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Microbial Degradation of Low-Level Radioactive Waste

Annual Report for FY 1993

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ABSTRACT

The Nuclear Regulatory Commission stipulates that disposed low-level radioactive waste (LLW) be stabilized. Because of apparent ease of use and normal structural integrity, cement has been widely used as a binder to solidify LLW. However, the resulting waste forms are sometimes susceptible to failure due to the actions of waste constituents, stress, and environment. This report reviews laboratory efforts that are being developed to address the effects of microbiologically influenced chemical attack on cement-solidified LLW. Groups of microorganisms are being employed that are capable of metabolically converting organic and inorganic substrates into organic and mineral acids. Such acids aggressively react with cement and can ultimately lead to structural failure. Results on the application of mechanisms inherent in microbially influenced degradation of cement-based material are the focus of this report. Sufficient data-validated evidence of the potential for microbially influenced deterioration of cement-solidified LLW has been developed during the course of this study. 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 of enumeration of the conditions necessary to support the microbiological growth and population expansion.

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INTRODUCTION

Regulatory Background

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

Concerns over the appropriateness of the TPWF tests for microbial degradation of cement containing LLW were voiced in 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 were 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 conclusion of the workshop was that "There appeared to be a clear need to specify more appropriate microbes for such [TPWF] tests" and that "This [need] might require a substantial research effort." (Tokar 1989).

Researchers familiar with the required TPWF biodegradation tests, American Society of Testing Materials (ASTM) G21 and G22 (ASTM 1981a; 1981b), have found them to be not 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 (ACNW) also has raised concerns about biodegradation testing of LLW. In a letter to the Commission dated September 6, 1990, the ACNW indicated that biodegradation testing should be specified for cementitious waste matrices using bacteria that are likely to affect cement as well as any organic component exposed during the degradative process.

Based on the NRC needs, the objectives of the project have been to (a) develop biodegradation test procedures that will allow for the determination of cement waste stability, (b) test those developed procedures on simulated nonradioactive waste and, if successful, continue with actual solidified LLW, (c) based on the best procedure developed, recommend modified test procedures for consideration as revisions to the TPWF, (d) investigate the environment of LLW sites to determine if microorganisms capable of

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

It is anticipated that the research conducted for this project will provide data, information, and recommendations that can be used by the NRC as a basis for (a) implementation of 10 CFR Part 61 requirements concerning the stability of cementitious LLW specimens when challenged by known cement-degrading microorganisms under optimal conditions for cement degradation, (b) modifications to the TPWF testing procedures to more realistically determine microbial effects on cement-solidified LLW, (c) guidance to licensees concerning determination of the susceptibility of cement-stabilized LLW to biodegradation, and (d) 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.

Technical Background

Three groups of organisms have been identified as principals in the creation of conditions conducive to the degradation of concrete integrity. They include some organic acid-producing bacteria, sulfur-oxidizing bacteria, and nitrifying bacteria. Species of each of these groups of organisms have been isolated from degrading concrete (Sand and Bock 1982; Bock et al. 1988; Karavaiko and Zhrebyat'eva 1989).

Microbially influenced degradation (MID) of concrete is thought to occur when microorganisms present in the environment produce mineral or organic acids that dissolve or disintegrate the cement matrix. Because the microorganisms are not metabolizing the cement matrix, the rate of degradation cannot be determined by measuring the generation of metabolically produced gases. The particular mechanisms of biological acid attack are consistent with those that have been associated with chemical attack. Mineral acids such as sulfuric and nitric acids are extremely destructive and are known to destroy concrete at rates much faster than physical weathering.

Organic acids, both water-soluble, low-molecular-weight acids and insoluble, high-molecular-weight acids will also degrade concrete. For example, acetic, formic, and lactic acids have been shown to be very aggressive in attacking concrete (Neville 1981). It appears that one of properties of concrete that determine its resistance to acid attack is the quality and amount of interstitial mortar (cement).

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 byproducts 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 acid conditions that can be less than pH 3.

Because of their diversity, heterotrophs can exist under a much wider range of environmental conditions than mineral acid-producing bacteria. Although the abundance of heterotrophic bacteria increases with the quantity of soil organic matter, they can survive in any environment in which even a minute carbon source exists. The amount of acid they are capable of producing depends on the organisms present and the environment in which they are growing. The phenomenon of heterotrophic biodegradation of concrete has not been well studied. Karavaiko and Zhrebyat'eva (1989) looked at heterotrophs in relation to effects on concrete and suggested that a community of organisms, not an individual species, was responsible for the process. It has been reported that at least 42 microbiological species have been identified in various environments where concrete degradation has occurred (Hall 1989).

In work conducted to determine the microbiological parameters associated with the English nuclear waste disposal effort (Dunk 1991), a wide

diversity of heterotrophic microorganisms were isolated from simulated, cement-solidified, plutonium-contaminated materials. Many of the microbes cultured were capable of growing in alkaline conditions (pH 11) common on the surface of concrete. Unfortunately, the studies were not carried out over a sufficient period of time to determine if growth of the organisms affected 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). Microorganisms used in these studies were isolated in a microbial growth media 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 media (pH 9). Also, these microbes grew well in the presence of cement and produced organic acids. A fungus and the bacterium *Pseudomonas cepacia* were selected for the concrete degradation studies. The degradation studies were conducted by exposing samples of concrete to a continuous source of cultured microbes for periods of up to 1 year. Samples of concrete were retrieved for evaluation at 1-month intervals. These samples were evaluated for strength, weight loss, loss of structural material (Ca and Al), and porosity as well as for changes in the structural matrix (x-ray diffraction). After 5 months, it was determined that samples exposed to the bacteria had decreased in weight by 12% (on a dry weight basis) while those exposed to the fungus showed a weight loss of 10%. In addition, the samples had a 5% decrease in Ca. Loss of Ca did not correlate to the amount of organic acid produced, which indicated that mechanisms other than organic chelation were responsible for the process of Ca depletion. At the end of 7 months, porosity had increased 11% in the samples. The increased porosity was probably directly related to the loss of $\text{Ca}(\text{OH})_2$ crystals. At the end of 11 months, there had been a 50 to 85% loss of $\text{Ca}(\text{OH})_2$ in the treated concrete samples, compared to control samples not exposed to the microbes, resulting in 80% loss of compressive strength. Additional studies were conducted involving the exposure of concrete samples to organic acid-producing fungi under optimum

growth conditions (Libert et al. 1993). Results from the 2-year study demonstrated that the organic acid-producing fungi were responsible for significant loss of calcium from a defined leach layer of 0.2 cm. The pH of the solution surrounding the test specimens was in the mid 4's.

Nitrifying Bacteria. The second group of microorganisms known to promote concrete degradation are the nitrifying bacteria. These bacteria (e.g., *Nitrosomonas* and *Nitrobacter*) are chemoaototrophs. They obtain energy through the oxidation of inorganic nitrogen compounds. These organisms have been isolated from a variety of soils, and many species of the ammonium oxidizers appear to be ubiquitous, becoming active under suitable conditions (Alexander 1977). They are an important part of the biological cycling of nitrogen within the ecosystem. Neutral-to-alkaline soils have the largest populations of nitrifying bacteria; however, optimum pH for individual isolates varies from 6.6 to 8.0 or higher.

Both the ammonium oxidizing *Nitrosomonas* sp. and the nitrite oxidizer *Nitrobacter* sp. have been isolated from degraded concrete. They have been demonstrated to be active in biological nitric-acid degradation of concrete during controlled experiments (Bock 1987; Bock and Sand 1993; Diercks et al. 1991). Bock et al. (1988) found that nitrifiers were the most abundant organisms contributing to deterioration of building stone. The outermost layer of the stone to a depth of 5 mm was heavily contaminated with these bacteria. The stone surface pH of 5 was directly attributable to the presence of nitric acid.

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. 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.

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Sulfur-Oxidizing Bacteria. Sulfur-oxidizing bacteria (genus *Thiobacillus*) are the microorganisms most often associated with the biological degradation of concrete structures. These organisms were found to be 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 CO₂ as their sole carbon source.

Ecologically, sulfur-oxidizing bacteria fill an important niche in the overall cycling of sulfur. In that cycle, there are several genera of bacteria that are capable of reducing oxidized forms of sulfur (organic sulfur compounds, inorganic sulfates) to sulfides and also, under the proper conditions, to elemental sulfur. In turn, these reduced sulfur compounds can then readily become the substrate for the sulfur-oxidizing bacteria resulting in the formation of sulfuric acid. Regardless of the initial form of sulfur (reduced or oxidized), the natural sulfur cycle provides the potential for sulfuric acid production.

Thiobacilli are versatile and can flourish under a range of environmental conditions. Islander (1991) proposed that a natural succession of different species of thiobacilli were involved in the degradation of concrete in sewer systems. The process begins with the neutralization of calcium hydroxide, which is present in the concrete. When the concrete surface reaches a pH of approximately 9, *Thiobacillus thioparus* actively begins to oxidize available forms of reduced sulfur. Products from this process contribute to a decrease in surface pH. Soon, the species *T. intermedius* and *T. novellus* become active and the surface pH continues to decrease. As the pH decreases to 6, *T. neapolitanus* becomes active. When the pH decreases to 3, *T. thiooxidans* becomes the dominant species, causing a further decrease of the surface pH to near 1 or below. Finally, a climax community develops that is dominated by

T. thiooxidans and perhaps acidophilic heterotrophs capable of utilizing excreted organic waste products. Because of the ability of *T. thiooxidans* to rapidly produce acid from available sources of sulfur, it has been suggested that its abundance is a better measure of corrosion rate than the actual pH (Sand and Bock 1984). Another thiobacilli that is capable of the production of extreme acid conditions is *T. ferrooxidans*. This species not only can oxidize reduced sulfur compounds but is able to utilize ferrous ion (Kuenen and Tuovinen 1981).

The ecology of thiobacilli in terrestrial environments (soil) is not as well documented and understood as it is in sewage systems. In general, it is assumed that sulfur oxidizers are ubiquitous in soil (Kuenen and Tuovinen 1981). This assumption is validated because a majority of soils that either contain reduced sulfur compounds or that have amendments of these compounds show overt evidence of thiobacilli activity. In the absence of oxidizable sulfur, mineral soils usually support 100 to 200 thiobacilli per gram (Alexander 1977). However, after addition of oxidizable sulfur compounds, a significant increase of thiobacilli in these soils is observed. Therefore, it is a common agricultural practice to add elemental sulfur to soils in order to improve their fertility. The practice of adding elemental sulfur to soil is essentially equivalent to the application of sulfuric acid. Studies have shown that treated soils have a rapid increase in the concentration of sulfate and, in the case of some neutral soils, a detrimental decrease in pH to as low as 2. Because of this effect, it has been found that sulfur additions can be used to reclaim alkali farm land. With controlled addition of free sulfur to alkali soils, the sulfuric acid generated can neutralize the alkalinity and increase soil productivity.

The above data suggest that, regardless of the initial populations of the various species of thiobacilli in soils, once the process of sulfur oxidation begins, *T. thiooxidans* or *T. ferrooxidans* will eventually become the dominate organisms. Although dominance depends on the environmental conditions, both of these species appear to be appropriate choices for use in MID evaluations.

MATERIALS AND METHODS

Development of Microbial Culture Collection at Disposal Sites

Preliminary data (Rogers et al. 1993) suggest that three different genera of bacteria are required 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. Possible sources from which the microorganisms could be isolated include samples from sites with natural microbial activity, actual or proposed LLW locations, or existing culture collections. While the literature suggests that the three types of microorganisms considered for this study are ubiquitous in the environment, it was considered important to demonstrate their presence in soils representative of present and proposed commercial LLW sites. The sources of microorganisms together with steps for confirming their viability in selective environments are detailed in Figure 1.

The main criteria for LLW sample sites was their location within the geographic area of

commercial disposal facilities or proposed state or state compact sites. At the time of collection, licensed disposal sites included Beatty, Nevada; Barnwell, South Carolina; and Hanford, Washington. Host states for proposed compact sites included Pennsylvania, Appalachian Compact; Nebraska, and Ohio, Midwest Interstate Compact; Connecticut and New Jersey, Northeast Interstate Compact; Washington, Northwest Interstate Compact; Nevada, Rocky Mountain Compact; South Carolina/North Carolina, Southeast Compact; California, Southwestern Compact; Nebraska, Central Interstate Compact; Texas and New York, Unaffiliated States (ANS 1992).

To aid in a rational selection, it was decided that the collection sites should also include at least four climatologically diverse environments: warm humid, cool humid, warm dry, and cool dry. Furthermore, selected sites needed to be within compacts that had reached maturity in the site selection process. When these conditions were overlain with the main criteria for sample sites and host locations, it is seen that candidate sites could be selected from the following compacts: Central Midwest, Illinois or Central Interstate,

Figure 1. Development of microbial culture collection.

Materials and Methods

Nebraska (cool humid); Northeast, New Jersey or New York (cool humid); Southeast, South Carolina\North Carolina (warm humid); Northwest, Washington (cool dry); Southwestern, California or Rocky Mountain Nevada (warm dry); and Texas (warm dry). The final selections

from the above list were Argonne and Urbana, Illinois; Richland, Washington; Beatty, Nevada; Ward Valley, California; El Paso, Texas; Barnwell, South Carolina; and New York State. Figures 2 and 3 are pictures of the Beatty and Ward Valley locations.

Figure 2. U.S. Ecology Low-Level Waste Facility, Beatty, NV.

Figure 3. Proposed U.S. Ecology Low-Level Waste Site, Ward Valley, CA.

As an overview, soils were collected from several locations (dependent on opportunity and availability) at each sampling site. At each location, a sufficient quantity of soil was retrieved with sterile implements and placed into specially prepared sterile collection bags and sealed. These bags were placed into an ice chest and sent to the laboratory for processing.

Soil Sample Collection

Specific methods for soil collection were as follows:

1. Select an area from which a sample was to be obtained at a designated sampling site.
2. Clear the area of loose soil and debris with a trowel.
3. Assemble the soil coring device (see Figures 4, 5, and 6). If a core was to be retrieved from the soil surface, the following procedures were used:
 - a. Remove adhering soil (with a kimwipe or brush) from the inside of the auger coring device.
 - b. Wash the interior of the auger with ethyl alcohol.
 - c. Select a sterilized, capped, stainless steel core liner and remove one of the caps (Figure 7).
 - d. Hold the liner by the remaining capped end and insert it uncapped end first into the auger coring device.
 - e. Remove the remaining liner cap and finish assembling the coring device by attaching the solid end plug.
4. Twist the coring device into the soil (Figure 8).
5. Remove the coring device when it is full.
6. Disassemble the coring device and expose soil core by removing the solid end of the coring device (Figure 9).
7. Wash the liner retrieving hook with ethyl alcohol.
8. Place the end of the hook into the hole in the end of the liner and retrieve the liner.
9. As the liner is removed from the auger, immediately place a cap (blue cap on the end with hole for retrieval) with a sterile stainless steel insert on the exposed ends of the liner.
10. Place the capped and sealed liner in a cooler.
11. Reassemble the coring device using the open-ended cap.
12. Using chalk, place a mark indicating the depth of the corer to the next sample on the rod connecting the coring device with the handle.
13. Put the coring device back into the started hole and twist until it has moved into the soil to the depth matching the chalk mark and the surface of the surrounding soil.
14. Retrieve the coring device from the hole and remove and dispose of the soil plug.
15. Continue moving the coring device into the soil profile to the depth of the next designated core.
16. Follow steps 3 through 10 to obtain the next soil core.
17. Record the depth of core retrieval by measuring the length of the rod submerged into the soil profile.

Figure 4. Disassembled soil coring equipment.

Figure 5. Assembled soil core retriever (extension rods are affixed for retrieving cores at depth).

Figure 6. Soil auger with attached extension rods for coring at depth.

Figure 7. Capped stainless steel core liner and casing device.

Figure 8. Twisting the coring device into the soil.

Figure 9. Removal of soil core from coring device.

The collected soils were processed in the following manner:

1. Process soil cores in a sterile work area such as a laminar flow hood.
2. Remove caps from the core liner.
3. Insert an extrusion tool into the liner on the lower end of the core (end opposite the extraction hole).
4. Extrude the first 2 cm of the soil core with a sterile spatula and discard that section.
5. Extrude the rest of the core into a sterile tray.
6. If more than one core was taken at the same depth (within the same site), extrude them also and place them in the tray together.
7. Homogenize these similar samples with repeated cutting and blending.
8. Place oricessed cores into sterile, tared bags.
9. Determine the sample weight.
10. Remove a 5-g sample of the soil to determine the percent moisture on a dry-weight basis.
11. Using an additional 5-g sample of the soil, prepare saturated paste to determine pH.
12. Finally, use a minimum amount of soil necessary for microbiological examination.
13. Place the remaining soil into refrigerated storage (~4 C).

Isolation of Microbes from Soils

Standard methods were used to isolate microbes from the soils. These are described in more detail later. These methods used bioassay methodologies to select those isolates that fell into the three genera of interest. Candidate organisms from this screening were isolated into pure

cultures and saved as part of a culture collection for latter evaluation.

In those soils in which a specific desired type of organism was not isolated on the first attempt (e.g., sulfur oxidizers), further processing steps included enrichment of the soils (baiting with substrates of interest such as reduced sulfur compounds and inorganic nitrogen compounds) followed by use of microbe-specific methods to isolate the microbes of interest. The protocol indicated that if a specific type of organism was not found after enrichment in soils from a LLW location, then samples of those soils could be inoculated with the bacteria of interest and a determination could be made of whether the soil had the potential to support the desired bacteria. Such an evaluation would be necessary since experience has shown that once soils contain the required substrates, they invariably become contaminated with microorganisms able to exploit the favorable conditions. This work also provided much needed information on the activity of candidate organisms under field conditions.

The above steps are consistent with environmental microbiology procedure, and similar protocols have been followed by the Canadians (Mayfield and Barker 1982), the French (Langomazino 1990 personal communication), and the English (Dunk 1991) in their work with microbial involvement in various aspects of LLW disposal.

Heterotrophic Bacteria. Methods used to isolate the various genera of microorganisms differed depending on the type of microbe. Heterotrophic organisms of interest tended to be readily isolated on selective media without the necessity of further enrichment. Isolates were obtained by first diluting 1-g quantities of each of the collected soils 10^2 to 10^6 times with sterile water. After dilution, 0.1 mL of each solution was spread on the solid surface of trypticase soy agar (TSA) with dextrose (40 g TSA, 5 g dextrose per liter DI H₂O) contained in petri plates. The inoculated plates were then incubated 48 hours at a temperature of 28°C. Those plates on which 30 to

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300 colonies of microorganisms were subsequently found to be growing were retained.

Morphologically different colonies were then evaluated with an agar plate assay (Katznelson and Bose 1959) for their ability to produce organic acid in sufficient quantities to effect the solubilization of an insoluble calcium phosphate compound. The assay was carried out by spotting the isolates on the surface of agar, which has been made opaque by the formation of insoluble dicalcium phosphate. Those isolates that were capable of solubilizing the insoluble compound form a clear zone or halo (see Figure 10). Microorganisms found by the test to be positive for organic acid production were retained in a culture collection.

Nitrifying Bacteria. Nitrifying bacteria are extremely slow growing in comparison to the heterotrophs. Their doubling time is typically 24 to 48 hours compared to a few hours for other microorganisms. Dilution techniques described in the above section could not be used to ascertain

the presence and approximate numbers of these bacteria in soils. However, rather than a direct application of diluted samples on to an agar plate, a "most probable number" (MPN) method described by Alexander (1982) was used. The specific MPN method modified for nitrifier enumeration was that developed by Schmidt and Belser (1982). The MPN method permitted a statistical estimation of the bacterial population without an actual, physical count of single cells or colonies. This method was based on an analysis of the presence or absence of the microorganism of interest in five replicates of each of three serial dilutions of candidate soils. A prerequisite for the technique was that the microorganism, whose population is to be determined, must be able to produce an easily recognizable transformation (e.g., a chemical change) in the medium into which it was inoculated. Because nitrifying bacteria carry out a two-step sequential chemical process of oxidizing ammonium to nitrite and then nitrate, it was necessary to use two different enumeration media to detect the formation of these compounds.

Figure 10. Examples of results of agar plate bioassay.

On the basis of probability theory, it was possible to calculate the most probable number of microorganisms in the various serial dilutions from the number of positive and negative occurrences of nitrite or nitrate. Using these combined data, a single value was obtained for estimation of the most probable number of nitrifying bacteria present in the original environmental sample.

The procedure used for the enumeration of nitrifying bacteria was as follows. Stock solutions of appropriate nitrifier media were prepared according to Schmidt and Belser (1982). Next, 5-g quantities of unprocessed soil from each collection site were then transferred into large culture tubes. Sterile phosphate buffer (45 mL) was added, and the mixture of soil and buffer was shaken vigorously for 1 minute. Subsequently, five 10-fold dilutions of the soil buffer mixture were carried out (1 mL to 9 mL sterile buffer) so that, eventually, a final sample dilution of 10^6 was achieved. One-mL aliquots of liquid from the five highest serial dilutions were then transferred to each of five replicate culture tubes containing 4 mL of the nitrifier media. The inoculated tubes were incubated in the dark for a period of 6 to 7 weeks. At the completion of the incubation period, the presence of ammonia-oxidizers was determined by confirming the formation of nitrite by use of a spot test. (This test used a 0.1-mL aliquot of tube contents mixed with one drop of diazotizing reagent [sulfanilamide] and one drop of coupling reagent [N-(1-naphthyl)-ethylenediamine dihydrochloride].) A resulting pink to red color was recorded as a positive for the presence of ammonia-oxidizers. The accumulation of nitrate, a presumptive indication of the presence of nitrite oxidizers, was determined by ion chromatography. The contents of those tubes shown to lack nitrite were further tested by a diphenylamine spot test for the presence of nitrate. This was done in the event that nitrite had been microbially oxidized to nitrate. In addition to the spot tests, subsamples of the tube contents were diluted and analyzed by ion chromatography to confirm nitrite and nitrate content.

Thiobacillus Sulfur-Oxidizing Bacteria. Thiobacilli, like nitrifying bacteria, are very slow

growing bacteria. Their doubling time is typically 3 to 4 days. The slow growth rate necessitates incubation and enrichment techniques that are unique to sulfur oxidizers. Therefore, these techniques precluded enumeration by direct isolation or MPN techniques. Also, obtaining pure isolates of these bacteria involves enrichment procedures rather than direct isolation. Enrichment procedures encourage growth and improve accurate assessment because of the increase in number. Many times, this procedure is the method of choice for reliable identification of sulfur oxidizers in soils that have a wide range of characteristics and microbial communities.

The various methods used for the isolation of thiobacilli included liquid culture, enrichment culture, and solid media. For liquid culture isolations, previously collected soil samples were inoculated into sterile liquid media selective for culturing sulfur-oxidizing microbes. Two media formulations were used for liquid culture of thiobacilli, Media S and Media B. Media S contained the salts ammonium sulfate, potassium phosphate, calcium chloride, and magnesium sulfate, and trace amounts of EDTA and metal salts, zinc sulfate, calcium chloride, manganese chloride, iron sulfate, ammonium molybdate, copper sulfate, and cobalt chloride. After sterilizing this solution in an autoclave, previously steam-sterilized elemental sulfur was added at a concentration of 10 g/L. Media B contained the salts ammonium chloride, potassium phosphate, magnesium chloride, and calcium chloride. Sodium thiosulfate was added at a concentration of 5 g/L, and the solution was sterilized in an autoclave. Both media were especially formulated without a carbon source. Each medium was dispensed in 50-mL portions into 125-mL erlenmeyer flasks. One-gram quantities of the respective soils were placed into flasks containing either Media S or B. The inoculated flasks were then incubated on a shaker at 30°C for 2 to 3 weeks or until evidence of microbial growth was observed either by turbidity or through microscopic examination.

After incubation, 5-mL aliquots of the growing cultures were transferred into other flasks containing 50 mL of fresh, sterile media of the same formulation previously used. Incubation then

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occurs as previously described. Two additional transfers using the above methodology were made for each soil culture when evidence of growth was observed. Three successive transfers of each culture were sufficient to ensure that metabolism by the isolated microbes was dependent on the reduced form of sulfur. This procedure ensured that the microbes present in the final culture were not species capable of utilizing residual energy sources (such as organic carbon) derived from the original soil sample. Thus, this method was very selective for isolation of only sulfur-oxidizing microorganisms.

When sulfur-oxidizing organisms were not readily isolated from a particular soil, enrichment procedures were used. To do this, a 5-g aliquot of each soil sample was weighed and aseptically transferred into a sterile 125-mL erlenmeyer flask. A solution of 20 g of sodium thiosulfate in 200 mL of deionized water was prepared and sterilized in an autoclave. Ten mL of this solution were added to each of the soils, resulting in 1 g of sodium thiosulfate per 5 g of soil. The soils were incubated at 30°C for 2 to 3 weeks. For each of these "enriched" soils, 1-g aliquots, were removed and inoculated into 50 mL of Medium B and 50 mL of Medium S, respectively. These

liquid cultures then underwent the same incubation and transfer according to the procedure described above.

Organisms selected from or enriched from soil in the liquid cultures were subsequently grown on solid medium selective for *Thiobacillus* sp. The medium was prepared using the same formulation as liquid Media B with 5 g/L gelrite added as the solidification agent. For each liquid culture from Medium S and Medium B, a 0.1-mL aliquot was spread onto a solid medium plate and incubated for 2 to 3 weeks at 30°C. Growth of organisms was monitored during this period of time.

Work was conducted to determine if microorganisms from selected existing culture collections had the potential to degrade cement LLW. Microbes were obtained and grown. Growth of the test microorganisms on selected media was monitored. Analytical methodology was used to detect the presence of microbially produced metabolites (mineral or organic acids), which have been reported to degrade concrete structures. Microbial isolates that were determined by the screening procedures to be potential candidates were identified and placed in a permanent culture collection. The process of selection is outlined in Figure 11.

Figure 11. Schematic of methods used for selecting candidate microorganisms.

Development of Procedures for Growing Candidate Microorganisms

Media necessary for the promotion and maintenance of active growth of the candidate microorganisms were obtained from the literature or developed. This involved determination of the appropriate energy and carbon source, mineral nutrients, pH range, and range of incubation temperatures. 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 considered. Efforts for this task are detailed in Figure 12.

Determination of Microorganisms Capable of Cement Degradation

A knowledge of the types of microorganisms that promote cement degradation is useful for selecting appropriate candidate organisms. There was no one prime candidate for a genera of heterotrophic bacteria used in the testing protocol. Several groups were determined capable of producing organic acids. A bioassay that was

sensitive to detect bacteria capable of dissolving calcium compounds was used to select candidate organisms. Only one or two species exhibiting extreme dissolution of calcium compounds were selected for additional studies.

A representative of *Nitrosomonas* sp. 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.

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

Figure 12. Development of procedures for growth and preservation of selected microbes.

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Acid production was used as the criteria for a final selection of both mineral acid and organic acid-producing bacteria. Strength of acid production was determined by the pH change of the solution in which the organism was being cultivated. Analytical methods were used to determine the amount of sulfate and nitrite production. In the case of mineral acids, the concentration of these anions could be related to the initial amount of available substrate.

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. Therefore, testing methods were devised and evaluated for a selected microorganism from each of the three different genera known to degrade concrete. The evaluation was designed using the optimum conditions of growth, exposure to the test specimen, and reaction time to promote degradation of simulated, cement-solidified LLW. Such an approach will provide the necessary flexibility to be able to conduct an evaluation of microbial activity, exposure methodology, specimen size, and test duration. The developed tests were intended to be very conservative. That is, they were to determine if microbial activity can affect the integrity of cement-solidified waste forms rather than speculate on whether degradation will occur under natural conditions.

A method was developed that provided candidate organisms with optimum growth conditions as well as providing necessary exposure time for the waste forms. This was accomplished by using a microbial propagation system coupled with a specimen exposure chamber (Figure 13). The candidate microorganisms were intended to be introduced to the test specimens in several ways including continual perfusion in which specimens were completely immersed in a continual flow of solution as well as intermittent immersion and intermittent misting. Each of these methods will be eventually evaluated; however, only results

Figure 13. Schematic of proposed methods for testing the effects of MID on cement waste forms.

from application of the first two are provided in this report.

During this phase of the study, it was necessary to conduct evaluations on cement specimens that were simulated waste forms. This provided the opportunity of determining how candidate organisms might react to actual solidified LLW. The type of cement that could be used as well as the type of simulated waste solidified were subjects for discussion.

Portland Type I, II, and III cements had been reported as being used as binders for the solidification of both liquid radioactive wastes (materials that are soluble in water, such as salts) and solid radioactive wastes (materials that are insoluble in water, such as nuclear-grade ion-exchange resins). Portland Type I cement has been the general purpose cement commonly used for construction applications in which sulfate attack from soil or water is minimal and where the heat generated during curing will not cause a critical rise in temperature. Portland Type II cement has been modified to have a lower heat of hydration than Type I and improved resistance to sulfate attack. Portland Type III cement has high initial strength and has been used when high strengths are desirable in relatively short times (from 1 to 3 days).

Colombo and Neilson (1979) stated that the type of Portland cement chosen for waste solidification probably depends more on availability and cost, thus favoring the selection of Type I cement. It has been common practice for additives to be mixed in proprietary quantities with Type I cement prior to its use as a solidification agent. The formulation of the cement binder used for this study was determined after a careful review of the available cementitious binders.

Comprehensive lists of waste formulations from boiling water reactors (BWRs) and pressure water reactors (PWRs) have been difficult to obtain. Colombo and Neilson (1979) compiled such a list over a decade ago. Because most operating power reactors were in operation at that time that their list was developed, it was reasonable to assume that it was representative of many present-day waste streams. Their data showed that reactor operations produced a variety of solid and liquid wastes that were compatible with cement solidification technology. Table 1 provides a listing of those materials. It appeared that for this project, the use of both a simulated solid waste (containing ion-exchange resin) and liquid waste (containing soluble salts) would provide a representative sample of LLW waste. The solid waste could be composed of ion-exchange resins in a formulation similar to 1b as listed in Table 1, while that of the liquid might be similar to 3b without the "crud." However, because of the difficulty in obtaining up-to-date formulations of LLW waste as well as cement solidification formulations, it was advisable to obtain simulated cement waste forms directly from interested vendors.

Representative formulations for the INEL-fabricated simulated LLW waste forms together with a cement mortar control waste form are found in Figure 14. The formulations for the waste forms were similar to those listed by Colombo and Neilson (1979). The formulation of the control cement mortar was as specified in ASTM "Standard Test Methods of Compressive Strength of Hydraulic Cement Mortars" (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. Commercial, vendor-supplied, simulated waste forms were obtained. They included both resin and boric acid salts formulations.

All of the simulated waste form specimens (including the ASTM formulation) manufactured at INEL were molded as cylinders 1.8 cm in diameter and 2.2 cm long. 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 methods to produce both simulated, solidified ion exchange resin and evaporator bottoms wastes forms molded into cylinders 5 × 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 vendor-simulated waste forms were then tested in a manner similar to the INEL-simulated waste forms using the testing matrix described in Figure 15. The two types of simulated waste forms and the control cement mortar were exposed to each of the three genera of selected microorganisms. Control solutions of sterile nutrient feed were also used for each set of experiments. Because the time requirement for observable effects was not known, exposure times had to be determined for each testing scenario. This initial work will have to be completed before the duration of a standardized test can be determined.

Development of Criteria for Evaluation of Test Effects

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 the INEL-fabricated and vendor-supplied, simulated waste

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Table 1. Typical formulations of waste types from BWR and PWR reactor sites with listed pH and temperatures prior to solidification (Colombo and Neilson 1979).

Material		Weight percent
1a.	<u>Bead resin waste (slurry)</u>	
	Water	50.0
	Bead resin (IRN-150) ^a	50.0
	Temperature	70°F
	pH	7
1b.	<u>Bead resin waste (dewatered)</u>	
	Water	35.0
	Bead resin (IRN-150) ^a	65.0
	Temperature	70°F
	pH	7
Material		Weight percent in filter cake
2a(1)	<u>BWR precoat filter cake with powdered resin (slurry)</u>	
	Water	50.0
	Anion powdered resin (PAO) ^b	20.0
	Cation powdered resin (PCH) ^b	20.0
	Crud ^c	5.0
	Sodium chloride	5.0
	Temperature	70°F
	pH	7
2a(2)	<u>BWR precoat filter cake with powdered resin (dewatered)</u>	
	Water	32.0
	Anion powdered resin (PAO) ^b	30.0
	Cation powdered resin (PCH) ^b	30.0
	Crud ^c	6.0
	Sodium chloride	2.0
	Temperature	70°F
	pH	7
2b(1)	<u>BWR precoat filter cake with diatomaceous earth (slurry)</u>	
	Water	75.0
	Diatomaceous earth	20.0
	Crud ^c	5.0
	Temperature	70°F
	pH	7

Table 1. (continued).

Material		Weight percent in filter cake
2b(2)	<u>BWR precoat filter cake with diatomaceous earth (dewatered)</u>	
	Water	60.0
	Diatomaceous earth	30.0
	Crud ^c	10.0
	Temperature	70°F
	pH	7
Material		Weight percent in evaporator bottoms
3a.	<u>BWR chemical regenerative waste of a forced recirculation evaporator</u>	
	Water	75.0
	Sodium sulfate	22.9
	Sodium chloride	2.0
	Crud ^c	0.1
	Temperature	170°F
	pH	6
3b.	<u>PWR chemical renenerative waste of a forced recirculation evaporator</u>	
	Water	73.4
	Sodium sulfate	14.9
	Ammonium sulfate	9.6
	Sodium chloride	2.0
	Crud ^c	0.1
	Temperature	170°F
	pH	2.5 to 4.0
3c.	<u>Boric acid waste of a forced recirculation evaporator</u>	
	Water	87.9
	Boric acid	12.0
	Crud ^c	0.1
	Temperature	70°F
	pH	3.5

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Table 1. (continued).

	Material	Weight percent in evaporator bottoms
3d.	<u>Decontamination waste of a forced recirculation evaporator</u>	
	Water	80.0
	Nutek NT-700 ^d	9.4
	EDTA	5.0
	Citric Acid	5.0
	Crud ^c	0.2
	Hydraulic oil No. 2	0.2
	Lubricating oil No. 20	0.2
	Temperature	170°F
	pH	5
4a.	<u>BWR chemical renenerative waste of a thin film evaporator</u>	
	Water	50.0
	Sodium sulfate	45.8
	Sodium chloride	4.0
	Crud ^c	0.2
	Temperature	150 to 250°F
	pH	6
4b.	<u>PWR chemical regenerative waste of a thin film evaporator</u>	
	Water	50.0
	Sodium sulfate	29.0
	Ammonium sulfate	16.8
	Sodium chloride	4.0
	Crud ^c	0.2
	Temperature	150 to 250°F
	pH	1.8 to 4.0
4c.	<u>Boric acid waste of a thin film evaporator</u>	
	Water	50.0
	Boric acid	49.8
	Crud ^c	0.2
	Temperature	150 to 250°F
	pH	2.5 to 3.5

Table 1. (continued).

Material		Weight percent in evaporator bottoms
4d.	<u>Decontamination waste of a thin film evaporator</u>	
	Water	50.0
	Nutek NT-700 ^d	20.0
	EDTA	9.8
	Citric acid	19.0
	Crud ^c	0.2
	Hydraulic oil No. 2	0.5
	Lubricating oil No. 20	0.5
	Temperature	150 to 250°F
	pH	5

a. Rohm and Haas Co., Philadelphia, PA 19105.

b. Ecodyne Corp., Union, NJ 07083.

c. Fine air cleaner test dust no. 1543094, AC Spark Plug Division, General Motors Corp. Flint, MI 48556.

d. Nuclear Technology Corp., Amston, CT 06231.

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. Other possibilities that could be examined include biological parameters

measured directly as a metabolic activity such as byproduct production (acid, etc.) or by use of consumables (oxygen, carbon, inorganic materials, etc.). These data could provide information on the activity of the microorganisms.

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Figure 14. Examples of possible composition of simulated low-level waste forms.

Figure 15. Matrix for testing simulated low-level waste forms.

RESULTS

Microorganism Availability

While the literature suggests that the three types of microorganisms considered for the study (sulfur oxidizing, nitrifying, and organic acid-producing bacteria) are ubiquitous in the environment, it was important to demonstrate that such types either be isolated from or tolerant to soils representative of present and proposed commercial LLW sites.

During the past 12 months, soils have been collected from a total of six locations. These included the University of Illinois, Urbana, and Argonne National Laboratory, Chicago, IL;

Savannah River National Laboratory, Aiken, SC; U.S. Ecology, Hanford, WA; U.S. Ecology, Beatty, NV; U.S. Ecology, Ward Valley, CA; regional, El Paso, TX. Additional information concerning sampling depths and pH values at each location are presented in Table 2. It should be noted that these samples represent a wide diversity of soil types. For example, soil pH values (obtained from 1:1 soil water paste) ranged from an acidic 4.2 to an alkaline 8.5.

Various methods were used to isolate candidate microorganisms from these soils. The types and, where possible, the numbers of bacteria isolated from the soils are listed in Table 3. Testing for the

Table 2. Physical description and pH of soils collected from the proximity of LLW and proposed LLW sites.

Location	Physical description	Depth (cm)	pH
Urbana, IL	Moderately drained crop land	0-17.8	4.5
		91.4-111.8	5.6
		172.7-193.0	6.8
Argonne, IL	Lysimeter site cut profile	0-15.2	6.6
		91-111.8	7.3
		116.8-147.3	7.5
Barnwell, SC	Disturbed soil underlying gravel	0-20.3	4.2
		91-111.8	7.0
		116.8-147.3	5.1
Hanford, WA	Undisturbed native vegetation	0-20.3	7.3
		121.9-142.2	8.2
		172.7-203.2	8.3
El Paso, TX 1	Crop land	0-20.3	8.2
		121.9-142.2	7.8
		182.9-203.2	7.8
El Paso, TX 2	Undisturbed underlying caliche	0-20.3	7.6
		81.3-101.6	7.7
Beatty, NV	Undisturbed desert pavement	0-10.2	8.2
		25.4-35.6	8.4
		Stockpile	8.0
Ward Valley, CA	Undisturbed desert pavement	0-20.3	8.3
		91.4-111.8	8.5

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Table 3. Types and numbers of microorganisms isolated from the proximity of LLW and proposed LLW sites.

Location	Depth (cm)	Heterotrophic bacteria		Nitrifying bacteria		Sulfur-oxidizing bacteria	
		Number	Presence of organic acid	Ammonia oxidizers	Nitrite oxidizers	Enrichment	Direct plating
Urbana, IL	0-17.8	7×10^6	+ ^a	7×10^2	1×10^3	—	—
	91.4-111.8	2×10^8	+	1×10^2	5×10^2	+	× ^d
	172.7-193.0	2×10^8	NT ^b	2×10^2	1×10^4	+	×
Argonne, IL	0-15.2			2×10^2	1×10^3	+	×
	91-111.8			1×10^1	5×10^1	+	×
	116.8-147.3			—	5×10^1	—	×
Barnwell, SC	0-20.3	6×10^6	+	1×10^2	1×10^4	+	—
	91-111.8	3×10^6	+	—	5×10^1	+	2×10^2
	116.8-147.3	6×10^5	— ^c	—	5×10^1	+	×
Hanford, WA	0-20.3	4×10^7	+	4×10^3	1×10^4	+	—
	121.9-142.2	9×10^5	+	7×10^2	3×10^4	—	1×10^2
	172.7-203.2	5×10^6	—	2×10^3	3×10^4	—	1×10^2
El Paso, TX 1	0-20.3	8×10^7	+	5×10^3	7×10^3	+	—
	121.9-142.2	7×10^5	+	1×10^1	2×10^2	+	—
	182.9-203.2	6×10^6	+	1×10^1	6×10^1	+	—
El Paso, TX 2	0-20.3			2×10^2	7×10^3	+	2×10^3
	81.3-101.6			7×10^2	2×10^2	—	
Beatty, NV	0-10.2	1×10^7	—	1×10^2	7×10^3	+	1×10^3
	25.4-35.6	3×10^7	—	1×10^2	3×10^3	—	—
	Stockpile	2×10^5	—	—	—	+	
Ward Valley, CA	0-20.3			1×10^1	3×10^2	—	—
	91.4-111.8			—	2×10^2	+	

a. Microbe present.

b. Not tested.

c. Microbe absent.

d. Thiobacilli present but individual colonies not distinguishable.

presence of heterotrophs was conducted selectively on soils from five of the eight collection sites. The evaluated soils were representative of all of the geographical locations that were sampled. Not surprisingly, heterotrophic microbes were isolated from all 15 soil samples collected at the five locations. Numbers of viable microbes ranged from 10^5 to 10^8 per gram of soil

(wet weight). Further, of these isolates, there were apparent organic acid-producing organisms obtained from four of the five locations evaluated.

There was evidence that representative soil samples from all locations contained a nitrifying bacterial population. The results of the

enumeration studies for these bacteria using the MPN method are presented in Table 3. These data show that both ammonia-oxidizers and nitrite-oxidizers were present in soils from all the sampling sites. 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. At two of these locations, there was occurrence at only one depth, two locations had the bacteria at two depths, while the remaining four showed activity at all depths. The numbers 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 numbers of these microbes ranged from 10^1 to 10^4 per gram of soil (wet weight) in the 21 soil samples they were found in.

Isolation and quantification of thiobacilli was more difficult. Using an enrichment technique, soil from all eight sampling locations had the presence of sulfur oxidizers confirmed in at least one of the soil profiles sampled. At three of the locations, the bacteria were present in at least two profiles, while another three locations had them occurring at all three depths. Data from a direct plating method confirmed the presence of thiobacilli in six of the eight soils. It appears that these bacteria could number approximately 10^2 to 10^3 per gram of soil. Perhaps more importantly, these data prove that given the required environmental conditions, these microbes will proliferate.

Because of the numbers 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 that group. This has been true for the nitrifying and thiobacilli species but not necessarily for the heterotrophic organisms. This was due to the large number of microbes capable of producing organic acids. In this case, a known, aggressive bacterium was selected.

Development of Testing Methodology

Total Immersion

Heterotrophic Bacteria. Preliminary work has been conducted with the continual perfusion method using lixiviant produced by representatives of the three types of candidate microorganisms. A strain of *Pseudomonas cepacia* was selected as representative of the organic acid-producing heterotrophs. This organism was selected because previous work demonstrated its capability for promoting the degradation of rock phosphate ore (Rogers and Wolfram 1992). The effect of this microbe on the integrity of both INEL and vendor waste forms appeared to be minimal. After 60 days of complete immersion in *Pseudomonas* lixiviant, the surface pH of the vendor-supplied, simulated cement waste forms were not significantly different from those immersed in sterile, neutral pH growth media. The surface pH of the resin formulation after lixiviant immersion was 9 compared to pH 10 after media immersion, and the surface pH of the evaporator bottoms formulation was 9 after lixiviant immersion compared to 9.5 after media immersion. The surface pH of the ASTM control cement formulation was slightly lower after immersion in the *Pseudomonas* lixiviant, pH 6 compared to pH 9 after immersion in the media.

Percent calcium leached from the resin, evaporator bottoms, and the ASTM formulations were 16.6%, 9.8%, and 24.7%, respectively, after immersion in *Pseudomonas* lixiviant. These results suggest that over the period of the test, the buffering capacity of the cement formulations is sufficient to resist chemical attack from the organic acids produced by *Pseudomonas*.

Nitrifying Bacteria. Although nitrifying bacteria produce acidity through their metabolic activity, it is known that most of these bacteria grow optimally in buffered systems in which the pH is maintained between 7.5 to 8.0 (Schmidt and Belser, 1982). The necessary buffering capacity in the case of natural sandstone is supplied by the continuous dissolution of the calcareous binding matrix (Mansch and Bock, 1992). Unlike

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thiobacilli whose metabolic activity results in overwhelming the buffering capacity of calcium hydroxide, the nitric acid formation by nitrifying bacteria is continuously being neutralized by the loss of the calcium constituent.

The continuous flow reactor system (similar to that used for the sulfur oxidizing bacteria) for application to the nitrifying bacteria using mixed cultures of *Nitrosomonas* and *Nitrobacter* was evaluated. It was found that the continuous flow reactor system was not suitable for optimum growth of these bacteria. Also, mixed cultures of the ammonia-oxidizing and nitrite-oxidizing bacteria were determined not to be necessary to achieve optimum mineral acid production. In addition, the growth of these bacteria under continuous bioreactor conditions requires the addition of a buffer to maintain the slightly alkaline growth conditions.

It was decided to use a fed batch bioreactor design. It has been demonstrated that fed batch reactor systems are particularly suited to the growth and monitoring of slow-growing microorganisms (Grady and Lim 1980; Baily and Ollis 1986). They combine the desirable aspects of continuous flow and batch reactor systems. With this method, it will be possible to monitor the solution concentrations of ammonia and nitrite, thus providing a quantitative assessment of acid production. Calcium concentration will be monitored to determine the quantity of cement-based Ca solubilized. The process will be implemented by inoculating specimens to be tested with pure cultures of the ammonia-oxidizing bacteria *Nitrosomonas* or *Nitrosovibrio*.

Thiobacillus Sulfur-Oxidizing Bacteria.

A test was devised in which salt and exchange resin specimens from each of the two formulations of simulated waste forms and the ASTM control waste forms could be exposed to lixiviant produced by *T. thiooxidans* and *T. ferrooxidans* isolates grown in separate continuous flow bioreactors. In addition to the thiobacilli lixiviants, replicate specimens were exposed to a continual

supply of the bacteria-free (sterile) medium used to support microbial growth. The intended duration of this continuous exposure test was 60 days. However, the vendor's waste forms were exposed for only 30 days. During the test, samples of the liquid that had come in contact with the INEL-simulated waste forms (effluent) was collected and analyzed by inductively coupled plasma (ICP) for Ca content and pH. In the case of the vendor's simulated waste form, the effluent was analyzed for Ca, Fe, Al, Mg, and Si. At the conclusion of the test, the waste forms were collected for physical examination to determine the extent of MID.

The extent of MID affecting the ASTM control cement specimens after 60 days of exposure to thiobacilli lixiviant can be seen in Figure 16. This figure pictures the ASTM control immersed for 60 days in the sterile medium (Figure 16a), ASTM control with *T. ferrooxidans* lixiviant (Figure 16b), and ASTM control with *T. thiooxidans* lixiviant (Figure 16c). The same sequence is followed in Figures 17 and 18 for the INEL-simulated cement-loaded evaporator bottoms and ion-exchange resin, respectively. There was little or no apparent physical damage to the ASTM control and evaporator bottoms waste form exposed to the sterile medium (Figures 16a and 17a), while there was extensive damage in the form of spalling, material loss, and lighting of color with those specimens that were in contact with the thiobacilli lixiviants (Figures 16b, 16c, 17b, and 17c). In the case of the ion-exchange-loaded waste forms (Figure 18), physical damage is seen with all the specimens. It is not uncommon for these types of specimens to disintegrate, even in distilled water, due to swelling of the resin beads. However, it should be noted in the photograph (Figure 18a) that the waste form exposed to the sterile medium still retains a cement matrix in which resin beads are firmly imbedded. However, the other two waste forms have lost their cement matrix as indicated by the presence of loose resin beads (Figure 18b and 18c).

Figure 16. ASTM control cement waste forms after being immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 17. INEL-simulated cement waste forms loaded with evaporator bottoms after being immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 18. INEL-simulated cement waste forms loaded with ion-exchange resin after being immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Chemical evidence for the effect of MID includes the extensive loss of Ca from the INEL-simulated waste forms exposed to thiobacilli lixiviant. The quantities of Ca removed during exposure to the two lixiviants and the sterile medium can be seen in Figure 19. The Ca loss from the specimens exposed to *T. ferrooxidans* lixiviant ranged from 30% for the ASTM control to 89% for the ion-exchange-loaded waste form. For the *T. thiooxidans* lixiviant and sterile medium, the corresponding values were 20 to 59% and 20 to 12%, respectively. These data support the assumption that loss of the cement matrix contributed to the deterioration of the simulated waste forms. The pH of the INEL waste form effluent was determined. These data show that the waste forms had little effect on moderating the acid environment supplied by the two lixiviants (Figure 20). The pH of the effluent was consistently 2 or lower during the test. On the other hand, considerable buffering was noted for the sterile medium effluent. The initial effluent pH was as high as 9 and then decreased over time to about 3.2, which was the pH of the fresh, sterile

Results

Figure 19. Quantities of Ca leached from INEL-simulated waste forms after 60 days of total immersion.

Figure 20. pH of lixiviants after exposure to INEL-simulated waste forms during the total-immersion study.

medium. The effect of a continual supply of low-pH, biologically produced lixivants can be seen in the resulting surface pH (as determined by pH-sensitive paper strips) of the test specimens (Table 4). Note that those specimens in contact with a lixiviant had very low surface pH's as compared to the sterile medium-treated or untreated specimens. Low pH values in these ranges are known to cause damage to cement materials.

As was mentioned earlier, the duration of the second study using the vendor-supplied, simulated cement waste forms was limited to 30 days. Neither of the specimens exposed to sterile media appeared to have any damage (Figures 21a and 22a). However, the simulated evaporator bottom waste form immersed in *T. ferrooxidans* lixiviant appeared to have swelled and was extensively damaged (Figure 21b). Also, exposure of the same type of waste form to the *T. thiooxidans* lixiviant caused similar swelling with most of the

physical damage appearing on the specimen's edges (Figure 21c). Both of the vendor-supplied, simulated ion-exchange resin waste forms were extensively damaged by the bacteria lixivants (Figures 22b and 22c). In comparison, these results were similar to those obtained from the evaluation of the INEL-simulated waste forms. That is, the specimens appeared to have lost much of their cement matrix, which resulted in the resin beads being loosely attached to the eroded surface.

As previously mentioned, effluent from this study was analyzed for the presence of Ca, Al, Si, Fe, and Mg. As observed for the INEL-fabricated waste forms, Ca was leached from the vendor waste forms as a result of exposure to the various liquids (Figure 23). The amount of Ca leached from the exchange resin wastes forms ranged from 17 to 46% with 7 to 29% loss for the evaporator bottoms specimens. Once again, those waste forms exposed to the bacterial lixivants lost substantially more Ca than those treated with only the

Table 4. Surface pH of INEL-simulated cement waste forms before and after immersion for 60 days in sterile media or microbially produced lixiviant.

Treatment	Simulated cement waste forms		
	Exchange resin, pH	Evaporator bottoms, pH	ASTM standard, pH
Untreated	9	13	11.5
Sterile media	10	10	7
<i>T. ferrooxidans</i>	0.5	4.5	3.0
<i>T. thiooxidans</i>	1.0	1.5	3.0

From: Colombo and Neilson, 1979.

Results

Figure 21. Vendor-supplied simulated cement waste forms loaded with evaporator bottoms after being immersed for 30 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 22. Vendor-supplied simulated cement waste forms loaded with ion-exchange resin after being immersed for 30 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 23. Quantities of Ca leached from vendor-supplied simulated waste forms after 30 days of total immersion.

sterile media. Calcium loss from the waste forms was approximately doubled by the presence of the *T. ferrooxidans* lixiviant and was more than tripled by the *T. thiooxidans* treatment, substantially more Ca than those treated with only the sterile media. Calcium loss from the waste forms was approximately doubled by the presence of the *T. ferrooxidans* lixiviant and was more than tripled by the *T. thiooxidans* treatment.

Loss of Al occurred only from those specimens exposed to the thiobacilli lixiviant (Figure 24). Amounts leached from the two types of simulated waste forms were similar within each treatment and ranged from 9 to 14% for the *T. ferrooxidans* treatment and 19 to 21% for specimens exposed to *T. thiooxidans* lixiviant. Only a minimal amount of Si (less than 0.2%) was removed from specimens regardless of the treatment (Figure 25). While the total initial quantity of Fe and

Loss of Al occurred only from those specimens exposed to the thiobacilli lixiviant (Figure 24). Amounts leached from the two types of simulated

waste forms were similar within each treatment and ranged from 9 to 14% for the *T. ferrooxidans* treatment and 19 to 21% for specimens exposed to *T. thiooxidans* lixiviant. Only a minimal amount of Si (less than 0.2%) was removed from specimens regardless of the treatment (Figure 25). While the total initial quantity of Fe and Mg was not determined for the vendor-supplied, simulated waste forms, these elements were shown to be leached from the specimens (Figures 26 and 27). More Fe was leached from those specimens exposed to the bacterial lixiviant than from the sterile media (Figure 26). Simulated waste forms containing exchange resin had a larger quantity of Fe removed than those containing evaporator bottoms material. Also, leaching was more apparent with the *T. thiooxidans* lixiviant. The Mg data (Figure 27) were unexpected since it was shown that more of the element, regardless of waste form type, appeared in the sterile media effluent than in that of the bacterial lixiviants. This was a curious finding for which no explanation was apparent.

Figure 24. Quantities of Al leached from vendor-supplied simulated waste forms after 30 days of total immersion.

Results

Figure 25. Quantities of Si leached from vendor-supplied simulated waste forms after 30 days of total immersion.

Figure 26. Quantities of Fe leached from vendor-supplied simulated waste forms after 30 days of total immersion.

Figure 27. Quantities of Mg leached from vendor-supplied simulated waste forms after 30 days of total immersion.

Like the case of the INEL-simulated waste forms, effluent pH data showed that the specimens had little effect on moderating the acid environment imposed by the thiobacilli lixivants. Over the course of the study, the average pH values of the two lixivants remained near 2 (Figure 28). The pH of the sterile media that were exposed to the vendor waste forms remained constant at about 3 during the first 7 days. Subsequently, the pH began to increase into the pH 6 and 7 range. These data are significantly different than those from the INEL material (Figure 20). It appears that the vendor waste forms contributed little to the initial buffering of the sterile media. Why buffering of the sterile media was delayed in this study was not ascertained.

The continual exposure of waste forms to thiobacilli lixivants had a marked influence on their final surface pH. Table 5 provides pH data showing that surface pH values of the untreated specimens decreased from alkaline (pH 9 to 13) to extremely acidic (pH 1 to 3). Although the sterile media had some effect, the surface pH's remained

alkaline. These results are similar to those obtained from the INEL materials.

Intermittent Immersion

Thiobacillus Sulfur-Oxidizing Bacteria.

A test similar to that described in the above section on total immersion was conducted using *Thiobacillus*. Both INEL-fabricated and vendor-furnished simulated evaporator bottoms and ion-exchange resin waste forms and ASTM controls were exposed to *T. ferrooxidans* and *T. thiooxidans* lixivants 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. This was accomplished by using 125-mL Soxhlet extraction columns (Figure 29). Each specimen was placed on top of a plastic pedestal inside an individual Soxhlet column. 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

Results

Figure 28. pH of lixiviants after exposure to vendor-supplied simulated waste forms during the total-immersion study.

Table 5. Surface pH of vendor-supplied, simulated cement waste forms, before and after immersion for 30 days in sterile media or microbially produced lixiviant.

Treatment	Exchange resin (pH)	Evaporator bottoms (pH)
Untreated	9	13
Sterile media	9.5	8.5
<i>T. ferrooxidans</i>	1.0	3.0
<i>T. thiooxidans</i>	3.0	3.0

the activation of a syphon. Approximately 100 mL of liquid passed through each column per day. Effluent from each Soxhlet column was collected to determine pH. In addition, effluents from the vendor-simulated waste-form specimens were analyzed for Ca, Fe, Al, Mg, and Si. At the conclusion of the test, the specimens underwent

physical examination, including determination of surface pH, to assess the extent of MID. The duration of the intermittent exposure test was 60 days.

The visual effect of MID on the INEL-prepared, simulated waste forms (photographed inside the Soxhlet columns) can be seen in Figures 30 through 32. The surface pH of the waste

Figure 29. Vessels (Soxhlet extractors) used to intermittently expose simulated waste forms to lixiviants.

forms exposed to the lixiviant was very low, ranging from 0.5 to 3.0 (Table 6). Those exposed only to sterile medium were pH 7 or above. Figures 30a, 30b, and 30c show the ASTM control after exposure to sterile media, *T. ferrooxidans* lixiviant, and *T. thiooxidans*, respectively. Figures 31 and 32 are arranged in the same sequence for the INEL-simulated evaporator bottoms and ion-exchange resin waste forms. There is a gradation of effects for those specimens exposed only to sterile medium (Figures 30a, 31a, 32a). Media exposure had no visible effect on the ASTM control while there was minor swelling and some cracking of the evaporator bottoms specimen; however, the waste form shape is still well defined. As with the total immersion sample, the ion-exchange resin specimen was totally crumbled. However, as noted previously,

individual resin beads appeared to be firmly embedded in the cement matrix. ASTM specimens that were in contact with the two thiobacilli lixiviants were light (white) in color, indicating that extensive leaching of Ca had taken place (Figures 30b and 30c). The evaporator bottoms waste forms exposed to the lixiviants became very light in color and lost some margin definition. Also, material loss was evident (Figures 31b and 31c), but no cracking or spalling appeared. Extensive, physical damage was seen in those ion-exchange resin forms exposed to lixiviants (Figures 32b and 32c). As discussed previously, these specimens lost their cement matrix as indicated by the appearance of loose resin beads. Due to funding considerations, there was no chemical analysis conducted on the lixiviant from these specimens.

Results

Figure 30. ASTM control cement waste forms after being intermittently immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 31. INEL-simulated cement waste forms loaded with evaporator bottoms after being intermittently immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 32. INEL-simulated cement waste forms loaded with ion-exchange resin after being intermittently immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Results of exposure of the vendor-supplied simulated waste forms to sterile medium and lixiviants are shown in Figures 33 and 34. The evaporator bottoms waste forms exposed to sterile medium were not physically degraded in any way (Figure 33a) while there was some damage to the ion-exchange resin specimen (Figure 34a). However, there was significant damage to all specimens in contact with the two lixiviants (Figures 33b, 33c, 34b, and 34c). Swelling with accompanying loss of material was noted for the evaporator bottoms waste forms. The ion-exchange resin specimens were completely disintegrated and loose, and unattached resin beads were evident (Figures 34b and 34c).

Analysis of the effluent from the vendor waste forms indicated that substantial percentages of Ca, Al, and Si were removed from specimens exposed to the thiobacilli lixiviants (Figures 35 through 37). Loss of Ca ranged from 70 to 90% by the thiobacilli lixiviants compared to about 30% by the sterile media control (Figure 35). The percentage of Al loss was 51 to 75% and

approximately 2% to the lixiviants and media control, respectively (Figure 36). Silica losses for those specimens exposed to thiobacilli lixiviants ranged from 19 to 28% and averaged about 13% for the control (Figure 37). Because initial concentrations of Fe and Mg were not available for these waste forms; only total quantities of these elements removed by the leachant are reported (Figures 38 and 39). Iron removal was observed from those waste forms exposed to the lixiviants (29 to 56 mg) while no loss was detected in the sterile media (Figure 38). There was active Mg removal only from those specimens in contact with the *T. thiooxidans* lixiviant (about 58 mg) with minor amounts to the sterile media (2 to 12 mg) (Figure 39).

Neither ion-exchange resin or evaporator bottoms waste form specimens had a predominant loss of elements. More Ca and Fe were removed from the ion-exchange resin specimens while more Al was leached from the evaporator bottoms

specimens. Silica loss was thiobacilli species-dependant. A greater percentage was removed from the evaporator bottoms waste form by exposure to the *T. ferrooxidans* lixiviant while slightly more Si was lost from the ion-exchange resin specimen exposed to the *T. thiooxidans* treatment. Release of Mg was the same from both waste form types.

Data for the cumulative percentage removal of Ca, Al, and Si from each waste form type is also presented (Figures 40 through 45). Element release rates were variable from the evaporator bottom samples (Figures 40 through 42). Although the total percent Ca loss was nearly the same for the two lixiviants, these data (Figure 40) indicate that the rate of release was different. A maximum amount of Ca was removed by the *T. ferrooxidans* lixiviant by day 35 while levels of Ca in the *T. thiooxidans* lixiviant were still increasing by day 58 (Figure 40); however, by this time, the total Ca leached was nearly the same as that of the other lixiviant. The rate of leaching

Table 6. Surface pH of INEL waste-form specimens exposed to lixiviant during intermittent immersion.

Treatment	Exchange resin (pH)	Evaporator bottoms (pH)	ASTM standard (pH)
Untreated	9	13	11.5
Sterile media	7	8	8
<i>T. ferrooxidans</i>	0.5	3.0	1.0
<i>T. thiooxidans</i>	0.5	1.5	1.5

Results

Figure 33. Vendor-supplied simulated cement waste forms loaded with evaporator bottoms after being intermittently immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 34. Vendor-supplied simulated cement waste forms loaded with ion-exchange resin after being intermittently immersed for 60 days in (a) sterile media, (b) *T. ferrooxidans* lixiviant, and (c) *T. thiooxidans* lixiviant.

Figure 35. Quantities of Ca leached from vendor-supplied waste forms after 60 days of intermittent exposure.

Figure 36. Quantities of Al leached from vendor-supplied waste forms after 60 days of intermittent exposure.

Figure 37. Quantities of Si leached from vendor-supplied waste forms after 60 days of intermittent exposure.

Results

Figure 38. Quantities of Fe leached from vendor-supplied waste forms after 60 days of intermittent exposure.

Figure 39. Quantities of Mg leached from vendor-supplied waste forms after 60 days of intermittent exposure.

Figure 40. Cumulative quantities of Ca leached from vendor-supplied evaporator bottoms waste forms during the intermittent-exposure study.

of Al was approximately the same for both lixivants until day 40 when a greater percent began to be released into the *T. thiooxidans* lixiviant (Figure 41). This resulted in more total Al being leached by this treatment. The cumulative data for Si leaching clearly show large initial release in the *T. ferrooxidans* treatment. This resulted in the total Si leached by this treatment to be substantially more than that for the *T. thiooxidans* treatment (Figure 42). These data show that the rate of release of elements from the ion-exchange resin specimens was nearly the same for the two thio-bacilli treatments (Figures 43 through 45). The majority of the Ca and Al release occurred during the first 30 days (Figures 43 and 44) while loss of Si was progressive with time (Figure 45). Also, the Ca data show that the final total Ca release (Figure 35) is not representative of the overall rate of leaching by the two lixiviant streams. Figure 43 shows that until about day 50, the rate of release was the same.

Average lixiviant pH for the study period (Figure 46) was similar to that of other studies. The pH was less than 2 by day 8 for the lixivants, and the sterile media pH gradually decreased and stabilized at the influent pH by day 31. Final surface pH values of these waste forms, ranging from 3 to 4, was not as acidic as the lixiviant to which they had been exposed (Table 7). Surprisingly, the surface pH of the specimens in contact with the sterile media were acidic (4 and 5) rather than alkaline.

Intermittent Misting. Intermittent misting is the third testing method under development. It will be used to expose test specimens to a lixiviant mist. The design is such that liquid is drawn away from the waste forms so that it does not puddle. To accomplish this, exposed lixiviant (that which comes in contact with waste forms) will be collected as it drips from specimen surfaces. In this way, the specimen will remain moist but will not at any time be submersed in lixiviant.

Results

Figure 41. Cumulative quantities of Al leached from vendor-supplied evaporator bottoms waste forms during the intermittent-exposure study.

Figure 42. Cumulative quantities of Si leached from vendor-supplied evaporator bottoms waste forms during the intermittent-exposure study.

Figure 43. Cumulative quantities of Ca leached from vendor-supplied ion-exchange resin waste forms during the intermittent-exposure study.

Figure 44. Cumulative quantities of Al leached from vendor-supplied ion-exchange resin waste forms during the intermittent-exposure study.

Results

Figure 45. Cumulative quantities of Si leached from vendor-supplied ion-exchange resin waste forms during the intermittent-exposure study.

Figure 46. pH of lixiviants after exposure to vendor-supplied waste forms during the intermittent-immersion study.

Table 7. Surface pH of vendor supplied waste forms specimens exposed to lixiviant during intermittent immersion.

Treatment	Exchange resin (pH)	Evaporator bottoms (pH)
Untreated	9	13
Sterile media	5	4
<i>T. ferrooxidans</i>	4	3
<i>T. thiooxidans</i>	3	3

The prototype for the misting chamber is seen in Figure 47. The body of the chamber is a 2.4-L plastic container. It comes fitted with a seal-tight snap lid. The lid has been modified to accommodate the motor (covered by the square perforated box) and misting generator from a "cool mist" vaporizer. Washed and sterilized glass marbles have been placed in the container in order to reduce the volume of lixiviant necessary to maintain a requisite height. Their weight also acts to "anchor" in place a modified 100-mL graduated cylinder (foreground; cut off at a height to allow collection of 60 mL of liquid). A teflon pedestal has been placed into the cylinder to serve as a stage for placement of a simulated waste form

(the dark cylindrical object directly above the top of the graduated cylinder).

The volume of lixiviant in the container is maintained at 300 mL through the continuous addition and measured withdrawal of lixiviant. The misting unit is timed to operate for 5 out of every 15 minutes. Mist that collects on the simulated waste form eventually runs off into the graduated cylinder where its cumulative volume (adjusted for the volume of the teflon pedestal) is recorded. The lixiviant will be recovered for later analysis. Because this is a prototype, it is expected that there could be several changes before a satisfactory design has been developed.

Results

Figure 47. Prototype for intermittent misting chamber.

CONCLUSIONS

An extensive literature review provided sufficient data to raise concerns that microbially influenced degradation (MID) could affect the stability of cement-solidified LLW. The presence of recognized MID-promoting bacteria has been discovered in soils from eight geographic locations representing a cross section of LLW and proposed LLW disposal facilities. Furthermore, using variations of a newly developed, accelerated laboratory test, it has been possible to document that LLW solidified with cement is susceptible to MID. An understanding of those conditions that trigger MID (i.e., MID-promoting microorganisms, reduced sulfur and nitrogen compounds, moisture, etc.) that could occur naturally in a disposal environment or be provided by the contents of a waste form are becoming more obvious. However, the rate-controlling processes of MID are not as well defined nor has the extent of radionuclide release been determined.

These findings validate the NRCs support for developing accelerated MID testing procedures. This report provides data showing that cementitious materials can be substantially damaged by the aggressive environment promoted by the action of two species of sulfur-oxidizing, thiobacilli bacteria. The physical damage to simulated INEL- and vendor-prepared waste forms (both those containing ion-exchange resin and evaporator bottoms) demonstrates that the stability of

cement-solidified waste forms could be compromised by the action of these microorganisms.

A possible mode of attack appears to be through a process that lowers the surface pH of the cementitious material. The decrease in pH promotes the removal of Ca (and other elements) from an exposed surface. Since binding strength of the cement primarily relies on the Ca-dominated cement paste, the loss of Ca causes a decrease of cement strength, resulting in a loss of waste form integrity. The data indicate that the severity of the attack could be moderated by the waste form composition. That is, test specimens containing less simulated waste would be more resistant to degradation than specimens with higher waste loadings. However, there are not enough data yet to make comparisons between the formulations currently used to manufacture waste forms.

Future work will include the development of additional methods for exposing simulated cement waste forms to not only the sulfur-oxidizing bacteria but also to nitrifying bacteria. Exposure methods will include the development and testing of an intermittent misting methodology. Data from the development effort will be used in the selection of a method that will be used for testing actual cement-solidified waste forms. This work will determine durability and the rate at which radionuclides are leached from this material.

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