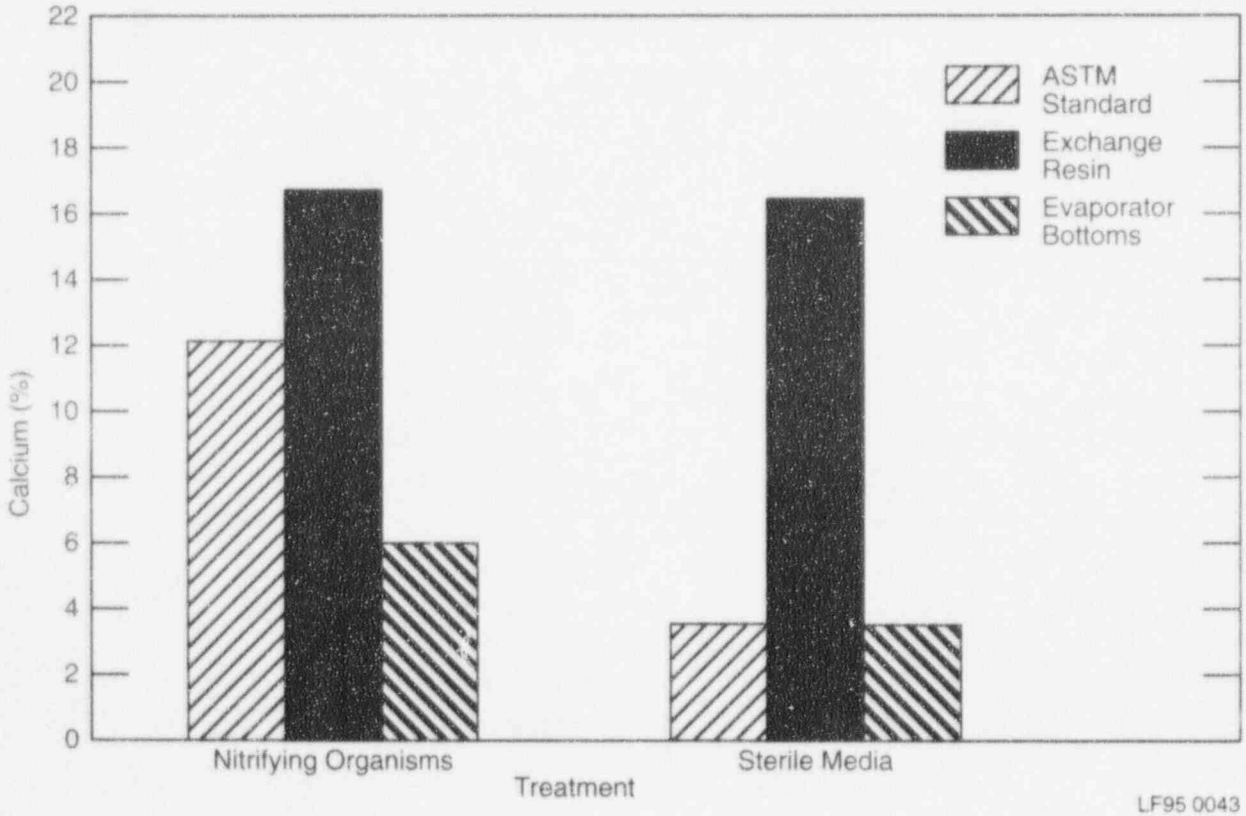


Figure 16. Quantities of Ca leached from INEL-supplied simulated waste forms after 60 days of total immersion (nitrifying organisms).

Following that, each of the treatment flasks (a total of three) was inoculated through the inlet tube with approximately 100 mL of an *N. europea* solution (strain "freitag" 1×10^7 cells/mL), which entirely immersed the pellets in culture solution. The pellets in the three control flasks were immersed in sterile medium. The *N. europea* strain had been cultured in the dark before pellet inoculation in 500 mL of ammonia-oxidizer growth medium (Schmidt and Belser 1982).

After 2 days to allow for pellet colonization, the solution from both inoculated and control reactor flasks was removed and replaced with fresh ammonia-oxidizer growth medium. After the specimens were incubated for an additional 17 days (day 17), there was a second 3-day period of colonization following the procedures described above. For the duration of the study, a regime was established of growth medium removal and replacement every 3 to 10 days.

Growth medium removed from both inoculated and control reactor flasks was analyzed for nitrite and Ca concentrations, pH, and microbial number. Solution concentrations of nitrite were quantitatively determined using a form of the Greiss-Ilosvay colorimetric test (Garret and Nason 1969) in which nitrite diazotizes sulfanilamide followed by coupling to N-(1-naphthyl)-ethylenediamine dihydrochloride. Microbial numbers were obtained by direct counting using a Hausser Scientific brightline hemacytometer with 400× phase contrast microscopy.

Besides periodic analysis of the liquid, one of the original five pellets was removed from each treatment and one from the control reactor (for a total of six samples) on days 26, 61, and 113. A sterilized chisel was inserted into the bisecting groove in each pellet to effect separation into two, approximately equal subsamples. After rinsing one subsample with sterile phosphate buffer to remove planktonic cells, the surface was scraped and then scrapings and pellet subsamples were sonicated for 5 minutes in 10 mL of phosphate buffer. A most-probable-number method (Schmidt and Belser 1982) was then used to enumerate ammonia-oxidizing bacteria present in the buffer solution. The second pellet subsample was prepared for viewing with a scanning electron microscope (SEM). This was accomplished by fixing it in 10 mL of 2% glutaraldehyde aqueous solution for 1 hour, followed by successive 15–20 minute treatments in 10 mL of 10%, 25%, 50%, and 75% ethanol in aqueous solution. The subsamples were air-dried and then sputter-coated with 30 nm of Au-Pd under high vacuum. Then, the SEM was used to inspect pellet subsample surfaces for the presence of nitrifying bacteria.

Figures 17 through 20 summarize data collected over 194 days. Figure 17 displays nitrite accumulation in inoculated and control reactor flasks. These data show that no nitrite was produced in any of the control reactors, whereas inoculated reactors had a very consistent accumulation of 1–2 mM nitrite between sampling events from about day 40 onward. This suggested that there was stable activity of *N. europea* in the inoculated reactors. A total of 5.9, 5.6, and 5.1 mM of nitrite were produced in the inoculated ASTM control, resin, and salts waste-form reactors, respectively. This corresponded to 0.37, 0.35, and 0.31 mL of concentrated nitric acid. These data are similar to day 66 and show little increase in the nitrite content. One possible explanation for this is that nitrite under prolonged exposure to conditions of low pH (<pH 5) can undergo chemical oxidation and be lost.

Figure 17 also shows the effect of reinoculation of the treated salts waste-form specimen. Nitrite production in this reactor had decreased to nearly nondetectable levels by day 19. The apparent reason for the lack of continued bacterial activity in this reactor was the initial high pH values, usually above 8.5 and some as high as 9.3 (Figure 18). The elevated pH was due to insufficient carbonization of the waste-form specimens. The inoculated reactor pH values ranged from 5.6 to 6.4 for most of the experimental period, whereas uninoculated controls were generally about 2.5 pH units higher. Results at day 194 clearly show the same

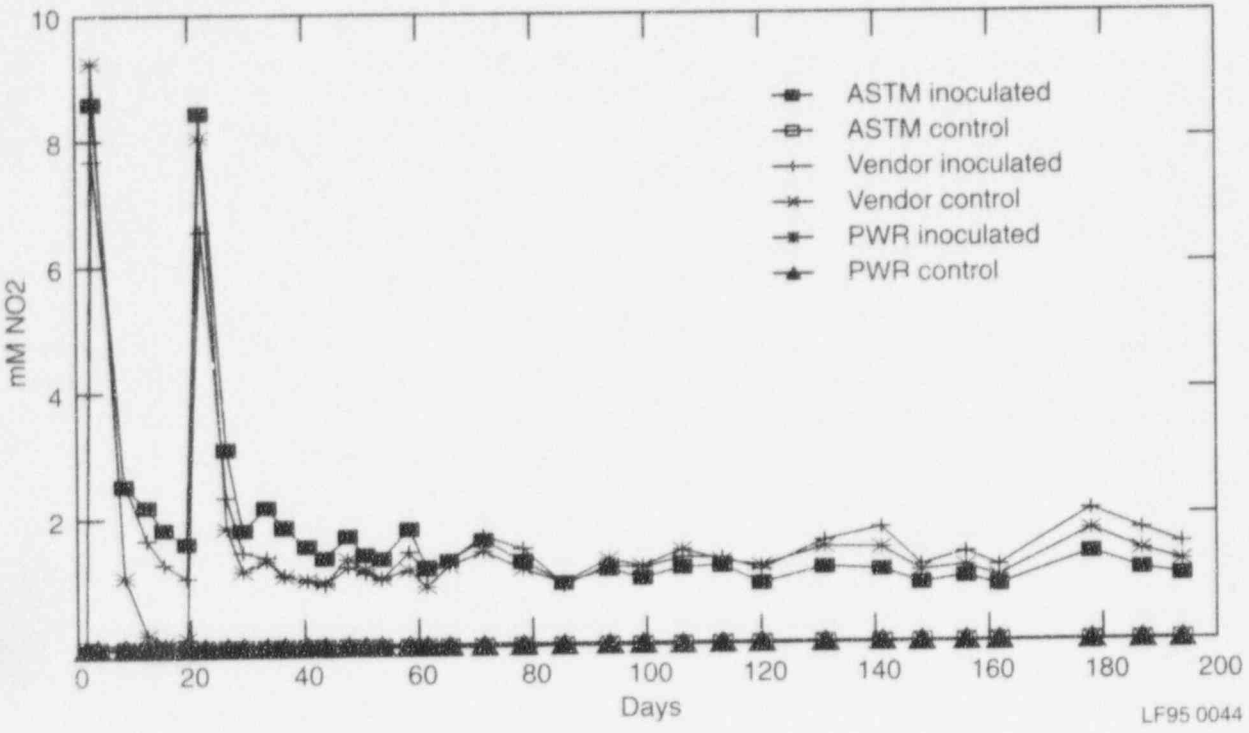


Figure 17. Nitrite content over time of medium containing nitrifying bacteria and vendor-supplied simulated waste-form specimens.

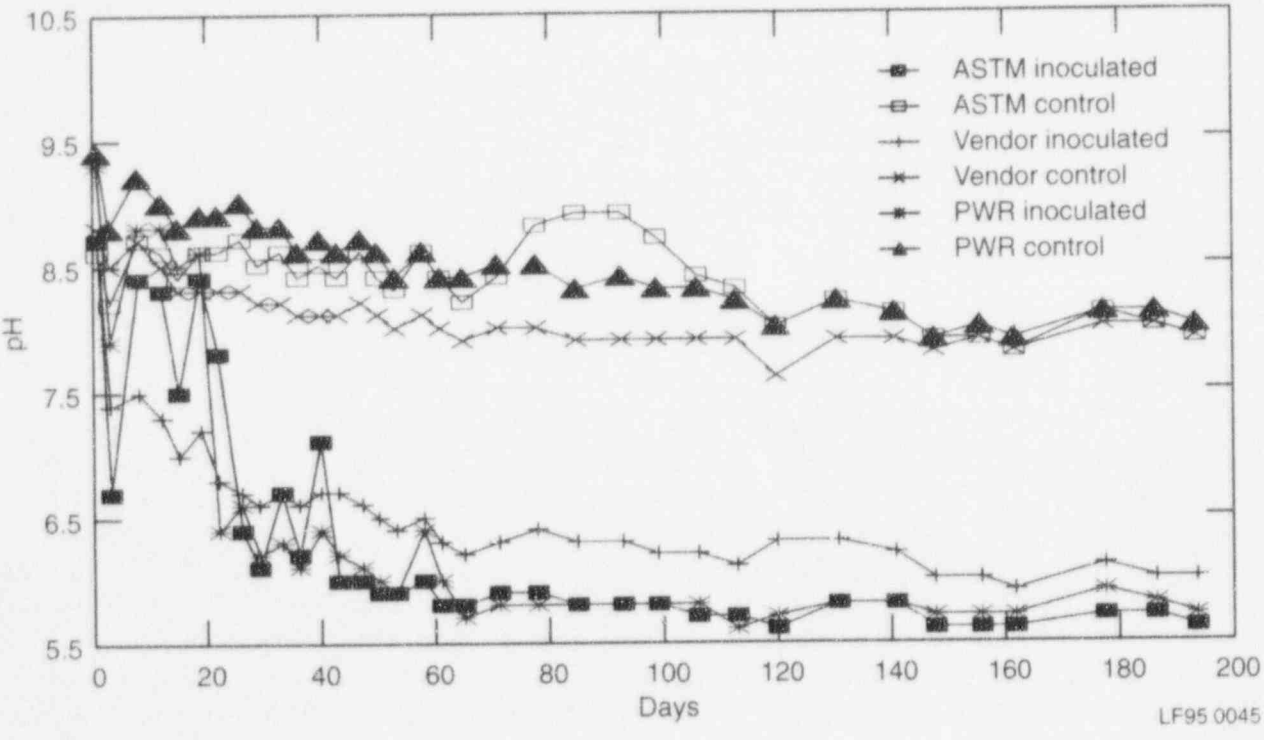


Figure 18. pH over time of medium containing nitrifying bacteria and vendor-supplied simulated waste-form specimens.

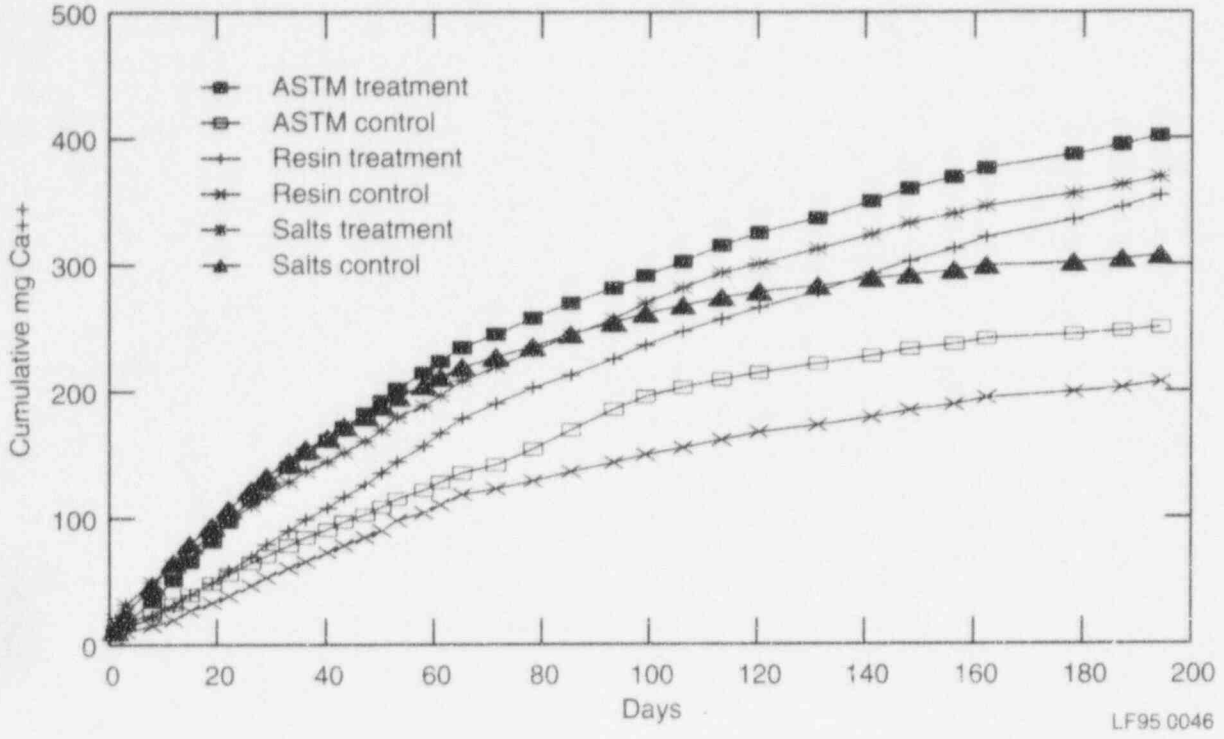


Figure 19. Cumulative Ca released from vendor-supplied simulated waste-form specimens exposed to nitrifying bacteria.

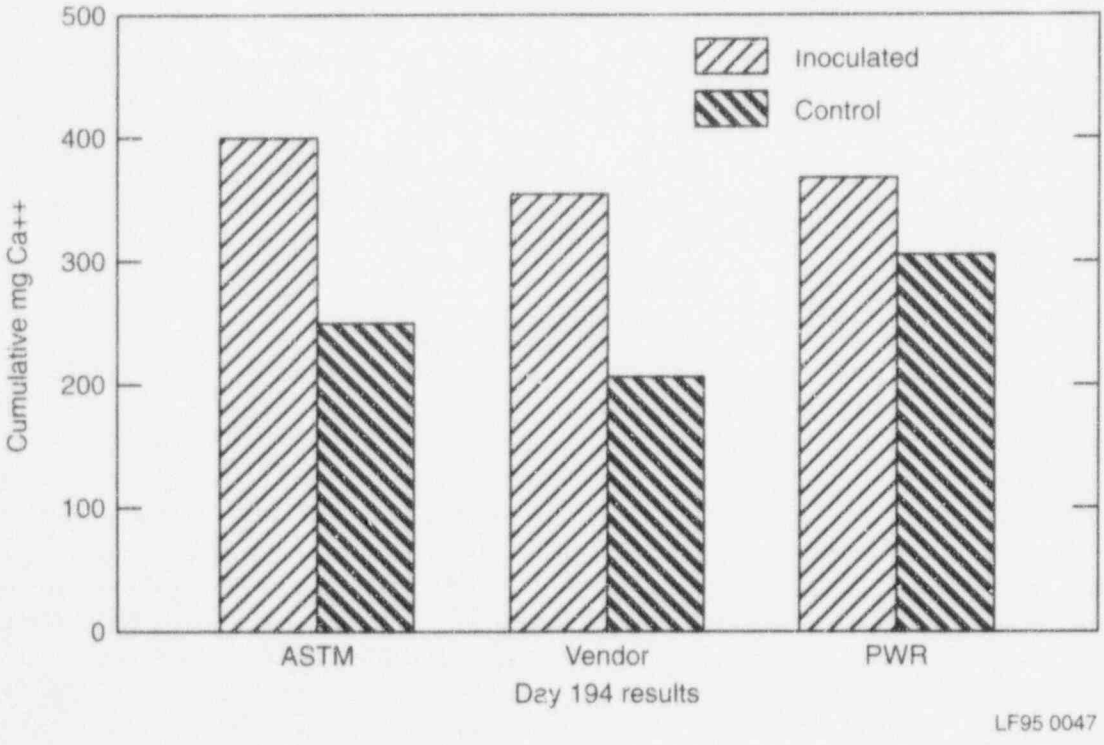


Figure 20. Total Ca released from vendor-supplied simulated waste-form specimens over a period of 194 days.

correlation between pH and nitrite production mentioned at the beginning of this section.

A comparison of the data for cumulative Ca solubilized in inoculated reactors to the data for the uninoculated controls shows that there was microbial influence (lower pH and high nitrite) responsible for an increase in Ca solubilization (Figures 19 and 20). This was particularly apparent for the ASTM and resin-containing pellet reactors. However, for the reactors containing the salts waste-form specimens, the control remained continually higher in cumulative solubilized Ca until about day 120, when levels began to decrease. Conversely, cumulative solubilized Ca continued to steadily increase throughout the study in the reactor that contained the inoculated salts waste-form specimen, finally overtaking the control at about day 135 (Figure 19).

The direct-count results that were used to detect microbial numbers in solution showed that the initial number of 1×10^7 cells/mL had dropped to $\sim 1 \times 10^4$. However, the three most-probable-number enumerations that were conducted on pellet samples removed on day 26, 61, and 113 showed the treated pellets to be heavily inoculated with nitrifiers. These results are shown in Table 1. The respective "> and <" numbers associated with two of the data points for the inoculated resin and with the controls reflect the statistical nature of the most-probable-number enumeration method. Absolute numbers are not expressed because serial dilutions were not carried out past the 10^{-6} dilution and because the 10^{-1} dilutions were not sampled for enumeration. For all intents and purposes, an actual value of zero was assumed for the controls since no nitrite production was ever detected in any of the reactors. It can be concluded from the data that the *N. europea* strain had colonized the pellet surfaces of the inoculated simulated waste-form specimens and the ASTM control by day 26. Day 61 bacterial numbers reflect a relatively stable surface population, which had already been established by day 26, although a 2.5-fold increase is indicated for the ASTM reactor. It is somewhat surprising that day 113 values indicate a general tenfold decrease in the surface population for all of the inoculated reactors. This magnitude of surface population decrease is not supported by the nitrite production data (Figure 17), which indicate relatively stable amounts of nitrite production in all inoculated reactors during days 61 through 113. It is possible that a systematic error involved with surface scraping or sonication effectiveness was responsible. This type of error would occur, for example, if bacterial "sticking efficiency" as a function of EPS or biofilm formation increased.

SEM imaging of respective complementary pellet subsamples also does not support a decrease in the surface bacterial population between days 61 and 113. These images are presented in Figures 21 and 22 and represent selected examples of SEM micrographs from the three respective chronological sampling times: days 26, 61, and 113. Because the SEM images represent only a very small area of the total pellet subsample surface and of the total surface area actually scanned and viewed by the SEM operator, subjective impressions of the operator become important and can provide useful insights in interpreting other relevant data. It should be noted that the ease of locating areas on all pellet surfaces suitably populated with bacteria for imaging increased steadily over the sampling period. For instance, at the first sampling time, it was difficult to find bacteria on the surface of the salts waste-form specimen. However, by day 61, bacteria were apparent, and by day 113, they seemed to be ubiquitous on the pellet surface.

Figure 21a shows an inoculated ASTM pellet and is a 3× magnified image of Figure 21b. In both images, the "pockmarks" and the presence of bacteria is unmistakable. It seems highly likely that a relationship exists between bacteria and pits because of their similar size (~1 μm). These images are similar to others obtained using ESEM imaging of ASTM pellets examined after our initial fed-batch experiment. Figure 21c shows the appearance after 26 days of an uninoculated vendor-supplied resin waste-form specimen. In an image 250× the magnification of Figure 21c, Figure 21d shows a well-colonized resin bead from an inoculated vendor-supplied waste form specimen. Many cells in this image appear to be dividing, suggesting cell viability and growth. Figure 22 shows the surfaces of pellet subsamples

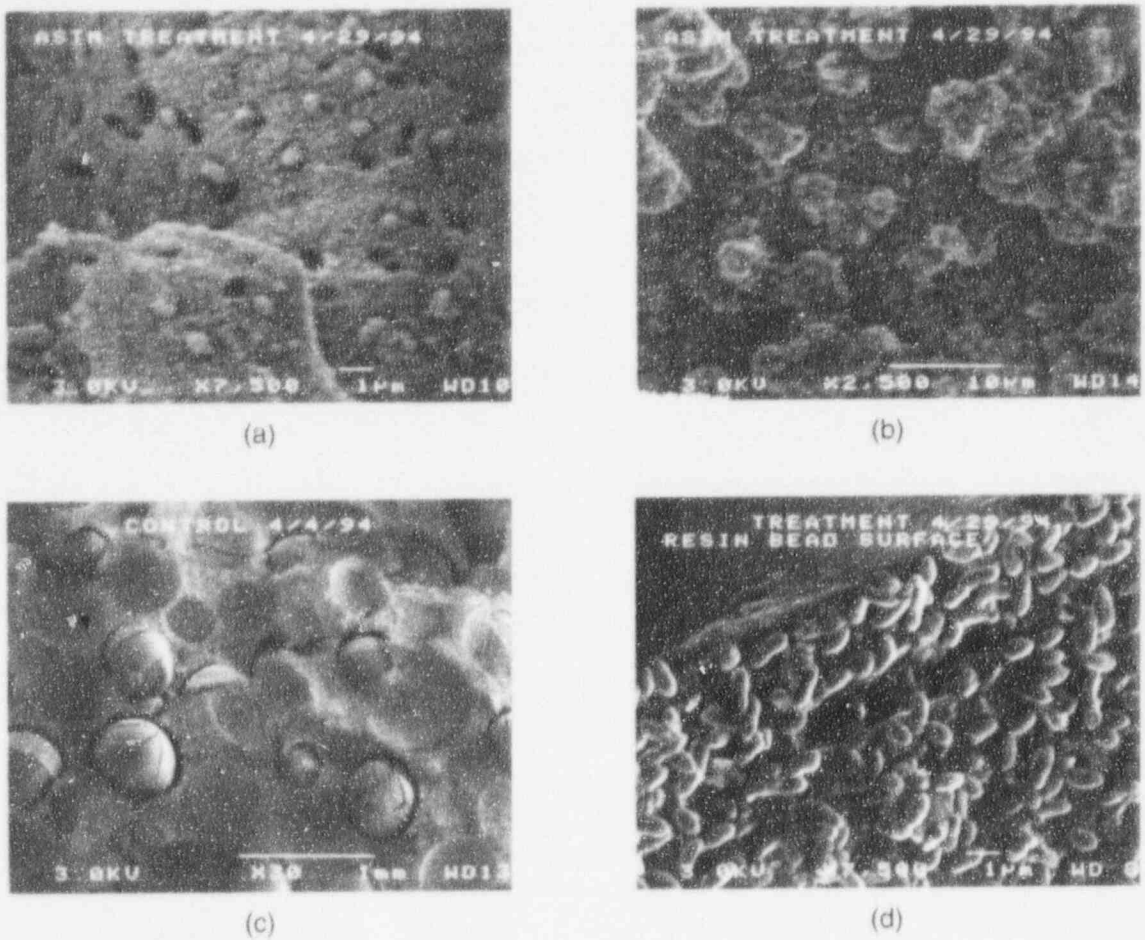


Figure 21. Electron micrographs of ASTM and vendor-supplied simulated waste-form specimens after 26 days exposure to nitrifying bacteria (a) ASTM treatment, (b) ASTM treatment, (c) vendor control, and (d) vendor treatment.

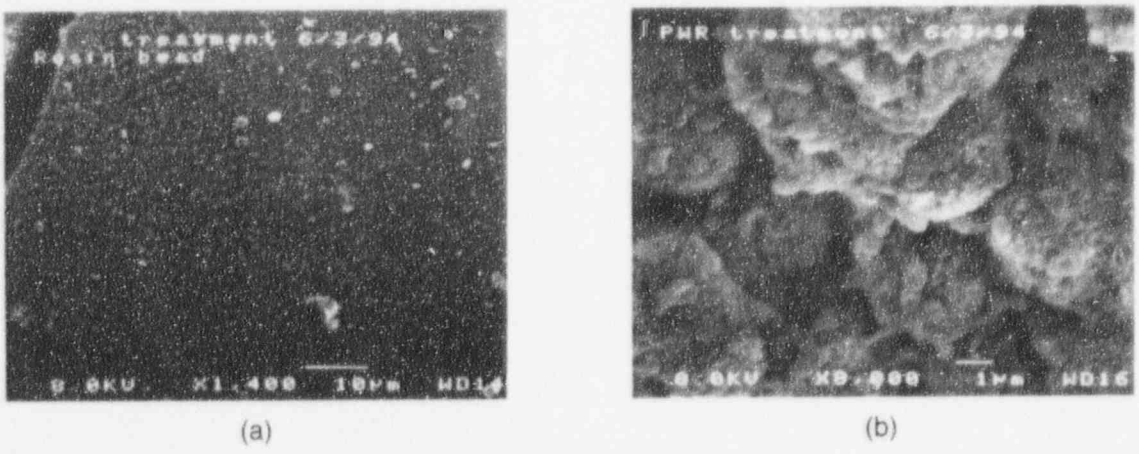
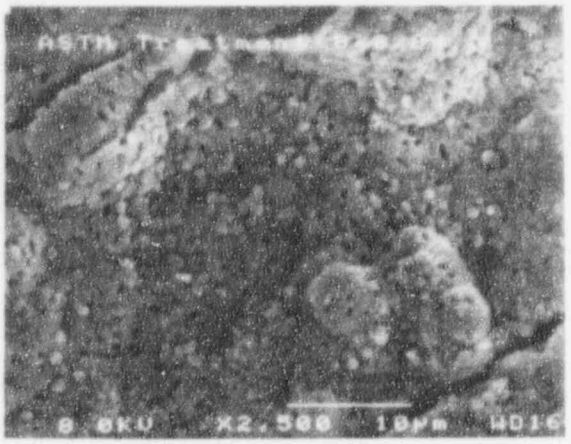


Figure 22. Electron micrographs of vendor-supplied simulated waste-form specimens after 61 days exposure to nitrifying bacteria (a) vendor treatment and (b) PWR treatment.

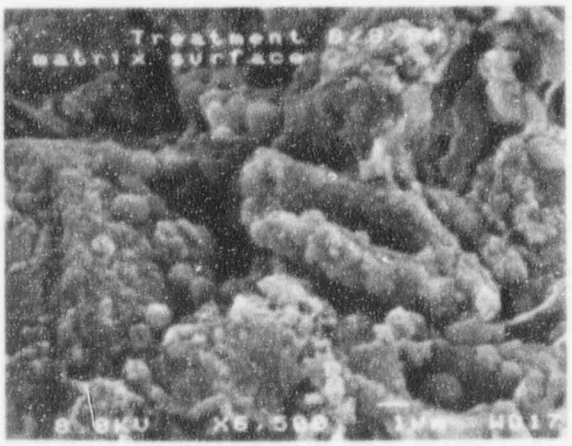
removed at day 61. Figure 22a clearly shows a colonization pattern on a resin bead surface, defined by a line from near the upper left corner to the lower right corner of the image. The bead surface is well colonized below this line, but bacteria are not present above it. It appears that a mineral deposit (not present below the line) may be preventing colonization of the upper area of the resin bead surface. Although bacteria were also observed on the cement matrix between resin beads, colonization was not as extensive. Figure 22b shows the type of general colonization pattern observed on the PWR salts waste-form pellet. Figure 23 shows SEM images of the surface of pellet subsamples removed from the three respective inoculated reactors on day 113. Figure 23a shows a similar bacterial colonization pattern for the ASTM pellet subsample surface as observed in Figure 21b, indicating a relatively stable surface population from days 26 to 113. It should be noted that the resin waste form cement matrix between resin beads appeared to be much more heavily colonized than previously observed, bacterial colonies usually being associated with pits or depressions in the matrix surface.

Sulfur-Oxidizing Bacteria. A test was devised in which evaporator bottoms and ion-exchange resin specimens from the INEL, vendor formulations of simulated waste forms, and the ASTM control waste form specimens were exposed to lixiviant produced by *T. thiooxidans* and *T. ferrooxidans* isolates grown in separate continuous flow bioreactors. Results of this work were reported last year (Rogers et al. 1994).

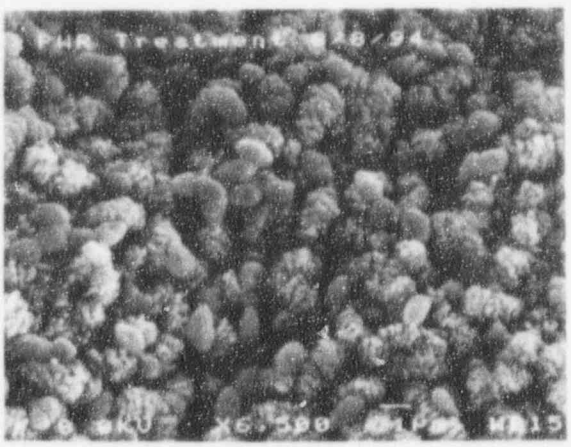
This year, an ASTM specimen and a vendor-supplied simulated evaporator bottoms waste-form specimen were exposed to *T. thiooxidans* lixiviant for 30 days, then examined for the presence of EPS and bacterial cells. The same procedure was used as described in the section on Nitrifying Bacteria. Fragments of both specimens examined by ESEM showed the presence of biofilm (Figures 24 and 25). Scrapings from the ASTM specimens were prepared and stained with alcian blue and acridine orange. The presence of



(a)



(b)



(c)

Figure 23. Electron micrographs of ASTM and vendor-supplied simulated waste-form specimens after 113 days exposure to nitrifying bacteria (a) ASTM treatment, (b) vendor treatment, and (c) PWR treatment.

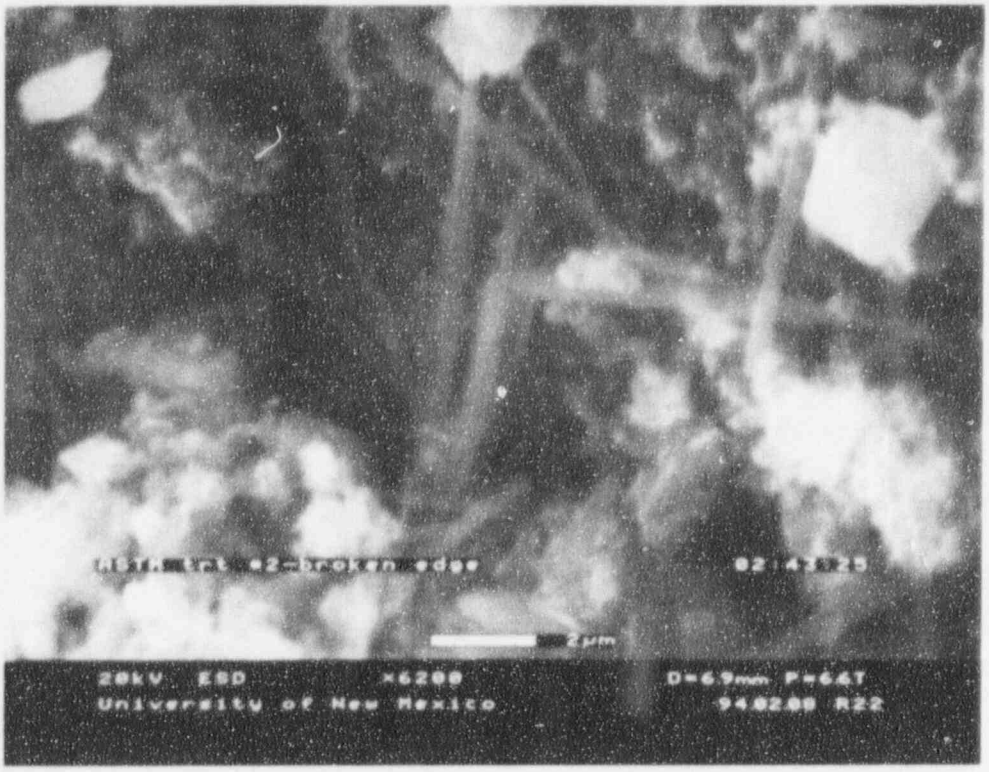


Figure 24. ESEM photograph of ASTM control waste-form specimen after exposure to sulfur-oxidizing organisms.

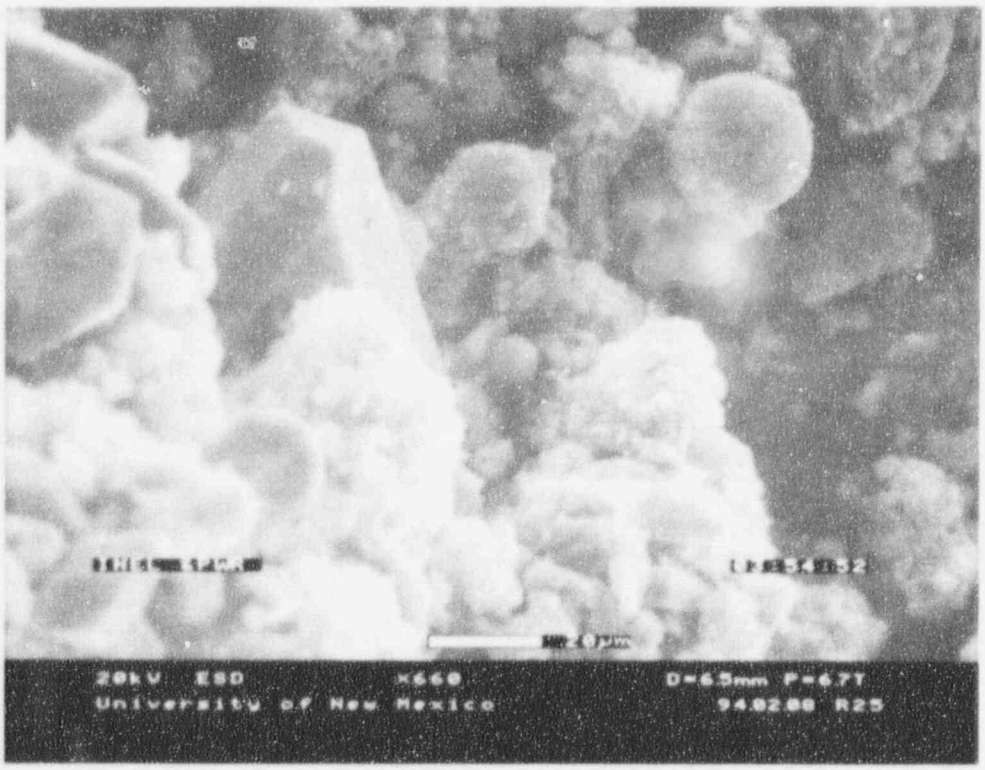


Figure 25. ESEM photograph of INEL-supplied simulated waste-form specimen after exposure to sulfur-oxidizing organisms.

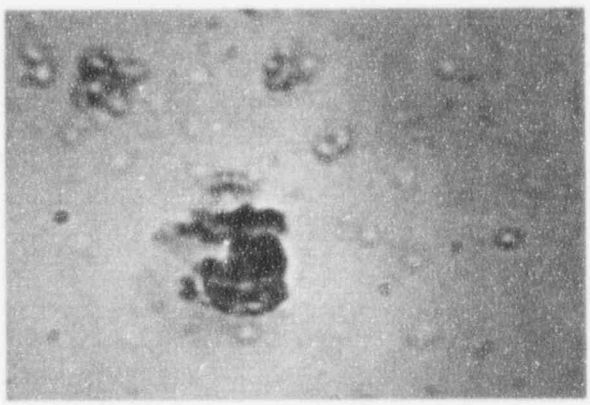
both EPS and bacterial cells was confirmed (Figure 26). Again, the background blue color confirms the presence of biofilm EPS material (Figure 26a) and the bright, fluorescing orange shows the presence of viable cells associated with the biofilm matrix (Figure 26b).

Intermittent Immersion. A test similar to that described in the section on total immersion was conducted using *Thiobacillus*. Both INEL-fabricated and vendor-supplied simulated evaporator bottoms, ion-exchange resin waste-form specimens, and ASTM controls were exposed to *T. ferrooxidans*, *T. thiooxidans* lixiviants, and the sterile microbial growth medium. This test differed from total immersion in that test specimens were fully immersed in the respective treatment liquid for only 6 hours out of every 12 hours. Results of these tests were reported last year (Rogers et al. 1994).

Intermittent Misting. Intermittent misting was the third testing method evaluated. It was used to expose test specimens to a lixiviant mist. The design was such that liquid was drawn away from the waste form specimens to prevent puddling. To accomplish this, exposed lixiviant (which contacted the specimens) was collected as it dripped from specimen surfaces. In this way, the specimen remained moist, but was not ever submersed in lixiviant.

The prototype for the misting chamber is seen in Figure 27. The body of the chamber was a 2.4-L plastic container. It came fitted with a seal-tight, snap lid. The lid was modified to accommodate the motor (covered by the square, perforated box) and the misting generator. The misting generator was taken from a "cool mist" vaporizer. Acid-bathed, washed, and sterilized glass marbles were placed in the container to reduce the volume of lixiviant necessary to maintain a requisite height. The marbles' weight acted as an "anchor" for the modified 100-mL graduated cylinder (foreground; cut off at a height to allow collection of 60 mL of liquid). The cylinder shown in Figure 27 had a teflon pedestal placed into it to serve as a stage for the placement of a simulated waste-form specimen (the dark, cylindrical object directly above the top of the graduated cylinder).

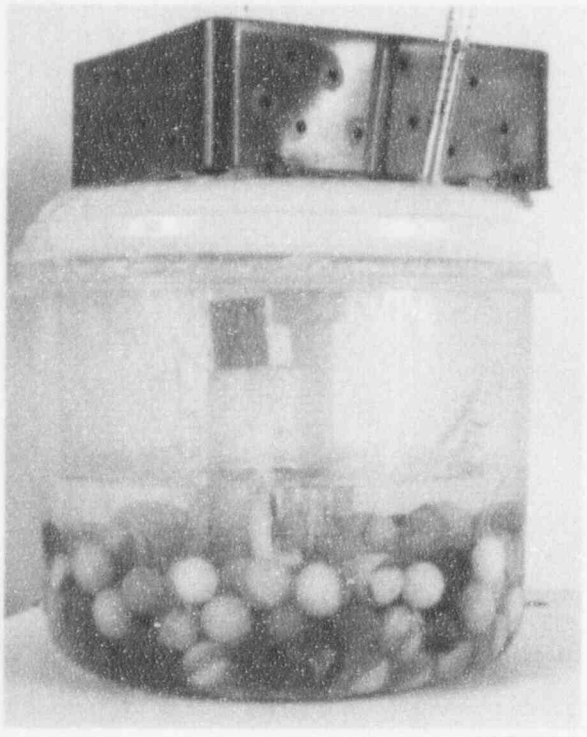
Two modifications were made to the prototype system before it was evaluated as a testing methodology. First, to help reduce differences due to treatment application, it was decided to use only one exposure chamber per lixiviant source. This was done so a specimen of each of the waste form types could be placed in the same chamber environment. Second, modified funnels were used as the physical support structure for the specimens (Figure 28). As mist collected on the specimens and eventually ran off, it was collected by the funnels into individual graduated cylinders. Lixiviant was then recovered from the graduated cylinders by a permanently installed tube that was part of a continuous effluent withdrawal system.



(a)

(b)

Figure 26. Biofilm staining of INEL-supplied simulated waste-form specimen after exposure to sulfur-oxidizing organisms (a) stained blue to demonstrate presence of biofilm EPS material and (b) fluorescent orange stain demonstrating presence of viable cells.



93-712-1-1

Figure 27. Prototype for intermittent misting chamber.

This system brought the effluent to collection flasks, and the contents of each collection flask were monitored daily.

The volume of lixiviant in the misting container was maintained at 300 mL by a continuous influent/effluent addition and withdrawal system for the main chamber and by the separate effluent systems from each of the three graduated cylinders. The misting unit was timed to operate for 5 out of every 15 minutes.

Specimens of the INEL-fabricated ASTM control, the vendor-furnished simulated evaporator bottoms (salts), and the ion-exchange resin waste form were exposed to mist generated from the *T. ferrooxidans* and the *T. thiooxidans* lixiviants and sterile growth medium. Lixiviant recovery was generally sporadic with an average range of 3 to 27 mL of effluent being collected from each graduated cylinder per day. Once collected, effluent from each treatment was analyzed for pH and the concentration of Ca, Al, and Si. The duration of the intermittent misting test was 60 days. At the conclusion of the test, the surface pH of each

93-1197-2-9

Figure 28. Waste form placement in misting chamber.

specimen was determined. Then part of each specimen was prepared for examination by an ESEM.

The visual effect of MID on the vendor-supplied simulated waste-form specimens can be seen in Figures 29 through 37. The surface pH of the specimens exposed to the lixiviant ranged from 2 to 4.5 (Table 2), while those exposed only to sterile medium were near pH 5. Figures 29, 30, and 31 show the ASTM control after exposure to sterile medium, *T. ferrooxidans* lixiviant, and *T. thiooxidans* lixiviant, respectively. Figures 32 through 37 are arranged in the same sequence for the vendor-supplied simulated evaporator bottoms and ion-exchange resin specimens. As an overview, there appeared to be minimal visual effects for the ASTM control and evaporator bottoms specimens exposed only to sterile medium, while the ion-exchange resin specimens showed deterioration (Figures 29, 32, 35). This was consistent with the results of the similar waste-form specimens used in the other two methods of exposure (intermittent and total immersion). While the ion-exchange resin specimen was eroded due to this treatment (Figure 35), individual resin beads, as noted in previous work, still appeared to be firmly embedded in the cement matrix. Also comparable to experience, ASTM specimens that were in contact with the two thiobacilli lixiviants had developed areas that were light (white) in color, which is indicative of Ca loss (Figures 30 and 31). This effect was more pronounced on the specimen exposed to *T. thiooxidans* (Figure 31). Examination of the evaporator bottoms specimens exposed to the lixiviants showed some evidence of swelling with deposits of leached salts on the surface (Figures 33 and 34). Margin definition on these waste-form specimens was not as distorted as seen at the conclusion of other treatments. However, it would appear that they were in the initial stages of deterioration.

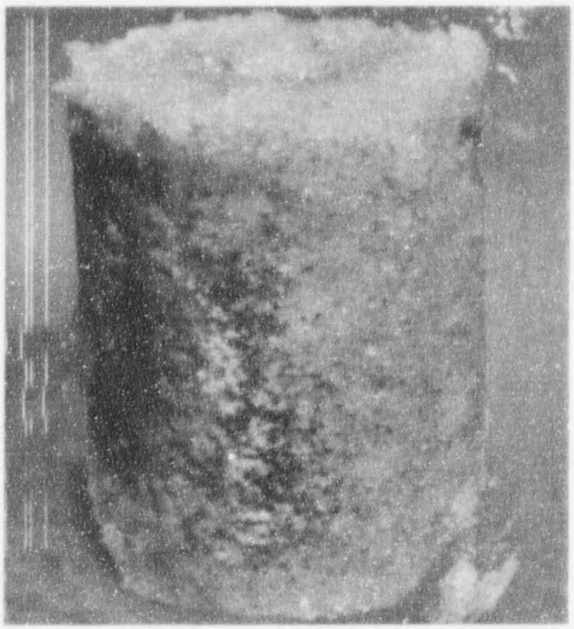
Physical damage to the ion-exchange resin specimens exposed to the lixiviants was similar to that of the control exposed to the sterile medium (Figures 36 and 37). However, it was significant that the treated specimens were beginning to loose the cement matrix as was evidence by the appearance of loose resin beads on the exposed surface. This same phenomenon is seen with this type of waste form when exposed to the other two treatments.

Results obtained from the exposure of the ASTM control and vendor-supplied simulated waste-form specimens to sterile medium and lixiviants are summarized in Table 2. This table displays the data from a variety of sources including physical, chemical, and microbial examinations and analyses. A discussion of the data in Table 2 follows. The effects of the intermittent misting treatment, generally, were not as pronounced as those from the other two preceding treatments. Undoubtedly, this could be attributed to the quantity of lixiviant that the waste-form specimens were exposed to during the misting procedure. After 60 days of exposure, the maximum quantity of lixiviant collected from the misting treatment was only one-sixth of that experienced in the two other test procedures (~1,000 mL versus ~6,000 mL). In addition, it can be seen that lixiviant exposure for the various specimens was not uniform in the misting chambers. The difference in lixiviant application appeared due to an uneven exposure pattern generated within the misting chambers.

Due to the irregularity of lixiviant deposition and subsequent recovery, it appeared advisable to normalize the loss of Si, Ca, and Al as percents per milliliter of collected lixiviant rather than presenting a cumulative total. When this is done, the effect of the individual treatments becomes evident (Table 2). For Ca, it is seen in every case that the waste-form specimens exposed to the lixiviant treatments had a greater loss per mL than did the sterile controls. These data are consistent with those discussed previously in conjunction with the specimens' physical appearance. Also, the Al leaching from the vendor-supplied simulated waste-form specimens was increased by the lixiviant treatment. The same is true for Si loss from the ion-exchange resin waste-form specimens, but somewhat confused for the evaporator bottoms specimens. With the evaporator bottoms, the loss did not coincide with the quantity of leachate. However, based on the overall data, it was concluded that biological activity was having a deleterious effect on the treated waste-form specimens.

93-1197-1-10

Figure 29. ASTM control waste-form specimens after being intermittently misted for 60 days with sterile medium.



93-1197-1-4

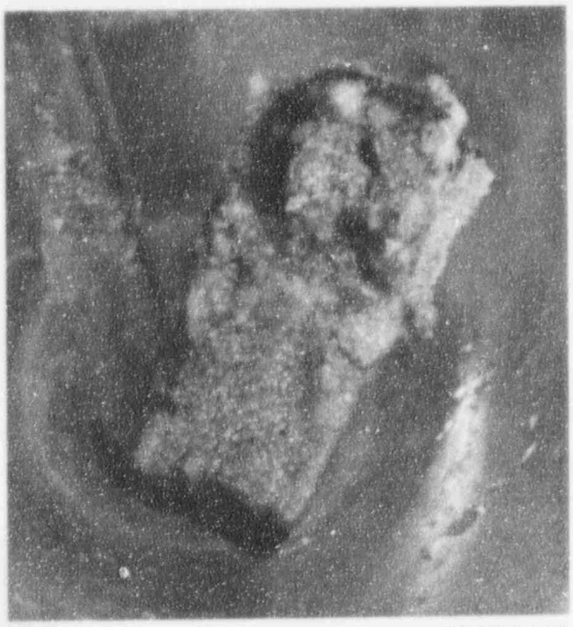
Figure 31. ASTM control waste-form specimens after being intermittently misted for 60 days with *T. thiooxidans* lixiviant.

93-1197-1-18

Figure 30. ASTM control waste-form specimens after being intermittently misted for 60 days with *T. ferrooxidans* lixiviant.

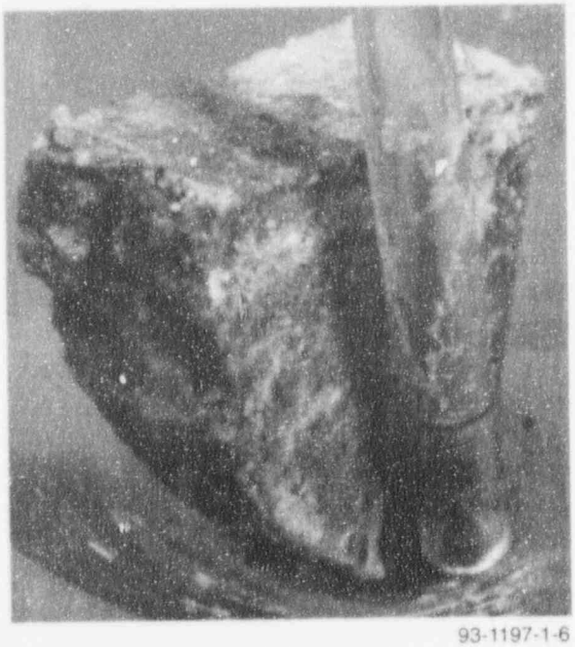
93-1197-1-12

Figure 32. Vendor-supplied simulated evaporator bottoms waste-form specimens after being intermittently misted for 60 days with sterile medium.



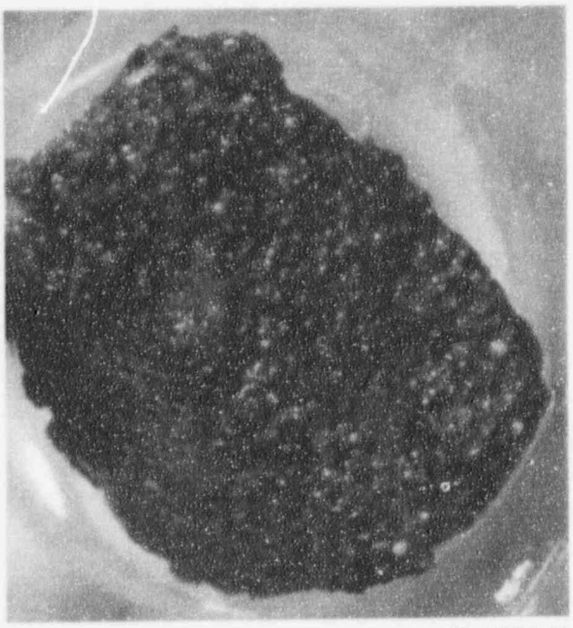
93-1197-1-17

Figure 33. Vendor-supplied simulated evaporator bottoms waste-form specimens after being intermittently misted for 60 days with *T. ferrooxidans* lixiviant.



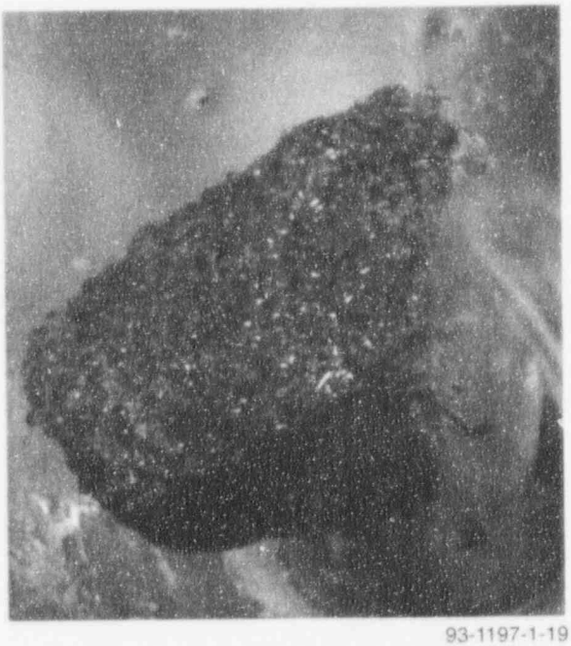
93-1197-1-6

Figure 34. Vendor-supplied simulated evaporator bottoms waste-form specimens after being intermittently misted for 60 days with *T. thiooxidans* lixiviant.



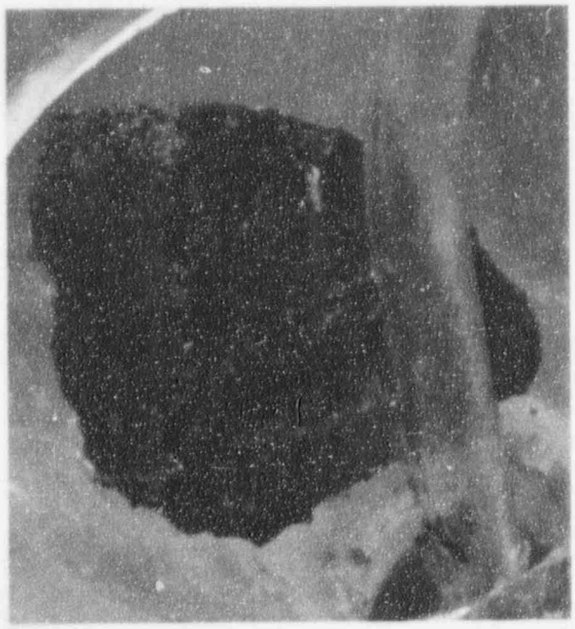
93-1197-1-11

Figure 35. Vendor-supplied simulated ion-exchange resin waste-form specimens after being intermittently misted for 60 days with sterile medium.



93-1197-1-19

Figure 36. Vendor-supplied simulated ion-exchange resin waste-form specimens after being intermittently misted for 60 days with *T. ferrooxidans* lixiviant.



93-1197-1-8

Figure 37. Vendor-supplied simulated ion-exchange resin waste-form specimens after being intermittently misted for 60 days with *T. thiooxidans* lixiviant.

The percent weight loss from the waste-form specimens usually appeared to correlate with the total volume of effluent that contacted the specimens. The exception, however, was the ion-exchange resin waste-form specimen exposed to *T. ferrooxidans* lixiviant. These data appear reasonable since this specimen was beginning to deteriorate (Figure 33).

Another aspect pursued in the examination of the subject waste forms was to determine if thiobacilli were present on the waste form surfaces. When scrapings from each of the waste-form specimens were incubated in the appropriate medium, all were positive for the presence of thiobacilli, except the ion-exchange resin specimen exposed to sterile medium. That meant that the specimens of evaporator bottoms and ASTM controls exposed to sterile medium had become contaminated with bacteria that were growing on the specimen surfaces. To confirm this finding, fragments of each waste-form specimen were submitted for ESEM analysis. This examination complemented the microbial isolation work. It

Table 2. Summary of data collected from waste-form specimens exposed to intermittent misting.

Waste type	Treatment	% wt loss	Surface pH	Bacterial presence	Visual rating	Si (%/mL)	Ca (%/mL)	Al (%/mL)	Volume (mL)
ASTM	Medium	8.43	5	+	–		0.007		1,339
ASTM	T. ferro	2.85	4.5	+	+		0.007		694
ASTM	T. thio	7.13	2	+	++		0.010		1,068
EB	Medium	14.44	5.5	+	–	0.005	0.005	0.002	671
EB	T. ferro	23.47	3	+	++	0.004	0.010	0.009	1,647
EB	T. thio	16.33	4.5	+	+	0.002	0.006	0.003	1,154
IER	Medium	12.47	4.5	–	+	0.003	0.004	0.001	1,122
IER	T. ferro	21.16	3.5	+	++	0.005	0.020	0.008	784
IER	T. thio	10.15	4	+	+	0.009	0.025	0.009	181

EB = Evaporator bottoms.

IER = Ion-exchange resin.

showed what appeared to be a biofilm formation on the evaporator bottoms and ion-exchange resin waste-form specimens, including the evaporator bottoms specimen exposed to sterile medium (Figures 38 through 41). The same formation was not seen on fragments of the other waste-form specimens. The data demonstrate that microorganisms were colonizing the surface of the waste-form specimens. Consequently, staining with alcian blue and acridine orange was not performed on these specimens.

Testing of Actual Waste Forms

Based on the stability of operational conditions and the consistency of data, the intermittent immersion exposure system was selected for testing of actual LLW. This selection was made because the intermittent immersion system was less likely to have loss of lixiviant through leakage or spills under present operating conditions. Therefore, it was considered more effective in controlling the spread of solubilized radioactive contaminants within the effluent. Also, it was suggested that conservative test conditions be used because the actual radioactive waste-form specimens represented a new, untested material. For this reason, the test was conducted over 60 days using thiobacilli. Based on our data, both species of thiobacilli were appropriate for use; however, *T. thiooxidans* was selected because it was the microorganism most often cited in the literature as being associated with MID of cement.

For this work, the method employed was similar to the one used for intermittent immersion of simulated waste-form specimens using *T. thiooxidans* (Rogers et al. 1994). Specimens used in this study were taken from two varieties of actual cement-solidified low-level waste forms (2 in. by 4 in.) made from power reactor wastes. One contained ion-exchange resin waste from Peach Bottom Atomic Power Station Unit 3, and the other contained filter sludge from Nine Mile Point Nuclear Plant Unit 1 (see Table 3 for waste stream analysis). In addition to these waste forms, an ASTM standard cement formulation (the same as used in all other studies) was used for comparison purposes. All treatments were replicated.

This test differed from total immersion in that test specimens were fully immersed in the respective treatment liquid for only 6 hours out of every 12 hours. This was accomplished by using 125-mL Soxhlet extraction columns (Figure 42). Each specimen was placed on top of a plastic pedestal inside an individual Soxhlet column. The filling of the columns was controlled by the influent flow rate. It required 6 hours for a specimen to become immersed. After the liquid level rose to a predetermined height, the column was emptied by the activation of a syphon. Approximately 100 mL of liquid passed through each column per day. Effluents derived from the radioactive waste-form specimens were analyzed for gross beta- and gamma-emitting isotopes. In addition, final composite samples were analyzed for Sr-90, Tc-99, and C-14. None of the specimens were examined for the presence of EPS or bacteria. Also, no nonradiochemical analyses (Ca, Al, etc.) were conducted on the lixiviant from these specimens. However, Ca concentration in the effluent from the ASTM specimens was determined.

The specimens were photographed midway through the test (days 20 and 30) and at the conclusion (60 days). The physical effect of MID on the radioactive waste-form specimens can be seen in Figures 43 through 48. Figures 43a through 43f show the ASTM control specimens in duplicate after exposure to sterile medium for 20, 30, and 60 days, respectively. Figures 44a through 44f show the ASTM control specimens after exposure to *T. thiooxidans* lixiviant after 20, 30, and 60 days. Figures 45 through 48 are arranged in the same sequence for the Peach Bottom (PB) and Nine Mile Point (NMP) waste-form specimens. As can be seen, there is a gradation of effects for those specimens exposed only to sterile medium (Figures 43, 45, and 47). Medium exposure had no visible effect on the ASTM control specimens (Figure 43), while there was minor swelling of the NMP specimens (the waste form shape is still well defined) (Figure 47). Unlike studies with other waste forms containing ion-exchange resin, the PB specimens were not totally disintegrated, with the exposed individual resin beads still appearing to be firmly embedded in the cement matrix (Figure 45). The ASTM specimens

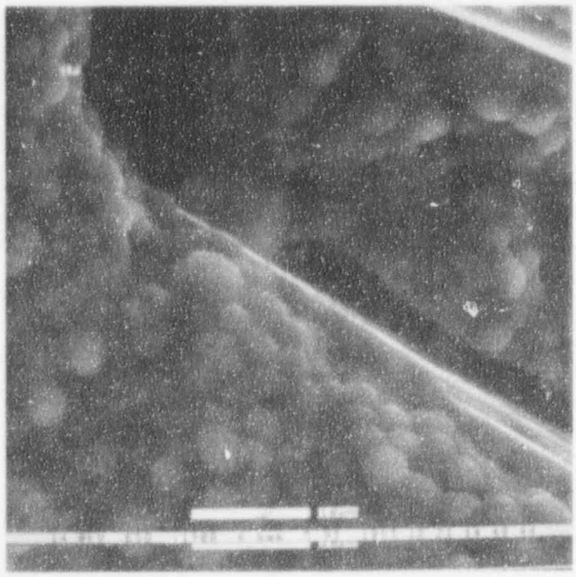


Figure 38. ESEM photograph of vendor-supplied simulated evaporator bottoms waste-form specimen after exposure to *T. ferrooxidans* lixiviant.

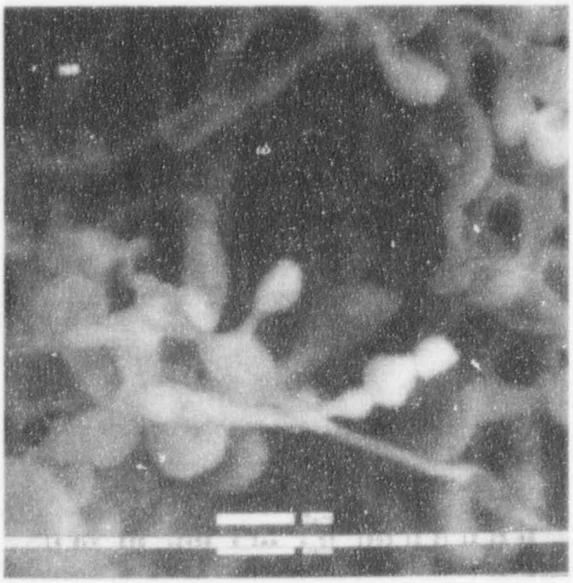


Figure 40. ESEM photograph of vendor-supplied simulated ion-exchange resin waste-form specimen after exposure to *T. thiooxidans* lixiviant.

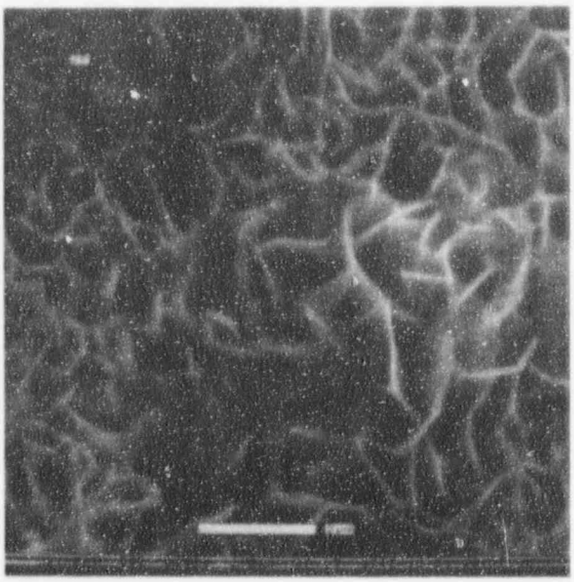


Figure 39. ESEM photograph of vendor-supplied simulated evaporator bottoms waste-form specimen after exposure to *T. thiooxidans* lixiviant.

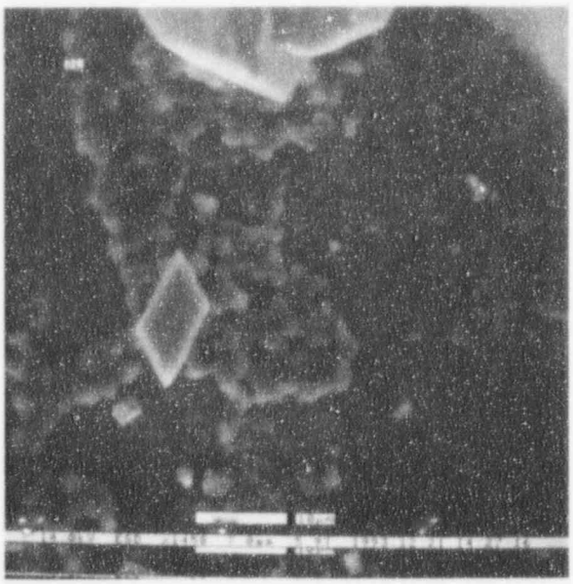


Figure 41. ESEM photograph of vendor-supplied simulated ion-exchange resin waste-form specimen after exposure to sterile medium.

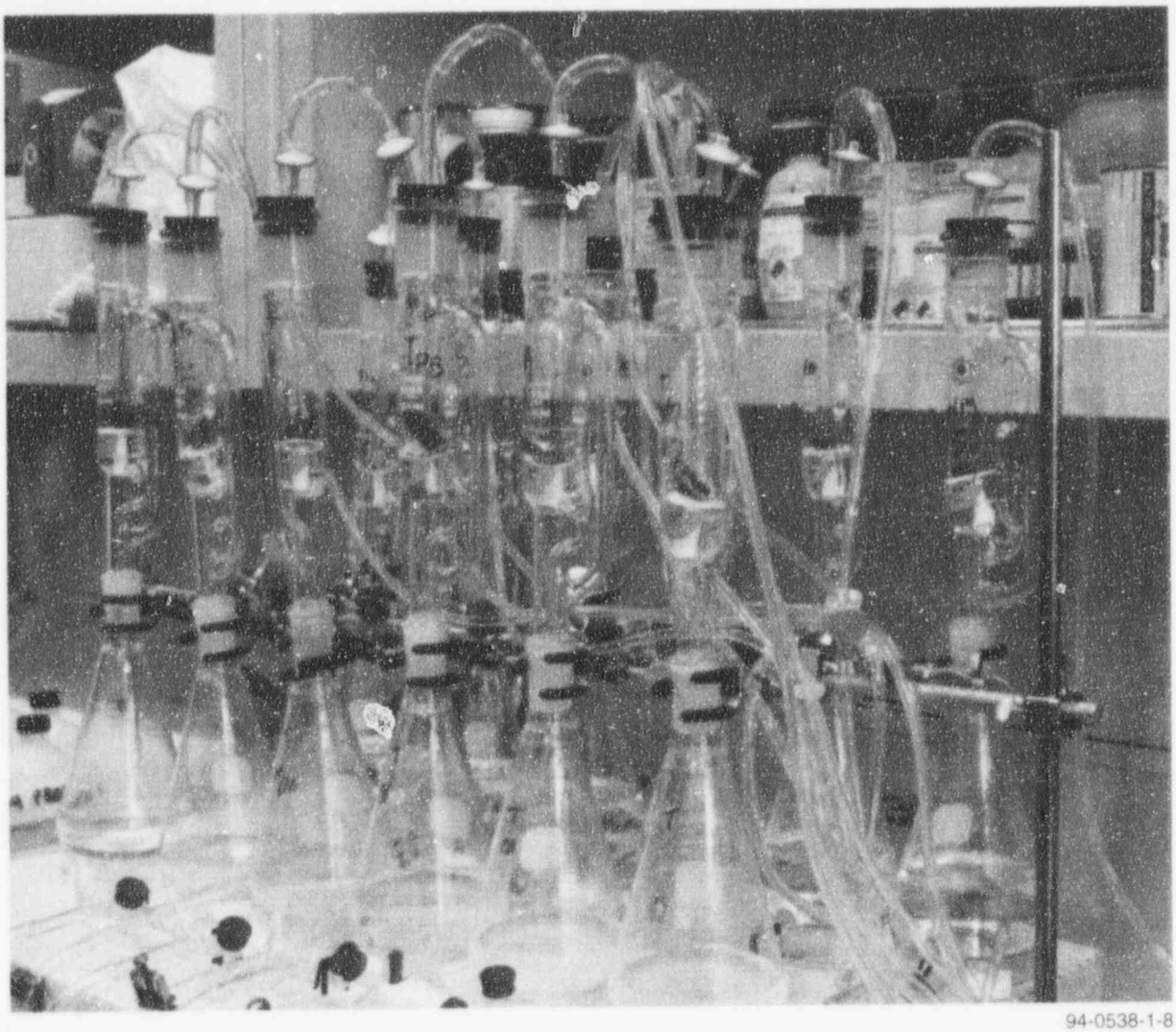
Table 3. Peach Bottom and Nine Mile Point waste stream analysis.

Radionuclide (m)	Peach Bottom resin (μCi/g on 10/24/89)	Nine Mile Point sludge (μCi/g on 3/14/91)
Mn-54	4.35E-2 ± 2.66E-3	7.16E-3 ± 6.67W-5
Fe-55	1.35E+0 ± 5.53E-2	4.50E-4 ± 8E-6
Co-58	—	2.65E-3 ± 2.20E-4
Co-60	4.64E+0 ± 1.16E-2	4.59E-1 ± 1.33E-3
Ni-63	7.62E-2 ± 6.85E-3	9.40E-2 ± 5E-3
Zn-65	5.92E-1 ± 1.13E-2	—
Sb-125	1.73E-2 ± 9.66E-4	3.66E-3 ± 3.84E-5
Cs-134	—	3.46E-3 ± 3.03E-5
Cs-137	3.53E-3 ±5.15E-4	4.20E-1 ± 1.92E-3
Eu-154	—	2.15E-4 ± 2.08E-5
Sr-89	—	4.03E-2 ± 3.67E-3
Sr-90	2.16E-5 ±2.3E-6	3.03E-3 ± 2E-4
Pu-238	6.61E-5 ± 1.66E-6	<1.0E-7
Pu-239	2.17E-5 ± 7.50E-7	<1.0E-7
Pu-241	1.75E-2 ± 4.24E-4	<1.0E-7
Am-241	5.18e-5 ± 1.40E-6	<1.0E-7
Cm-242	9.46E-6 ± 9.43E-7	<1.0E-7
Cm-244	1.30E-4 ± 1.59E-4	<1.0E-7
C-14	8.74E+0 ± 8.7E-2	1.05E+0 ± 1.1E-1
Tc-99	3.71E-2 ± 8.61E-4	8.11E-3 ± 1.63E-4
I-129	<5E-6	<5E-6
Chromium[a]	650 ppm ± 20 ppm	64 ppm ± 66 ppm
Iron[a]	3,000 ppm ± 160 ppm	2,430 ppm ± 130 ppm
Zinc[a]	158 ppm ± 12 ppm	98 ppm ± 42 ppm
Nickel[a]	730 ppm ± 30 ppm	20 ppm ± 4 ppm
Borate[b]	—	9 ppm ± 4 ppm
Phosphate[b]	—	None detected
Sulfate[b]	—	18.4 ppm ± 1.8 ppm
Picolinic acid[c]	<5E-6	—

a. Analyses were performed using inductively coupled plasma spectroscopy elemental analysis methods.

b. Analyses were performed using ion chromatography.

c. Analysis was performed using picolinic acid titration.



94-0538-1-8

Figure 42. Setup for intermittent immersion testing of actual vendor-supplied waste-form specimens.

exposed to the thiobacilli lixiviant were light (white), indicating that extensive leaching of Ca had taken place (Figure 44). The NMP evaporator bottoms specimens exposed to the lixiviant gradually crumbled, and material loss was evident (Figure 48). Physical damage was also seen in the PB ion-exchange resin specimens exposed to lixiviant (Figure 46). As discussed previously (Rogers et al. 1994), the loss of the cement matrix in these specimens is indicated by the appearance of the loose resin beads on the surface.

A direct comparison between the waste-form specimens before and after exposure to the sterile medium and thiobacilli lixiviant can be seen in Figures 49 and 50 for PB and NMP materials, respectively. Figures 49a and 50a show unexposed PB and NMP specimens. All the specimens that were exposed to the sterile medium retained their physical integrity. The PB specimens appeared to have some exposure of their ion-exchange resin beads (Figures 49b and 49c), but the NMP specimens did not appear to be physically degraded in any way (Figures 50b and 50c). Both types of specimens appeared to be lighter in color than the originals, suggesting some leaching action. The specimens that were

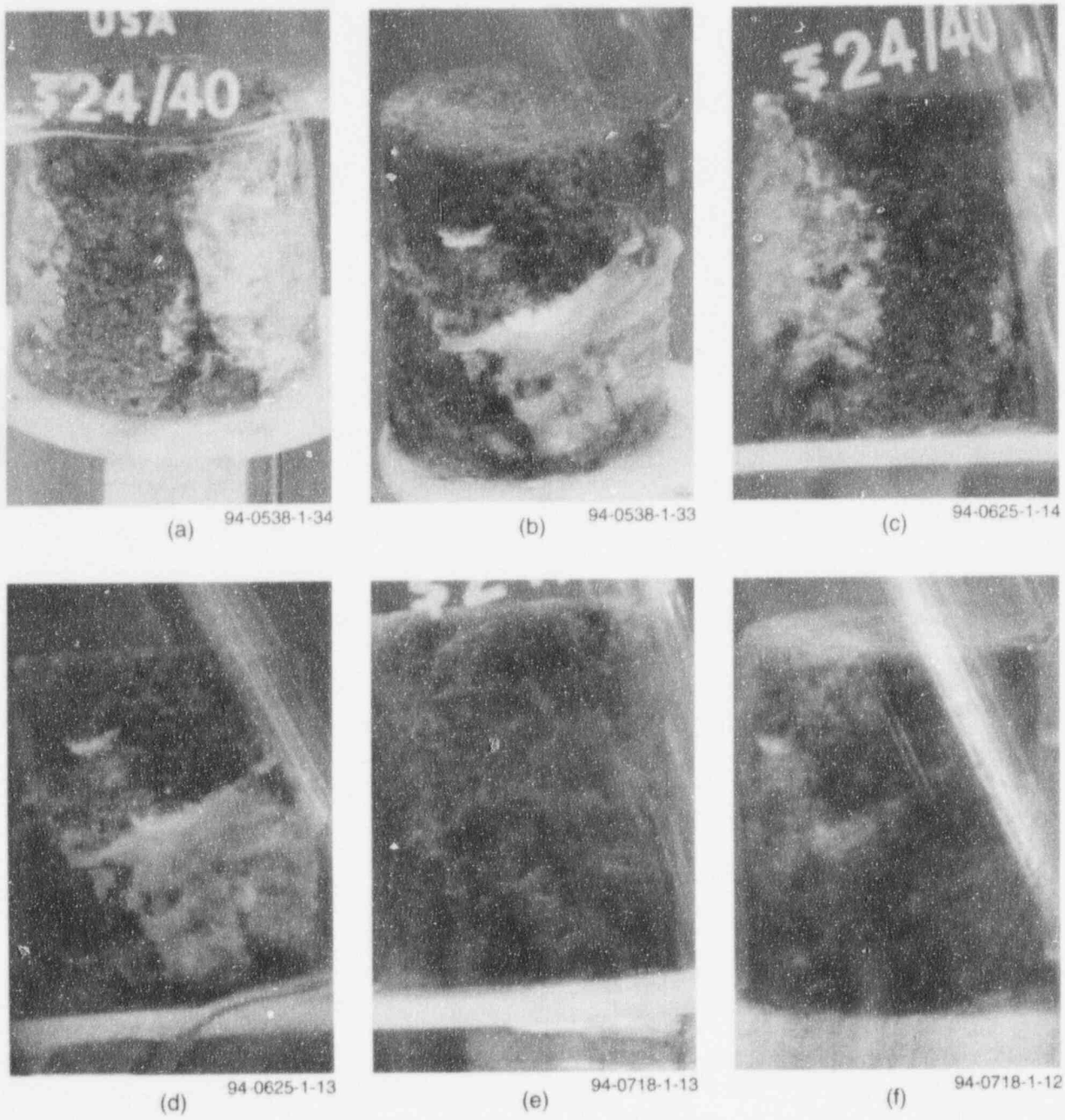


Figure 43. ASTM control waste-form specimens exposed over time to sterile medium (a) ASTM duplicate 1 at day 20, (b) ASTM duplicate 2 at day 20, (c) ASTM duplicate 1 at day 30, (d) ASTM duplicate 2 at day 30, (e) ASTM duplicate 1 at day 60, and (f) ASTM duplicate 2 at day 60.

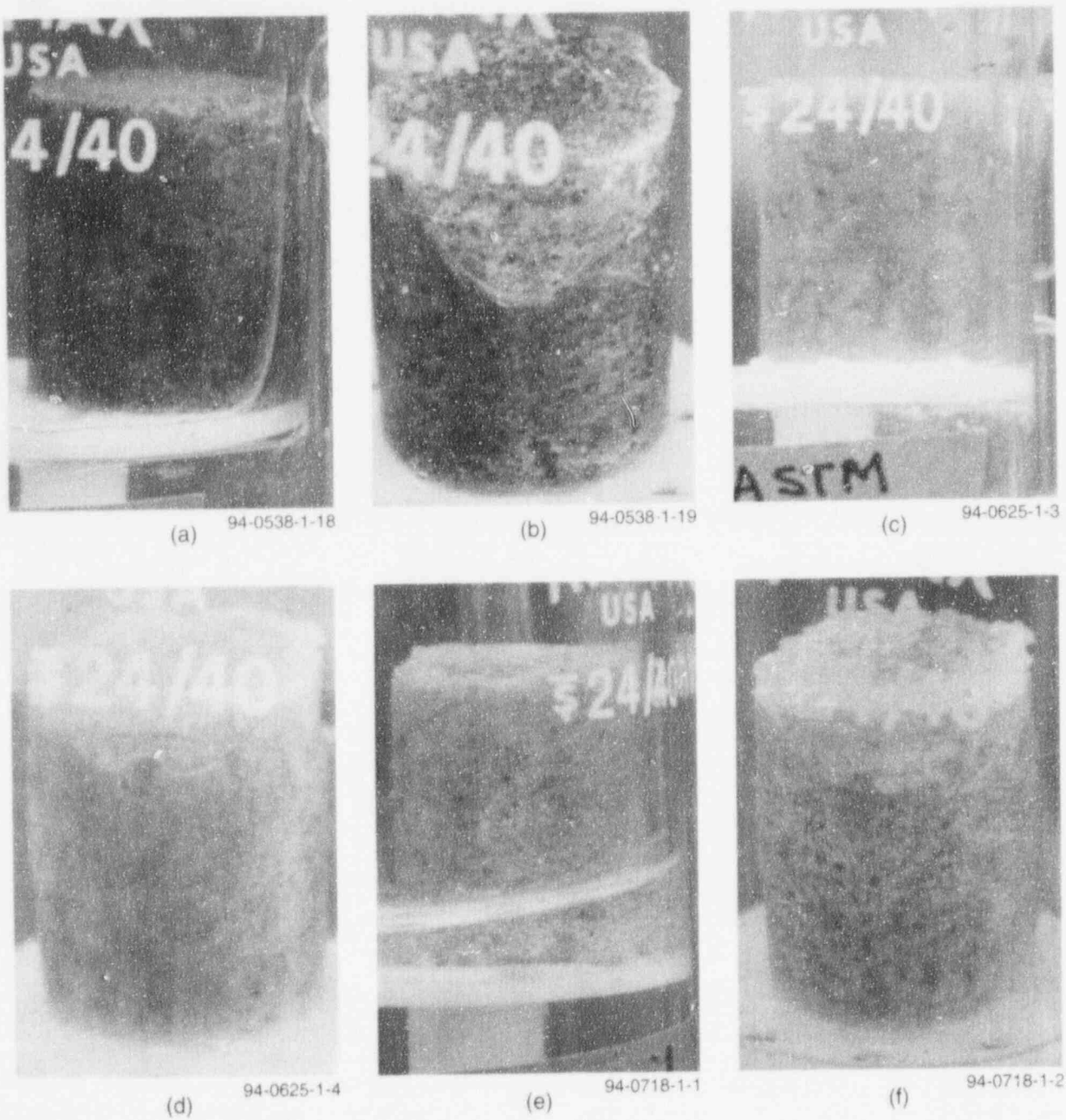


Figure 44. ASTM control waste-form specimens exposed over time to thiobacilli lixiviant (a) ASTM duplicate 1 at day 20, (b) ASTM duplicate 2 at day 20, (c) ASTM duplicate 1 at day 30, (d) ASTM duplicate 2 at day 30, (e) ASTM duplicate 1 at day 60, and (f) ASTM duplicate 2 at day 60.

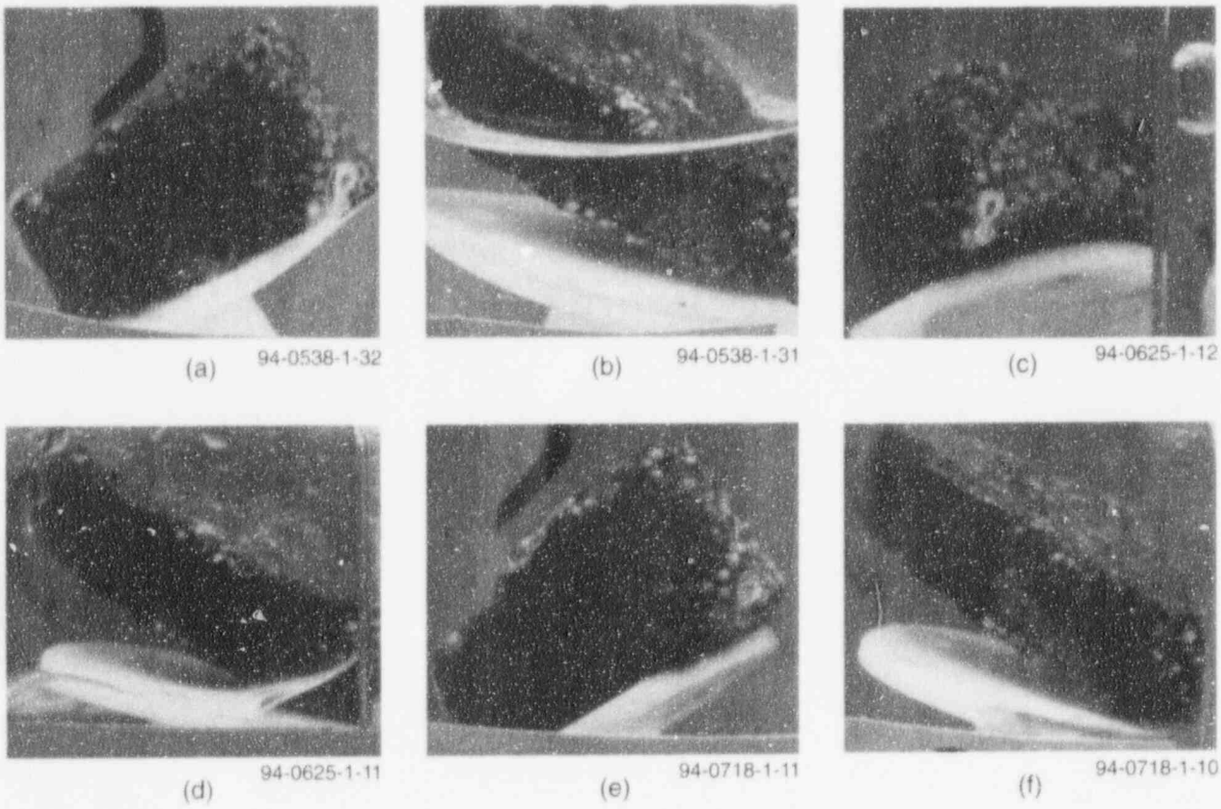


Figure 45. Vendor-supplied waste-form specimens exposed over time to sterile medium (a) Peach Bottom duplicate 1 at day 20, (b) Peach Bottom duplicate 2 at day 20, (c) Peach Bottom duplicate 1 at day 30, (d) Peach Bottom duplicate 2 at day 30, (e) Peach Bottom duplicate 1 at day 60, and (f) Peach Bottom duplicate 2 at day 60.

exposed to the lixiviant had more extensive damage than was noticed before they were removed from the Soxhlet columns. The PB specimens had crumbled, with evidence of unattached resin beads (Figures 49d and 49e), and the NMP specimens were completely disintegrated (Figures 50d and 50e).

In general, when effluent from the power reactor samples was analyzed for the presence of gross beta- and gamma-emitting nuclides, the PB effluents were found to have Co-60 and Cs-137; the effluents from the NMP samples contained these nuclides as well as Cs-134; also, C-14, Tc-99, and Sr-90 were found in the composite effluents from both plants. Total quantities of leached nuclides are tabulated in Tables 4, 5, and 6. The data show that the waste-form specimens exposed to the thiobacilli lixiviant had a greater loss of Cs-137, Cs-134, Co-60, C-14, Tc-99, and Sr-90 than did the medium-treated controls (Tables 4, 5, and 6).

The quantity of Co-60 lost from both types of waste-form specimens exposed to the thiobacilli lixiviant was an order of magnitude greater than that lost due to the sterile medium treatment (Tables 4 and 5). Comparable amounts of Co-60 (~1 to 3 million pCi) were released from both types of specimens, although the quantity recovered from the NMP specimens was about twice that of the PB types. Further, the data show that

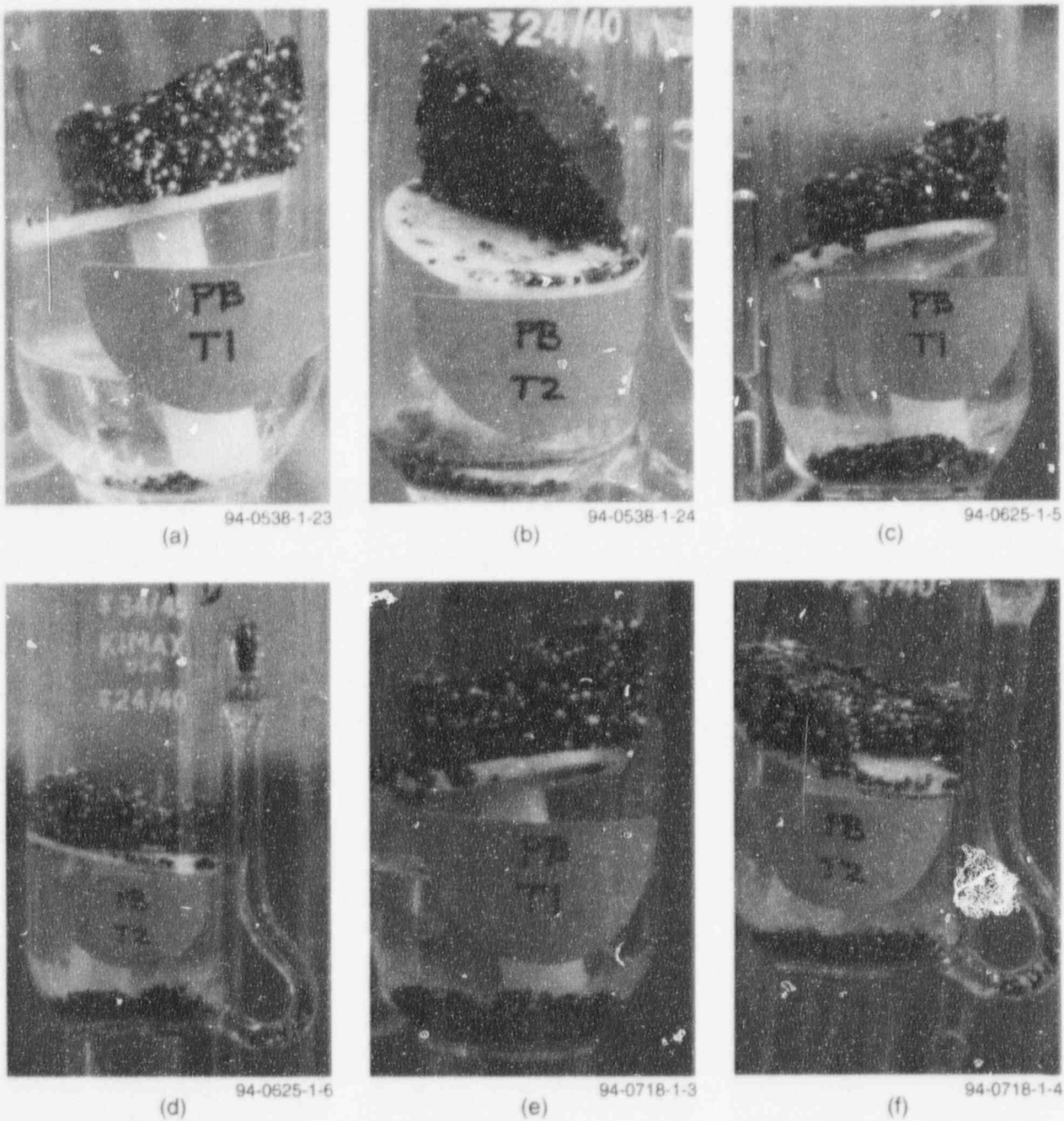


Figure 46. Vendor-supplied waste-form specimens exposed over time to thiobacilli lixiviant (a) Peach Bottom duplicate 1 at day 20, (b) Peach Bottom duplicate 2 at day 20, (c) Peach Bottom duplicate 1 at day 30, (d) Peach Bottom duplicate 2 at day 30, (e) Peach Bottom duplicate 1 at day 60, and (f) Peach Bottom duplicate 2 at day 60.

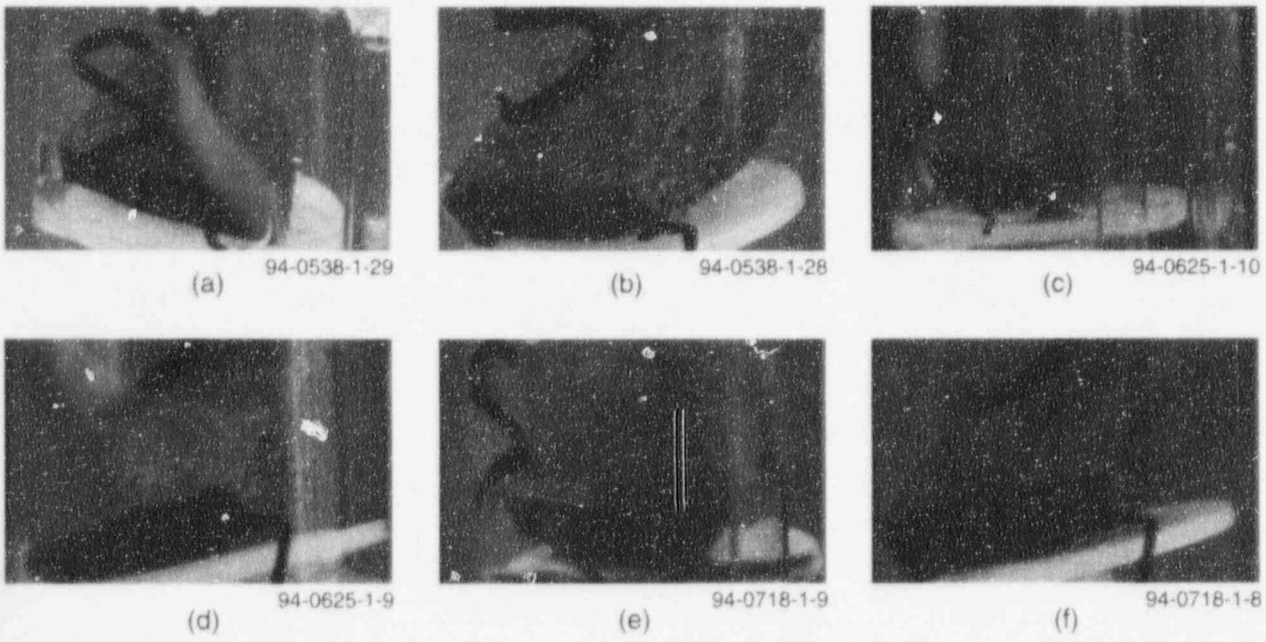


Figure 47. Vendor-supplied waste-form specimens exposed over time to sterile medium (a) Nine Mile Point duplicate 1 at day 20, (b) Nine Mile Point duplicate 2 at day 20, (c) Nine Mile Point duplicate 1 at day 30, (d) Nine Mile Point duplicate 2 at day 30, (e) Nine Mile Point duplicate 1 at day 60, and (f) Nine Mile Point duplicate 2 at day 60.

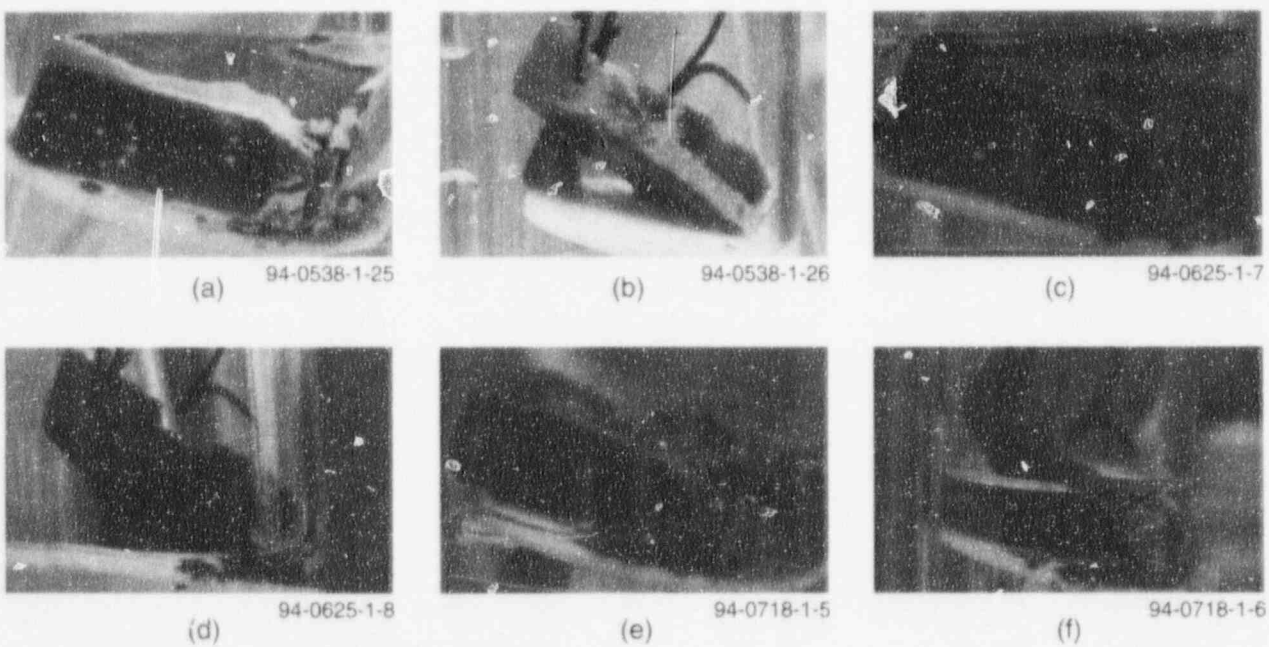


Figure 48. Vendor-supplied waste-form specimens exposed over time to thiobacilli lixiviant (a) Nine Mile Point duplicate 1 at day 20, (b) Nine Mile Point duplicate 2 at day 20, (c) Nine Mile Point duplicate 1 at day 30, (d) Nine Mile Point duplicate 2 at day 30, (e) Nine Mile Point duplicate 1 at day 60, and (f) Nine Mile Point duplicate 2 at day 60.

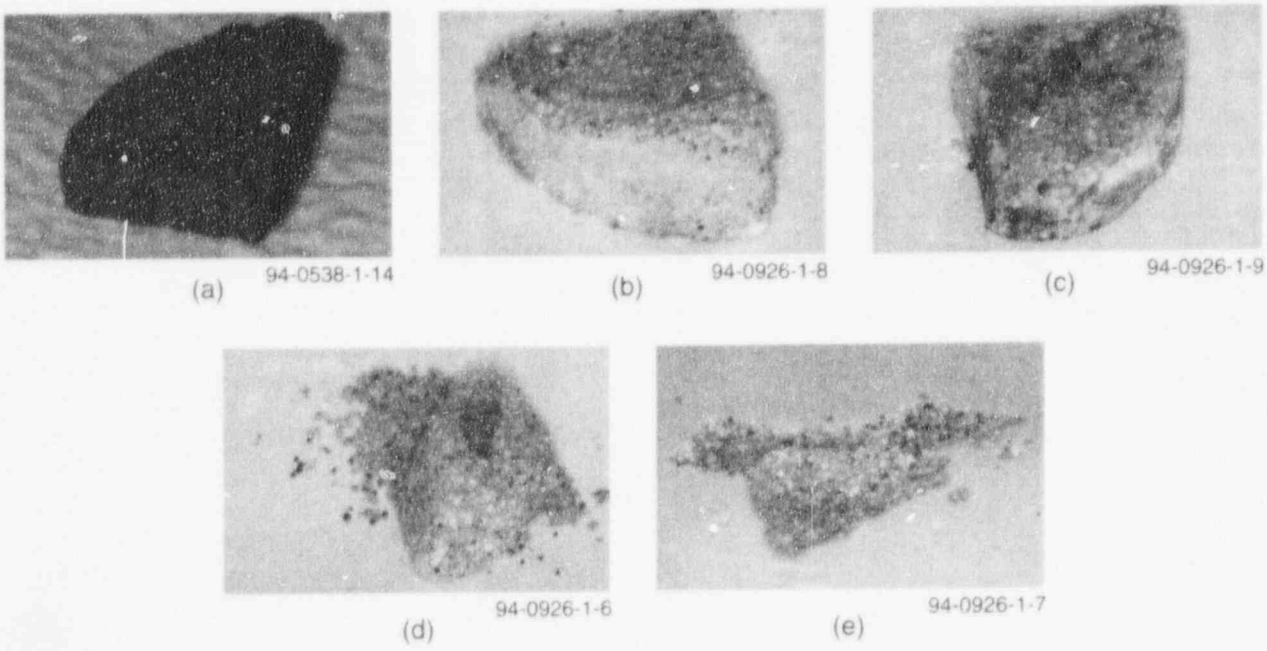


Figure 49. Recovered vendor-supplied Peach Bottom waste-form specimens (a) before exposure, (b) duplicate 1 after 60-day exposure to sterile medium, (c) duplicate 2 after 60-day exposure to sterile medium, (d) duplicate 1 after 60-day exposure to thiobacilli lixiviant, and (e) duplicate 2 after 60-day exposure to thiobacilli lixiviant.

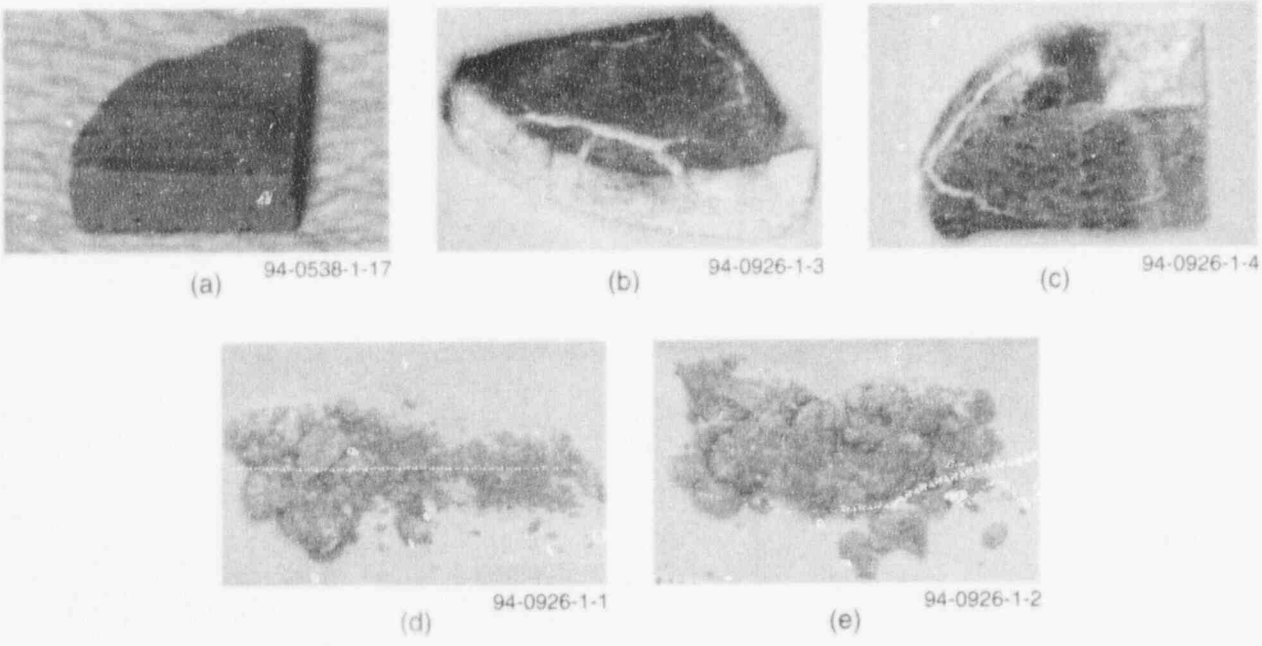


Figure 50. Recovered vendor-supplied Nine Mile Point waste-form specimens (a) before exposure, (b) duplicate 1 after 60-day exposure to sterile medium, (c) duplicate 2 after 60-day exposure to sterile medium, (d) duplicate 1 after 60-day exposure to thiobacilli lixiviant, and (e) duplicate 2 after 60-day exposure to thiobacilli lixiviant.

Table 4. Quantities of Co-60, Cs-137, and Cs-134 leached over time from Peach Bottom cement-solidified low-level waste-form specimens as a result of the accelerated biotest.

		Total pCi nuclide					
		Co-60		Cs-137		Cs-134	
Treatment	Week	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Medium	1	60372.0	54470.0	15652.0	31980.0	0.0	0.0
	2	25882.5	22620.0	362.3	1566.5	0.0	0.0
	3	126000.0	26180.0	1351.0	840.0	0.0	0.0
	4	13370.0	11550.0	171.5	371.0	0.0	0.0
	5	15960.0	13140.0	503.5	234.0	0.0	0.0
	6	6040.0	5200.0	240.0	244.0	0.0	0.0
	7	7150.0	7975.0	250.0	253.0	0.0	0.0
	8	166750.0	135000.0	355.3	546.8	0.0	0.0
Lixiviant	1	374000.0	777150.0	20000.0	45193.5	0.0	0.0
	2	265500.0	287500.0	2955.0	1717.5	0.0	0.0
	3	373500.0	242250.0	1183.5	641.3	0.0	0.0
	4	165000.0	174000.0	0.0	632.0	0.0	0.0
	5	525000.0	434000.0	0.0	917.0	0.0	0.0
	6	303000.0	364000.0	0.0	1361.8	0.0	0.0
	7	323000.0	273750.0	952.0	0.0	0.0	0.0
	8	225000.0	391500.0	0.0	733.5	0.0	0.0

Table 5. Quantities of Co-60, Cs-137, and Cs-134 leached over time from Nine Mile Point cement-solidified low-level waste-form specimens as a result of the accelerated biotest.

		Total pCi nuclide					
		Co-60		Cs-137		Cs-134	
Treatment	Week	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Medium	1	8100.0	7725.0	1507500.0	1582500.0	4005.0	3840.0
	2	4277.0	4550.0	338000.0	351000.0	845.0	877.5
	3	6221.6	5075.0	532000.0	469000.0	1414.0	1176.0
	4	3206.0	3451.0	150500.0	175000.0	402.5	465.5
	5	5814.0	3543.0	266000.0	183750.0	725.8	418.3
	6	2907.0	3701.8	165325.0	187000.0	412.3	501.5
	7	2667.5	2392.5	187000.0	165000.0	555.5	380.6
	8	47385.0	56980.0	188500.0	238000.0	531.1	588.0
Lixiviant	1	169000.0	238000.0	2080000.0	2401250.0	5330.0	5992.5
	2	69000.0	75000.0	325500.0	379500.0	837.0	958.5
	3	175500.0	114750.0	643500.0	429250.0	1390.5	93
	4	31500.0	67500.0	117000.0	193500.0	272.3	45
	5	227500.0	53625.0	234000.0	46540.0	0.0	0.0
	6	168000.0	153000.0	105000.0	99000.0	257.4	345.0
	7	150000.0	165000.0	81000.0	82500.0	0.0	0.0
	8	187000.0	96000.0	89250.0	80000.0	0.0	0.0

Table 6. Total quantity of C-14, Tc-99, and Sr-90 leached over 8 weeks from Peach Bottom and Nine Mile Point cement-solidified low-level waste-form specimens as a result of the accelerated biotest.

		Total pCi nuclide					
		C-14		Tc-99		Sr-90	
Treatment	Rep	PB	NMP	PB	NMP	PB	NMP
Medium	1	202	59	84	238	2,423	51,480
	2	243	66	124	147	4,969	33,191
Lixiviant	1	313	773	745	853	15,283	90,860
	2	358	452	1,024	1,302	29,804	92,660

there was a considerable release (over 3 million pCi) of Cs-137 from the NMP specimens. This release appeared to be independent of treatment type, although the thiobacilli treatment did have nearly a 10% greater release. The same was also true for Cs-134, although the difference between treatment and control appear indistinguishable. On the other hand, the quantity of Cs-137 leached from the PB specimens was not as consistent. Total Cs-137 removed from the duplicated PB samples due to the thiobacilli lixiviant was 51,196 and 25,000 pCi, while those of the medium samples was 18,849 and 36,035 pCi (Table 4). However, when these data are averaged, based on treatment, the thiobacilli treatment appears to have released more Cs-137 than the medium (38,143 versus 27,442 pCi). While there may not be a significant difference between these averages, they follow the trend established in which more nuclides were leached due to the thiobacilli treatment than by the medium. Analyses of the composite effluent samples showed that more total quantities of C-14 and Sr-90 were removed from the treated NMP specimens than from the treated PB specimens (Table 5). The amount of liberated Tc-99 was comparable for both waste form types (Table 6). Again, the waste-form specimens exposed to the thiobacilli lixiviant had more nuclides removed than the control specimens. On average, there was approximately 1.5, 8.5, and 6.1 times more C-14, Tc-99, and Sr-90, respectively, leached from the PB specimens treated with the thiobacilli lixiviant than from the samples treated with the control medium. For the NMP specimens, these values were 9.9, 5.6, and 2.2, respectively.

Data for the cumulative removal of Co-60, Cs-137, and Cs-134 over time from each treatment and type of waste-form specimen is presented in Figures 51 through 55. Figures 51 and 53 show that the release of Co-60 from both types of specimens was dependent on the thiobacilli treatment and are very similar. Action by the sterile medium occurred at almost a negligible rate when compared with that of the treatment. The release of Co-60 from all the specimens was being maintained at a constant rate by the end of the eighth week. There was a distinctly different pattern of Cs release compared to that of the Co-60, as seen in Figures 52, 54, and 55. First, it is noted that Cs release was not entirely dependent on the treatment. There was a parallel Cs release for treatments exposed to lixiviant and sterile medium. Next, for the PB specimens (Figure 52), the majority of Cs-137 loss occurred within the first week, while for the NMP specimens exposed to the biotreatment, the rate of Cs-137 and Cs-134 leaching decreased after the third week (Figures 54 and 55). The total amount of Cs leached due to both treatments stabilized by the end of the eighth week. The figures, however, show that the rate of the leaching process was greater in the thiobacilli treatments.

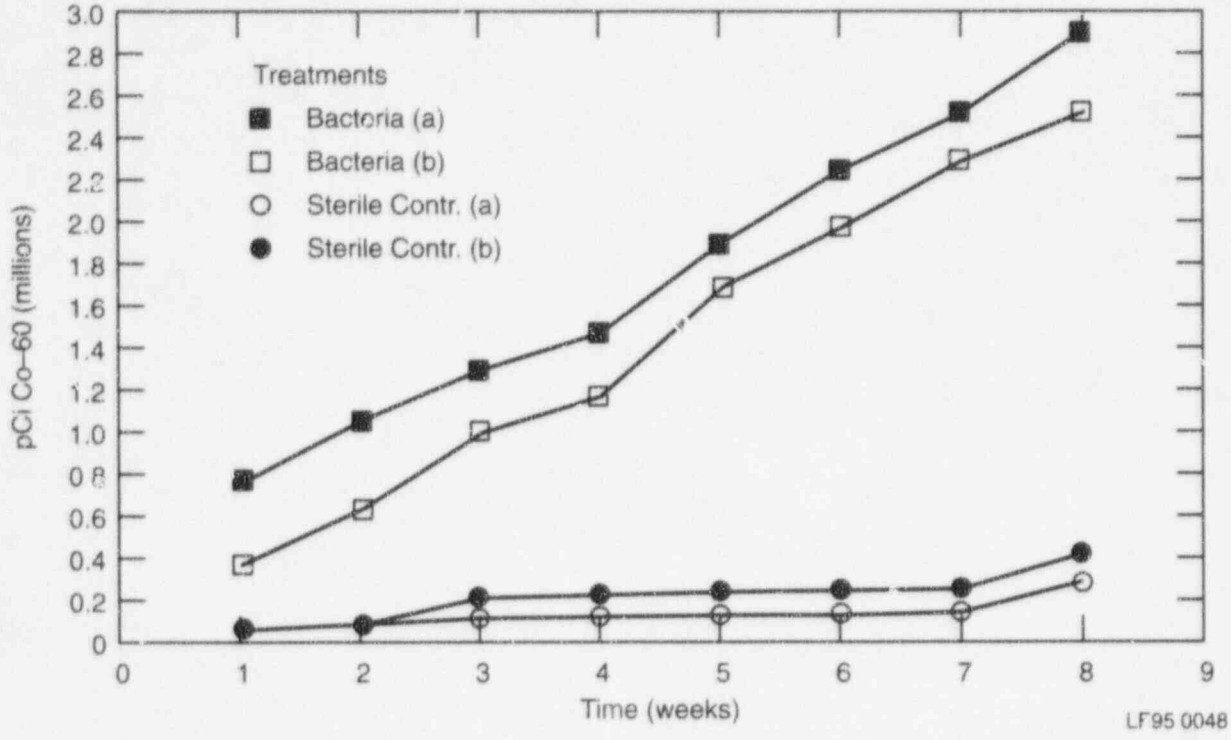


Figure 51. Cumulative Co-60 leached over time from Peach Bottom waste-form specimens exposed to either sterile medium or thiobacilli lixiviant.

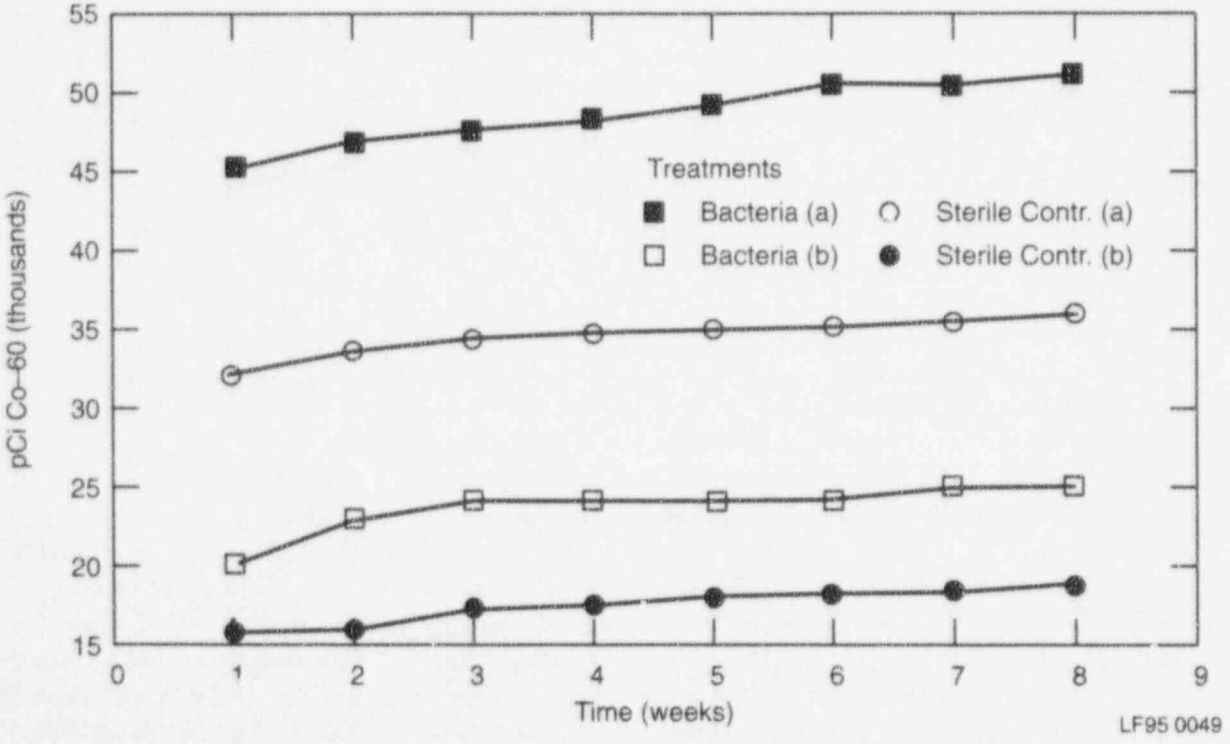


Figure 52. Cumulative Cs-137 leached over time from Peach Bottom waste forms exposed to either sterile medium or thiobacilli lixiviant.

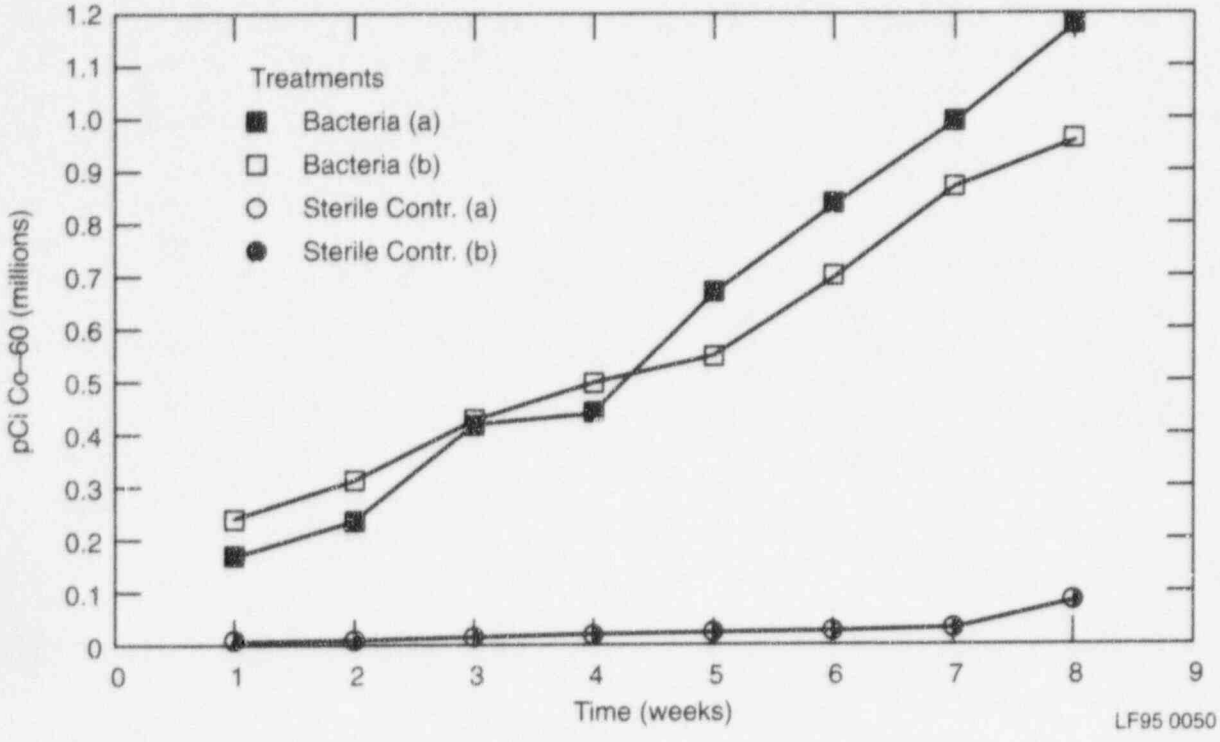


Figure 53. Cumulative Co-60 leached over time from Nine Mile Point waste-form specimens exposed to either sterile medium or thiobacilli lixiviant.

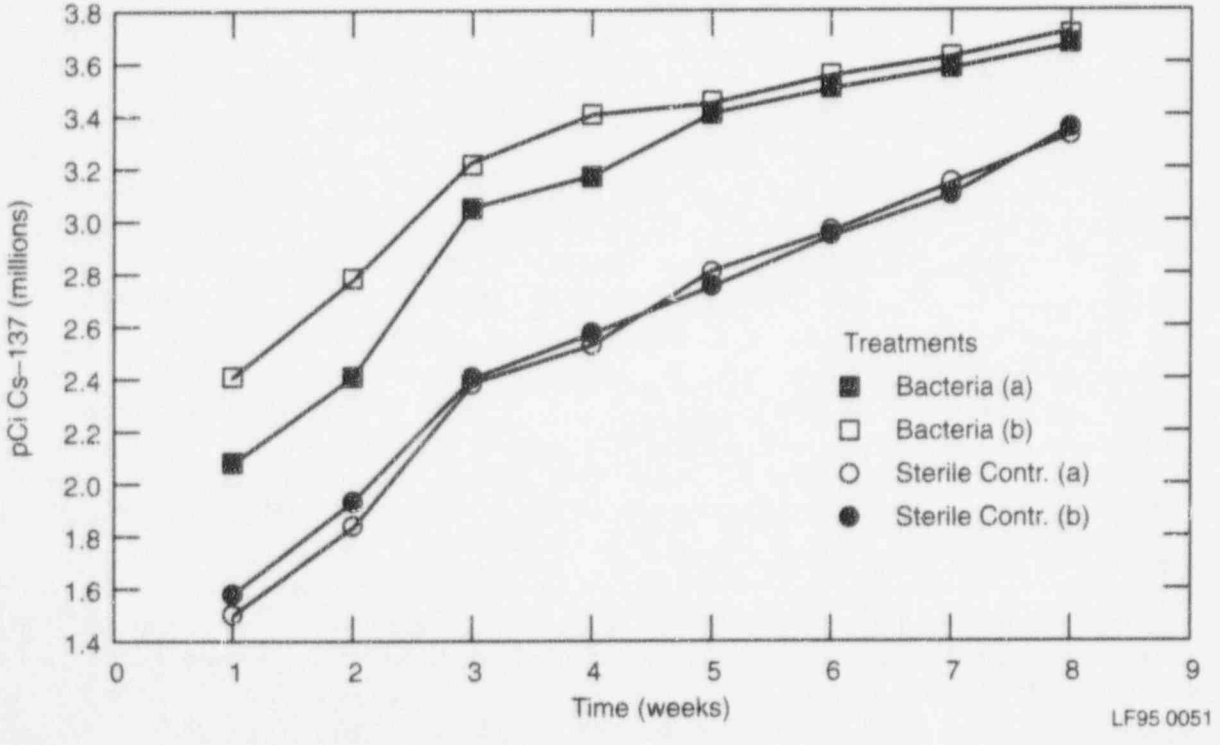


Figure 54. Cumulative Cs-137 leached over time from Nine Mile Point waste-form specimens exposed to either sterile medium or thiobacilli lixiviant.

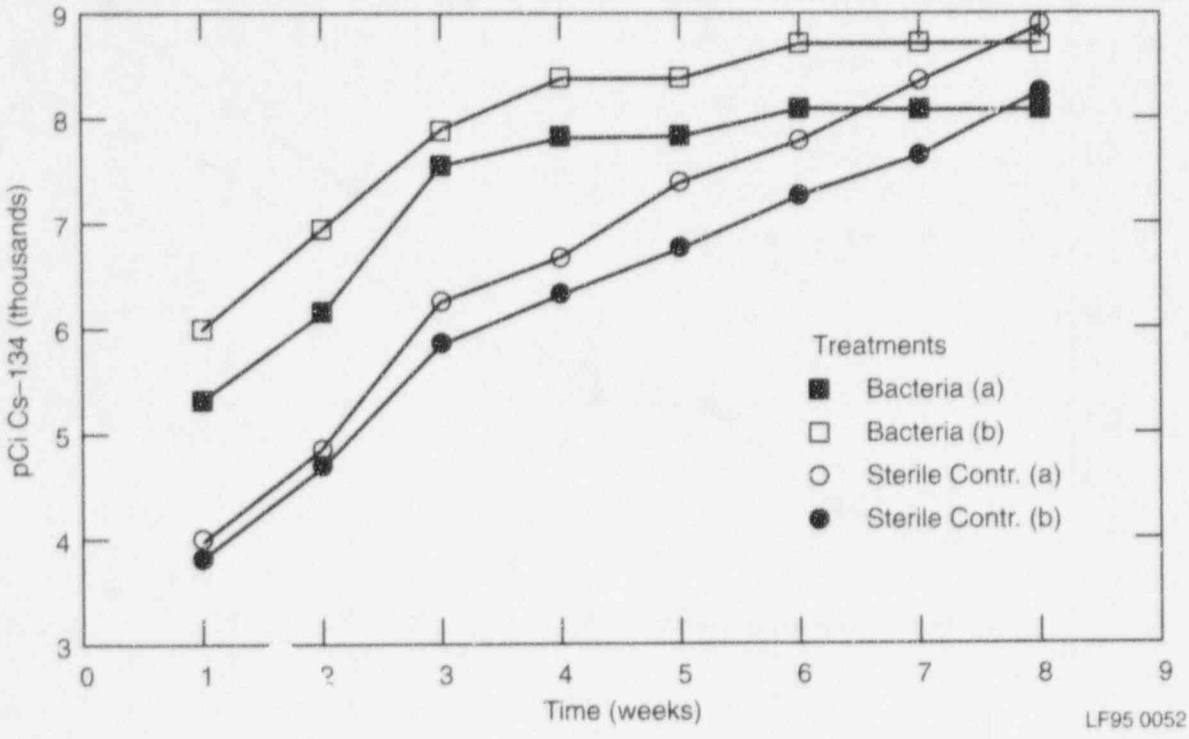


Figure 55. Cumulative Cs-134 leached over time from Nine Mile Point waste-form specimens exposed to either sterile medium or thiobacilli lixiviant.

Other Activities

During the reporting period, the following conferences were attended and presentations made:

- A paper titled "Microbially Influenced Degradation of Cement-Solidified Low-Level Radioactive Waste Forms" by R. D. Rogers, M. A. Hamilton, R. H. Veeh, and J. W. McConnell, Jr., was presented by M. A. Hamilton at the ASTM Symposium, November 2, 1993, in Williamsburg, VA. The paper will be published as a chapter in the peer reviewed conference proceedings.

- A presentation titled "Activity of Sulfer Oxidizers at Low-Level Radioactive Waste Sites" by R. D. Rogers, M. A. Hamilton, R. H. Veeh, and J. W. McConnell, Jr., was made by M. A. Hamilton at the American Soil Science Society Annual Meeting, November 11, 1993, in Cincinnati, OH.

- A paper titled "Development of Methodology to Evaluate Microbially Influenced Degradation of Cement-Solidified Low-Level Radioactive Waste Forms" by R. D. Rogers, M. A. Hamilton, R. H. Veeh, and J. W. McConnell, Jr., was presented by R. D. Rogers at the Fall 1993 Materials Research Society Meeting, December 1, 1993, in Boston, MA.

- R. D. Rogers visited Germany, France, and England, and met with researchers in those countries working on microbial degradation of concrete and LLW. Those meetings were held from May 11–17, 1993.

SUMMARY

The literature provides data showing that there are microorganisms that can influence the degradation of cement. Three groups have been identified as principals in the creation of conditions conducive to the degradation of concrete integrity. They include representatives of heterotrophic, nitrifying, and sulfur-oxidizing bacteria. These microbes appear to be ubiquitous in the environment. Work conducted for other tasks of this program has demonstrated that it is possible to isolate these species of microbes and grow them under laboratory conditions. Representatives of the three genera of MID bacteria have been used in the development of prototype testing methodologies for exposing waste-form specimens to microbial effects. The evaluated methods include exposure of waste-form specimens in constant flow solutions containing microbial biomass and metabolic products (lixiviant) using total immersion, intermittent immersion, intermittent lixiviant mist, and by a fed batch system.

Demonstration of the first three methods has been completed with the sulfur-oxidizing bacteria *T. thiooxidans* and *T. ferrooxidans*. When used with these microorganisms, the exposure methods appear to be very conservative in nature because they expose simulated waste-form specimens to an environment that promotes active microbially influenced degradation (MID). Extensive damage to tested, simulated waste-form specimens occurred from each exposure methodology by the end of 60 days. Based on test results, it was determined that the intermittent immersion method, using Soxhlet columns for exposure cells, provided more control of lixiviant delivery to the test specimen and subsequent effluent recovery.

As previously reported, exposure to heterotrophic bacteria did not have the same effect as the thiobacilli. Effects similar to those described above were not seen after 60 days of total immersion of specimens in heterotrophic lixiviant. In addition, initial work with the nitrifying bacteria using the fed batch method showed that exposure periods over 60 days were suggested before effects comparable to the thiobacilli are obtained.

It was found with both the nitrifier and thiobacilli that the presence of the cement-solidified waste did not hinder the active growth of planktonic microbes. Further, it was established that the microbes could colonize the cement waste-form specimen surface and initiate the formation of a biofilm. This information was confirmed by both selective staining and SEM. These data were considered important since the presence of biofilm EPS on a waste form surface indicates that the microorganisms are not only tolerating the chemical conditions of the surface, but they are also able to proliferate. The implication of these findings is that biofilm formation with accompanying microbial activity would contribute to degradation of infected waste forms. This is an important finding because MID microorganisms have been shown to exist at actual and proposed LLW disposal sites (Rogers et al. 1994), and it indicates that in a natural, low-level waste disposal setting, microbes could proliferate at the soil/waste form interface or waste form surface. Under such intimate conditions, the MID microorganisms would be protected from the environment while challenging the integrity of the waste form.

In general, this work has shown that production of acid as a function of obtaining energy for metabolism can cause a significant decrease in the pH of both waste form surfaces and solutions. This is obvious from the strong inverse correlation between oxidation of reduced forms of nitrogen and sulfur and the pH decrease observed in inoculated treatments and uninoculated controls. The increase in solubilized Ca associated with the biological treatments shows that the presence of MID bacteria contributed to an increase in degradation of the cement matrix of both INEL and vendor-supplied waste-form specimens and the ASTM control formulation.

For the nitrifying bacteria, most-probable-number enumeration of treated waste form types and the ASTM control demonstrates the presence

of a generally stable population of *Nitrosomonas europea* on the respective pellet surfaces. SEM imaging of the inoculated pellet surfaces corroborates bacterial colonization.

Work with the thiobacilli has shown that the effects of MID on treated waste forms can be ascertained by physical, chemical, and biological examination. Physically, exposed waste forms appear to be in various degrees of degradation ranging from eroded surfaces to complete disintegration. The extent of damage appears to depend on the specimen composition and the time of lixiviant exposure. Chemical evidence of MID includes a decrease in exposed lixiviant pH and a decreased surface pH of the test specimen. Final pH of both solutions and surfaces can range from 1 to ~3. Also, it was found that a substantial number of elements composing the cement matrix of the specimen were solubilized, indicating a loss of waste form integrity.

Based on the stability of operational conditions and the consistency of data, the intermittent immersion exposure system using *T. thiooxidans* was selected for testing waste-form specimens containing radionuclides. The conservative test conditions were used because these waste forms represented a new, untested material. Waste forms used in this study were two varieties of actual cement-solidified low-level waste forms made from power reactor wastes. One contained ion-exchange resin waste from the Peach Bottom (PB) Atomic Power Station Unit 3, and the other contained sludge from the Nine Mile Point (NMP) Nuclear Plant Unit 1. Effluents derived from the testing of these specimens were analyzed for gross beta- and gamma-emitting isotopes. In addition, final composite samples were analyzed for Sr-90, Tc-99, and C-14.

Medium exposure had no visible effect on the ASTM control, while there was minor swelling of the NMP specimens (the waste form shape is still well defined). Unlike some INEL studies with other waste-form specimens containing ion-exchange resin, the PB samples were not totally disintegrated, and the exposed individual resin beads appeared to be firmly embedded in the cement matrix. NMP evaporator bottoms waste-form specimens that were exposed to the thiobacilli lixiviants gradually crumbled, and material loss was evident. Similar physical damage was seen in the PB ion-exchange resin specimens exposed to sterile lixiviant. Upon close examination, however, there was more extensive damage to all specimens in contact with the lixiviant than was first observed. The NMP specimens were completely disintegrated, and the PB waste-form specimens had crumbled, with evidence of unattached resin beads.

When effluent from the power reactor samples was analyzed for the presence of gross beta- and gamma-emitting nuclides, the PB effluents, generally, were found to have Co-60 and Cs-137; the effluents from the NMP samples contained these nuclides and Cs-134. In addition, C-14, Tc-99, and Sr-90 were found in the composite effluents from both types of waste-form specimens. The data show that the specimens exposed to the thiobacilli lixiviant had a greater loss of Co-60, C-14, Tc-99, and Sr-90 when compared to the controls treated with the sterile medium, while those effects on Cs-137 and -134 were less noticeable. Analysis of the composite effluent samples showed that more total quantities of C-14 and Sr-90 were removed from the treated NMP specimens than from the PB specimens, while the amount of liberated Tc-99 was comparable for both waste form types. Again, those specimens exposed to the thiobacilli lixiviant had more nuclides removed than did the controls. On average, the PB waste forms leached approximately 1.5, 8.5, and 6.1 times more C-14, Tc-99, and Sr-90, respectively, when treated with the thiobacilli lixiviant than they did when treated with the control medium. For the NMP specimens, these values were 9.9, 5.6, and 2.2, respectively.

These data support the continued development of appropriate tests necessary to determine the resistance of cement-solidified LLW to microbially induced degradation that could impact the stability of the waste form. They also justify the continued effort to define the conditions necessary to support the microbiological growth and population expansion.

CONCLUSIONS

This report provides data from three developed biodegradation test methodologies that are suitable for testing cement-solidified low-level waste. Data from several testing scenarios have demonstrated conclusively that cementatious materials can be substantially damaged by the aggressive environment promoted by the activity of specific microorganisms. Of the three genera of bacteria used for testing, the two species of sulfur-oxidizing thiobacilli bacteria were the most aggressive. Use of these bacteria in three different waste form exposure scenarios (total immersion, intermittent immersion, and intermittent misting) has demonstrated that their presence can promote physical damage to simulated waste forms (those containing exchange resin or evaporator bottoms). Previous work has shown that, under conditions of total immersion (the only exposure method used on these genera), the heterotrophic bacteria can initiate cement degradation. This action occurs over an extended period and is much less effective than the thiobacilli. It was also demonstrated that the nitrifying bacterium used in a fed batch system can initiate cement degradation. It was shown that both the thiobacilli and the nitrifying bacteria *N. europea* can form biofilms on exposed surfaces of waste forms. (The surface activity of the heterotrophic bacteria was not evaluated.) The active colonization of the waste form surface and resulting formation of a biofilm showed that the bacteria were not adversely affected by cement chemistry. These data indicate that the colonization of the surfaces of cementatious waste forms could occur under natural conditions. It is therefore concluded that prolonged exposure to microbial activity could compromise the integrity of cement-based waste forms. Furthermore, work with specimens of actual waste forms has demonstrated the accelerated release of radionuclides due to microbial action. In an accelerated test, thiobacilli would be the candidate organism of choice.

Based on stability of operational conditions and consistency of data, the intermittent immersion exposure system should be used for testing of actual LLW. Also, under present operating conditions, the intermittent immersion system is less likely to have loss of lixiviant through leakage or spills, and therefore will be most effective in controlling the spread of solubilized radioactive contaminants. Because samples of actual radioactive waste forms will represent new, untested material, it is suggested that the conservative test conditions be used for the purposes of the Branch Technical Position testing. This will necessitate that the tests be conducted for at least 60 days and that a thiobacilli be used. While both species of thiobacilli would be appropriate, *T. thiooxidans* has been selected because it is the microorganism most often cited in the literature associated with MID of cement.

REFERENCES

ASTM (American Society for Testing and Materials), 1981a, "Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi," ASTM G21-70, *1981 Annual Book of Standards*, Part 35.

ASTM, 1981b, "Standard Practice for Determining Resistance of Plastic to Bacteria," ASTM G22-76, *1981 Annual Book of Standards*, Part 35.

ASTM, 1990, "Standard Test Methods of Compressive Strength of Hydraulic Cement Mortar," ASTM C109-90, *1990 Annual Book of ASTM Standards*.

Bailey, J. E. and D. F. Ollis, 1986, *Biochemical Engineering Fundamentals*, McGraw-Hill, Inc., New York.

Colombo, P. and R. M. Neilson, Jr., 1979, *Properties of Radioactive Wastes and Waste Containers*, NUREG/CR-0619, BNL-NUREG-50591, Brookhaven National Laboratory.

Diercks, M., W. Sand, and E. Bock, 1991, "Microbial Corrosion of Concrete," *Experientia 47*, pp. 514–516.

Dunk, M., 1991, *Influences of Microbiology on Nuclear Waste Disposal*, DOE/HMIP/RR/91/033, HMIP Department of the Environment.

Francis, A. J., S. Dobbs, and R. J. Nine, 1980, "Microbial Activity of Trench Leachates from Shallow-land Low-level Radioactive Waste Disposal Sites," *Applications of Environmental Microbiology, 40*, pp. 108–113.

Garret, R. H. and A. Nason, 1969, "Further Purification and Properties of Neurospora Nitrate Reductase," *Journal of Biological Chemistry, 244*, pp. 2870–2882.

Grady, C. P. L., Jr. and H. C. Lim, 1980, *Biological Wastewater Treatment: Theory and Applications*, Marcel Dekker, Inc., New York.

Islander, R. L., J. S. Devinny, R. Mansfeld, A. Postyn, and H. Shih, 1991, "Microbial Ecology of Crown Corrosion in Sewers," *Journal of Environmental Engineering, 177*, pp. 751–770.

Libert, M. F., R. Sellier, G. Jouquet, M. Trescinski, and H. Spor, 1993, "Effects of Microorganisms Growth on the Long-Term Stability of Cement and Bitumen," *Proceedings of the Materials Research Society: Scientific Basis for Nuclear Waste Management XVI Symposium: Boston MA*, Volume 294, C. G. Interrante and R. T. Pabalan (eds.), pp. 267–272.

Mansch, R. and E. Bock, 1992, "Simulation of Microbiologically and Chemically Influenced Corrosion of Natural Sandstone," *Proceedings of the International Symposium on Microbiologically Influenced Corrosion (MIC) Testing, Miami, FL, November 1992*, Special Technical Publication (STP) "MIC Testing."

Mori, T., T. Nonada, K. Tazake, M. Koga, Y. Hikosaka, and S. Hoda, 1992, "Interactions of Nutrients, Moisture, and pH on Microbial Corrosion of Concrete Sewer Pipes," *Water Resources, 26*, pp. 29–37.

NRC (U.S. Nuclear Regulatory Commission), 1987, *Licensing Requirements for Land Disposal of Radioactive Waste*, Title 10, Code of Federal Regulations, Part 61, U.S. Federal Register, Vol. 46, No. 142, July 24, 1987.

NRC, 1991, *Technical Position on Waste Form*, Revision 1, Low-Level Waste Management Branch, Washington, D.C., January 1991.

Rogers, R. D. and J. W. McConnell, Jr., 1988, *Biodegradation Testing of TMI-2 EPICOR-II Waste Forms*, NUREG/CR-5137, EGG-2540, Idaho National Engineering Laboratory, June.

Rogers, R. D. and J. H. Wolfram, 1992, "Biological Separation of Phosphate Ore," *Mineral Bioprocessing*, 1992.

Rogers, R. D., M. A. Hamilton, and J. W. McConnell, Jr., 1993, *Microbial-Influenced Cement Degradation—Literature Review*, NUREG/CR-5987, EGG-2695, Idaho National Engineering Laboratory, March.

Rogers, R. D., M. A. Hamilton, R. H. Veeh, and J. W. McConnell, Jr., 1994, *Microbial Degradation of Low-Level Radioactive Waste, Annual Report for FY 1993*, NUREG/CR-6188, EGG-2730, Vol. 1, Idaho National Engineering Laboratory, April.

Sand, W. and E. Bock, 1988, "Biogenic Sulphuric Acid Attack in Sewage Systems," *7th International Biodeterioration Symposium: Cambridge England*, D. R. Houghton, R. N. Smith, and H. O. W. Eggins (eds.), pp. 113–117.

Schmidt, E. L. and L. W. Belser, 1982, "Nitrifying Bacteria," *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*, Chapter 48, pp. 1027–1042, *Agronomy Monograph No. 9*, ASA-SSSA, Madison, WI.

Tokar, M., 1989, *Workshop on Cement Stabilization of Low-Level Radioactive Waste*, NUREG/CR-0103, NISTIR 89-4178, October.

NRC FORM 335 (2-89) NRCM 1102, 3201, 3202

U.S. NUCLEAR REGULATORY COMMISSION

BIBLIOGRAPHIC DATA SHEET

(See instructions on the reverse)

1. REPORT NUMBER (Assigned by NRC, Add Vol., Supp., Rev., and Addendum Numbers, if any.)

NUREG/CR-6188
INEL-95/0153
Vol. 2

2. TITLE AND SUBTITLE

Microbial Degradation of Low-Level Radioactive Waste
Annual Report for FY 1994

3. DATE REPORT PUBLISHED

MONTH	YEAR
August	1995

4. FIN OR GRANT NUMBER

L1807

5. AUTHOR(S)

R. D. Rogers, M. A. Hamilton, R. H. Veeh, J. W. McConnell, Jr.

6. TYPE OF REPORT

Technical

7. PERIOD COVERED (Inclusive Dates)

8. PERFORMING ORGANIZATION – NAME AND ADDRESS (If NRC, provide Division, Office or Region, U.S. Nuclear Regulatory Commission, and mailing address; if contractor, provide name and mailing address.)

Idaho National Engineering Laboratory
Lockheed Idaho Technologies Company
Idaho Falls, ID 83415

9. SPONSORING ORGANIZATION – NAME AND ADDRESS (If NRC, type "Same as above", if contractor, provide NRC Division, Office or Region, U.S. Nuclear Regulatory Commission, and mailing address.)

Division of Regulatory Applications
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission
Washington, D.C. 20555 -0001

10. SUPPLEMENTARY NOTES

11. ABSTRACT (200 words or less)

The Nuclear Regulatory Commission stipulates in 10 CFR 61 that disposed low-level radioactive waste (LLW) be stabilized. To provide guidance to disposal vendors and nuclear station waste generators for implementing those requirements, the NRC developed the Technical Position on Waste Form, Revision 1. That document details a specified set of recommended testing procedures and criteria, including several tests for determining the biodegradation properties of waste forms. Cement has been widely used to solidify LLW; however, the resulting waste forms are sometimes susceptible to failure due to the actions of waste constituents, stress, and environment. The purpose of this research program is to develop modified microbial degradation test procedures that will be more appropriate than the existing procedures for evaluating the effects of microbiologically influenced chemical attack on cement-solidified LLW. Groups of microorganisms indigenous to LLW disposal sites are being employed that can metabolically convert organic and inorganic substrates into organic and mineral acids. Such acids aggressively react with cement and can ultimately lead to structural failure. Results over the past year on the application of mechanisms inherent in microbially influenced degradation of cement-based material are the focus of this annual report. Data-validated evidence of the potential for microbially influenced deterioration of cement-solidified LLW and subsequent release of radionuclides has been developed during this study.

12. KEY WORDS/DESCRIPTORS (List words or phrases that will assist researchers in locating the report.)

Concrete, Cement, Low-Level Radioactive Waste, Microbial Degradation

13. AVAILABILITY STATEMENT

Unlimited

14. SECURITY CLASSIFICATION

(This Page)
Unclassified

(This Report)
Unclassified

15. NUMBER OF PAGES

16. PRICE

Federal Recycling Program

UNITED STATES
NUCLEAR REGULATORY COMMISSION
WASHINGTON, D.C. 20555-0001

120555139531 1 1AN1CC1CJ1C01
US NRC-OADM
DIV FOIA & PUBLICATIONS SVCS
TPS-PDR-NUREG
2WFN-6E7
WASHINGTON DC 20555