Biodegradation Testing of TMI-2 EPICOR-II Waste Forms

Prepared by R.D. Rogers, J.W. McConnell, Jr.

Idaho National Engineering Laboratory
EG&G Idaho, Inc.

Prepared for
U.S. Nuclear Regulatory Commission
NOTICE

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, or any of their employees, makes any warranty, expressed or implied, or assumes any legal liability of responsibility for any third party's use, or the results of such use, of any information, apparatus, product or process disclosed in this report, or represents that its use by such third party would not infringe privately owned rights.

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, 1717 H Street, N.W. Washington, DC 20555
3. The National Technical Information Service, Springfield, VA 22161

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 Office of Inspection and Enforcement 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 GPO Sales Program: formal NRC staff and contractor reports, NRC-sponsored conference proceedings, 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 and periodical 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 Division of Information Support Services, Distribution Section, U.S. Nuclear Regulatory Commission, Washington, DC 20555.

Copies of industry codes and standards used in a substantive manner in the NRC regulatory process are maintained at the NRC Library, 7920 Norfolk Avenue, Bethesda, Maryland, and are available there for reference 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.
Biodegradation Testing of TMI-2 EPICOR-II Waste Forms

Manuscript Completed: May 1988
Date Published: June 1988

Prepared by
R.D. Rogers, J.W. McConnell, Jr.
Idaho National Engineering Laboratory
Managed by the U.S. Department of Energy
EGG Idaho, Inc.
P.O. Box 1625
Idaho Falls, ID 83415

Prepared for
Division of Engineering
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission
Washington, DC 20555
NRC FIN A6876
Under DOE Contract No. DE-AC07-76ID01570
ABSTRACT

Waste-form testing is being conducted by the Three Mile Island Unit 2 (TMI-2) EPICOR-II Resin/Liner Investigation; Low-Level Waste Data Base Development Program of the U.S. Nuclear Regulatory Commission (NRC) in accordance with the NRC Branch Technical Position on Waste Form. Waste forms which were tested contain ion exchange resins solidified with vinyl ester-styrene and Portland Type I-II cement. This report describes the biodegradation testing of those waste forms, presents the test results, and provides recommendations for alternate test methodology.
SUMMARY

ASTM biodegradation tests were conducted on waste forms containing high specific activity ion exchange resins from EPICOR-II prefilters. Those tests were part of a program to test waste forms in accordance with the NRC Branch Technical Position on Waste Form. Small waste forms were manufactured using two different solidification agents, Portland Type I-II cement and vinyl ester-styrene (VES). Ion exchange material was taken from two EPICOR-II prefilters; PF-7, which contained all organic material, and PF-20, which contained organic resins and a layer of inorganic zeolites.

Test results showed that the VES waste forms supported microbial growth, while cement waste forms did not support that growth. Growth was also observed adjacent to some VES waste forms. Radiation levels found in the ion exchange resins used in this study were not found to inhibit microbial growth.

The extent of degradation of the waste forms could not be determined using the ASTM tests specified by the NRC Branch Technical Position on Waste Form. As a result of this work, a different testing methodology is recommended, which would provide direct verification of waste form capabilities. That methodology would evaluate solidification materials without using the ASTM procedures or subsequent compression testing. The proposed tests would provide exposure to a wide range of microbial species, use appropriately sized specimens, provide for possible use of alternate carbon sources, and extend the test length. Degradation would be determined directly by measuring metabolic activity or specimen weight loss.
CONTENTS

ABSTRACT ................................................................. ii
SUMMARY ................................................................. iii
INTRODUCTION .......................................................... 1
MATERIALS AND METHODS ............................................. 2
  Description of Waste Forms ......................................... 2
  Procedures Specified for Determining Biodegradation .......... 2
RESULTS AND DISCUSSION ............................................ 6
  Results of ASTM 022 Bacteria Testing ............................ 6
  Results of ASTM 021 Fungal Testing .............................. 6
  Results of Compression Testing .................................... 14
  Discussion of ASTM 021 Results .................................... 14
CONCLUSIONS AND RECOMMENDATIONS .............................. 24
REFERENCES ............................................................ 25

FIGURES

1. Fungal growth on a powdered unirradiated VES waste form .......... 7
2. Close-up view (10x) of fungal growth on a powdered unirradiated VES waste form .......... 8
3. Close-up view (10x) of fungal growth on wafer cut from VES waste form (after 60 days) .......... 9
4. Close-up view (10x) of fungal growth on wafer cut from unirradiated VES waste form (after 60 days) .......... 10
5. Fungal growth on wafer cut from unirradiated VES waste form (after 18 months) .......... 11
6. Close-up view (10x) of fungal growth on wafer cut from unirradiated VES waste form (after 18 months) .......... 12
7. Fungal growth on NS agar covering unirradiated VES waste form .......... 13
8. Fungal growth on PDA containing irradiated cement waste form C1A-30 (control) .......... 15
10. Fungal growth associated with irradiated VES waste form D1-9 (first ASTM test) .......... 17
11. Fungal growth on PDA containing irradiated VES waste form D2A-7 (control) .................. 18
14. Fungal growth associated with irradiated VES waste form D2A-3 (second ASTM test) ........ 21
15. Fungal growth associated with irradiated VES waste form D2A-4 (second ASTM test) ........ 22

TABLES

1. Contact gamma dose for waste forms used in biodegradation study ......................... 5
2. Support of fungal growth by individual components of Dow polymer (VES) ............... 6
3. Compressive strengths of some EPICOR-II cement waste forms used for biodegradation testing ................................................................. 23
BIODEGRADATION TESTING OF TMI-2 EPICOR-II WASTE FORMS

INTRODUCTION

EPICOR-II prefilters expended in the clean-up of Three Mile Island Unit 2 (TMI-2) liquid wastes contain organic and inorganic ion exchange resins which are loaded with gamma- and beta-producing radionuclides. The Idaho National Engineering Laboratory (INEL) is using the waste ion exchange resins to obtain information on survivability of waste forms composed of the resins solidified in matrices of Portland cement and vinyl ester styrene (VES). Emphasis has been placed on how these waste forms meet the requirements of U.S. Nuclear Regulatory Commission (NRC) regulation 10 CFR Part 61, "Licensing Requirements for Land Disposal of Radioactive Waste,"\(^1\) using methods specified in the Branch Technical Position on Waste Form\(^2\) (TP) of the NRC Office of Nuclear Material Safety and Safeguards.

One of the structural stability requirements for waste forms in 10 CFR Part 61 is resistance to biodegradation. This report provides results obtained from testing of solidified EPICOR-II resins using the initial biodegradation methods specified by the TP.
MATERIALS AND METHODS

Description of Waste Forms

Waste forms used in the biodegradation test were composed of solidified EPICOR-II prefilter resin wastes. Two different types of resin waste mixtures were solidified. Type A is a mixture of synthetic organic ion exchange resins from prefilter PF-7 (phenolic cation, strong acid cation, and strong base anion resins), while Type B is a mixture of synthetic organic ion exchange resins and inorganic zeolite from prefilter PF-20 (strong acid cation and strong base anion resins). Portland Type I-II cement and vinyl ester-styrene (VES) were used to solidify both types of resin wastes. VES is a proprietary solidification agent developed and supplied by the Dow Chemical Company and is composed of a binder (styrene monomer and vinyl ester resin), a catalyst, and a promoter. Individual waste forms were manufactured by allowing a mixture of solidification agent and resin waste to solidify in 0.048-m-diameter by 0.102-m-high polyethylene molds. Enough of the mixture was added to each vial to produce waste forms with an average dimension of 0.048 m diameter by 0.076 m high, or a volume of 0.1375 L. A complete description of waste form manufacture is given in Reference 3.

Procedures Specified for Determining Biodegradation

The NRC TP specifies that procedures from the American Society for Testing Materials (ASTM) be used to determine the resistance of solidified waste forms to fungi and bacteria. Those procedures can be found in ASTM G21,4 “Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi,”11 and ASTM G22,5 “Standard Practice for Determining Resistance of Plastics to Bacteria.” If the results from either of those tests indicate that a waste form is capable of supporting microbial growth, the TP states that a more conclusive procedure like that described by Bartha and Pramer must be initiated to determine if biodegradation will occur.

Three general types of tests are used for assessing the potential for microbial deterioration of materials: (a) tests to determine whether or not the material will support microbial growth; (b) tests simulating natural environments; and (c) field studies. While environmental simulations and field studies provide definitive answers, they are most often not used due to the length of time for testing and the resulting expense. The most widely used test procedures involve some method to determine the potential of a material to support microbial growth. Results of those tests then serve as input in the process of evaluating biodegradation potential.

Biodegradation potential tests can be subdivided into two classes:7 those which determine ready biodegradability and the ones which measure inherent biodegradability. Ready biodegradability tests are characterized by:

- The use of a non-specific analytical method to indicate the extent to which a particular substance is mineralized;
- Exposure of a candidate material to a small number of microbial species in the absence of other carbon sources, and;
- The short duration of the test (normally 3 to 4 weeks).

The ASTM G21 and G22 procedures fit the protocol of those tests which determine ready biodegradability except that they do not provide data on the extent of mineralization.

In an evaluation of the biodegradation tests specified by the TP, Bowerman et al.7 points out that tests of ready biodegradability suffer from several limitations. These include the inability of the tests to provide for microbial adaptation and the inability to determine if the co-metabolism of a readily degradable carbon source would produce enzymes which could attack the candidate compound. In addition, the tests are normally of too short a duration to show that biodegradation of a candidate compound is occurring.

Bowerman et al.7 continues by pointing out that the comparatively low surface-to-volume ratio of the compression test specimens as recommended in the TP may not be suitable for an accelerated biodegradation test. This observation is supported by work which has shown that when the ratio of the specimen perimeter to surface area is maximized, the rate of biodegradation is also maximized.8

a. Mention of specific products and/or manufacturers in this document implies neither endorsement or preference nor disapproval by the U.S. Government, any of its agencies, or EG&G Idaho, Inc., of the use of a specific product for any purpose.
Increased biodegradation apparently occurs because the distance from any point on the specimen to the perimeter (where oxygen and nutrients are available) is minimized as the ratio of perimeter to surface area is increased. He concluded that while the ASTM test conditions assess ready biodegradability, "no growth may not be sufficient proof of resistance to biodegradation." and "Sample sizes suitable for compressive strength testing following the biodegradation tests may not be appropriate for use in short-term biodegradation screening tests."

Finally, there is an obvious problem in applying a test designed for evaluating biodegradation of organic polymers to that of testing biodegradation of cement. The most that can be gained would be to show that cement has a biocidal effect on those organisms that can degrade plastic. This is not to say that cement cannot be eroded by microbial-influenced corrosion (MIC) under certain environmental conditions. Under aerobic conditions, cement is potentially open to attack from the action of both sulphur-oxidizing bacteria (in particular the \textit{Thiobacillus} species) and nitrifying bacteria (represented by the \textit{Nitrobacter} species). These organisms are widely distributed in most soils and are known to produce sulfuric and nitric acids, respectively.\textsuperscript{9}

A scenario on how these organisms attack cement follows. Fresh cement has a pH in the region of 12-13 which gradually falls to around pH 9 as a result of the influx of carbon dioxide and the leaching of alkalis. At that point, the organisms are able to colonize on the cement. The \textit{Thiobacilli} will then oxidize available elemental sulfur, which has been biologically or chemically formed from various sulfur compounds. The resulting sulfuric acid destroys cement.\textsuperscript{10} Nitrifying organisms oxidize reduced nitrogen compounds to either nitrous or nitric acid which, in turn, attacks cement.\textsuperscript{11} It is also possible for nitrate to be reduced to nitrite\textsuperscript{12} and then be oxidized to form nitric acid. How susceptible the cement used to solidify waste is to microbial-induced degradation is not known. The Germans have begun to develop a biotesting protocol to determine the resistance of various types of cement.\textsuperscript{10,11} The degree of degradation appears to depend on cement hardness, porosity, permeability, and chemical nature of the aggregate which, in this case, would include the waste material.

An evaluation involving a multiorganism approach is appropriate for waste materials. As in the case of VES, organisms which have shown some propensity for degrading the study material should be used. \textit{Thiobacillus} and \textit{Nitrobacter} species would be organisms of choice for cement, since they have been implicated both by observation and experiment\textsuperscript{10,11,13} as having deleterious effects on cement and are assumed to be present in soil at disposal sites. Because such work is not routine, no standard method for the biodegradation testing of cement is available in the U.S. However, the components of such a test would include using wafer-thin specimens cut from waste forms which have been sufficiently "aged" so that they have a surface pH of 9 or less. They should be exposed to selected \textit{Thiobacillus} and \textit{Nitrobacter} species in a supportive media (liquid, soil, or agar) for several months. In order to determine what type of support media to use, as well as the period of incubation, some initial investigations are required. An evaluation of the effects could be made by comparing the final sample weight to that of the initial weight. Appropriate controls without organisms also should be used to determine what, if any, effects the culture conditions might have.

Both ASTM procedures are described in detail as referenced and will only be highlighted here. Five of the fungal cultures specified for the ASTM G21 procedure were obtained from the American Type Culture Collection (ATCC), Rockville, MD, and the sixth, designated ILF-1, was isolated in the laboratory from a VES waste form. The ATCC fungal strains were \textit{Aspergillus niger} (9642), \textit{Penicillium funiculosum} (9644), \textit{Chaetomium globosum} (6205), \textit{Gliocladium virens} (9645), and \textit{Aureobasidium pullulans} (9348). Cultures of these fungi were received freeze-dried and were revived following ATCC instructions. Stock cultures were maintained on potato dextrose agar plates and recultured as necessary. Stock cultures were kept in a refrigerator at 278 K; cultures used to produce inoculum were maintained at 301 K.

As specified by ASTM G22, \textit{Pseudomonas aeruginosa} (ATCC 13388) was used for bacterial testing. Freeze-dried cultures were revived and cultured on nutrient agar salts. Stock cultures were refrigerated at 278 K, while the culture used for inoculum was maintained at 301 K.

All procedures used to prepare the inoculum and to culture and maintain organisms were as specified by ASTM G21 for the fungi or ASTM G22 for the bacteria. Agar used for the waste-form testing was the ASTM nutrient salts (NS) formulation. This medium contained inorganic nutrients but no organic carbon sources and was intended to supplement those organisms which could utilize the waste forms as a carbon source.
As defined by ASTM G21, several preliminary investigations were performed on VES and VES mixed with unirradiated exchange resin obtained from Epicor Inc. before the irradiated waste forms were evaluated. Sequentially, though not necessarily chronologically, work with unirradiated VES was conducted on the individual components used to make the VES polymer (binder, catalyst, promoter), powder made from waste form water, wafers cut from a waste form, and a whole waste form. Work with the polymer components was conducted with both NS agar and NS liquid. A quantity of 0.2 mL of each of those VES components was placed on a separate section of agar in each of six Petri dishes. Each of the agar surfaces was inoculated with one of the fungal species used in the ASTM G21 test. After 20 days of incubation, the agar surface was examined to detect growth of the fungi. For the liquid study, 1 mL of each of the polymer components was added to 20 mL of sterilized NS liquid contained in a 50-mL Erlenmeyer flask. Each of the three flasks was then inoculated with 0.2 mL of the fungal spore suspensions and sealed with a sterile rubber stopper. VES containing exchange resin was ground to pass through a 35-mesh sieve (0.00042 m), and the powder was sprinkled onto the surface of inoculated NS agar. In this case, duplicate plates with VES and an inoculated control were used. The wafers were obtained from dry-cutting 0.0030-m sections. A whole unirradiated waste form was also used.

All work was conducted with 0.1 x 0.015-m Petri dishes except that with the full-sized waste forms which required 0.100 x 0.080-m culture dishes. The diameter and height of the larger dishes were such that they could each accommodate a waste form laid on its side. After a waste form was placed in a dish, 106 mL of molten agar, cooled to 328 K, was poured in, thus submerging all but the upper one-fifth of the form.

Radionuclide contents of irradiated waste forms used in this study are described in Table 1. Because of the high radiation doses emanating from the waste forms, three separate biodegradation test setups were required at different times to accommodate the waste forms with the available shielding. In the first evaluation, waste forms C1A-28, C1A-29, D1-8, and D1-9 were tested under the ASTM G21 procedure, while D1-11, D1-12, C1A-31, and C1A-32 were used with ASTM G22. In addition, D1-10 and C1A-30 were incubated under ASTM G21 conditions except that the ASTM NS agar was replaced with a carbon-rich potato dextrose agar (PDA). The purpose of using PDA was to provide controls to determine if there were inherent properties of the waste forms (i.e., chemical or radiation) which would prevent or reduce growth of test organisms. The second set of tests used waste forms D2A-3 and D2A-4 for ASTM G21 tests, D2A-5 and D2A-6 for ASTM G22 tests, and D2A-7 for the carbon-rich PDA control. The third and final test used waste forms C2A-22 and C2A-23 for ASTM G21, C2A-25 and C2A-31 for ASTM G22, and C2A-32 for the carbon-rich control. For each testing period as specified by ASTM G21, sterile paper strips were placed on NS agar (in the absence of waste forms) and sprayed with the fungal inoculum. In addition, the surface of an NS agar plate was sprayed with inoculum to determine if either the spore suspension or the NS agar could support fungal growth.

After 20 days of incubation, the waste forms and associated agar were inspected as detailed in ASTM G21 or G22. When dealing with the irradiated forms, a close, prolonged inspection was not possible; determination of the extent of the growth had to be ascertained from color photographs of each waste form in its agar matrix.
### Table 1. Contact gamma dose for waste forms used in biodegradation study

<table>
<thead>
<tr>
<th>Waste Form ID&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Contact Gamma Dose&lt;sup&gt;b&lt;/sup&gt; (R/h)</th>
<th>Waste Form ID&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Contact Gamma Dose&lt;sup&gt;b&lt;/sup&gt; (R/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1A-28</td>
<td>2.2</td>
<td>D1-8</td>
<td>4.0</td>
</tr>
<tr>
<td>C1A-29</td>
<td>2.2</td>
<td>D1-9</td>
<td>3.7</td>
</tr>
<tr>
<td>C1A-30</td>
<td>2.1</td>
<td>D1-10</td>
<td>3.6</td>
</tr>
<tr>
<td>C1A-31</td>
<td>2.1</td>
<td>D1-11</td>
<td>3.4</td>
</tr>
<tr>
<td>C1A-32</td>
<td>2.0</td>
<td>D1-12</td>
<td>3.1</td>
</tr>
<tr>
<td>C2A-22</td>
<td>11.0</td>
<td>D2A-3</td>
<td>16.0</td>
</tr>
<tr>
<td>C2A-23</td>
<td>11.0</td>
<td>D2A-4</td>
<td>14.5</td>
</tr>
<tr>
<td>C2A-25</td>
<td>11.0</td>
<td>D2A-5</td>
<td>13.5</td>
</tr>
<tr>
<td>C2A-31</td>
<td>11.0</td>
<td>D2A-6</td>
<td>13.5</td>
</tr>
<tr>
<td>C2A-32</td>
<td>11.0</td>
<td>D2A-7</td>
<td>11.5</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> C = Portland cement; D = vinyl ester styrene; 1 = Type A waste; 2 = Type B waste; and A = the first of two batches.

<sup>b</sup> Waste forms have the following curie contents:

<table>
<thead>
<tr>
<th></th>
<th>134Cs</th>
<th>137Cs</th>
<th>90Sr</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>4.38 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>66.22 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>3.92 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>74.52 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>D2A</td>
<td>18.22 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>275.45 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.64 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>294.31 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>C1A</td>
<td>2.95 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>44.58 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>2.64 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>50.17 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2A</td>
<td>13.53 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>204.59 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.47 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>218.59 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Results of ASTM G22 Bacteria Testing

Unirradiated specimens of VES and cement were found not to support the test organisms *Pseudomonas aeruginosa* under ASTM G22 conditions. The same was also true for those tests conducted on eight irradiated waste forms (four VES and four Portland cement). These data do not support the earlier work of Piciulo et al., who reported that various mixtures of VES and simulated waste (including a deionized water control) supported the bacteria. However, because the bacterial growth in the referenced study was determined by an indirect method not specified by the ASTM G22 procedure, the results have been considered inconclusive.7

Results of ASTM G21 Fungal Testing

Results of the initial investigations will be presented first, followed by those on the irradiated waste forms. In those tests involving VES components, all of the NS agar areas to which components had been added supported at least one of the fungal species in addition to fungi which were able to grow at the component agar interface (Table 2). An inoculated control plate, which consisted of NS agar without a carbon source, had no traces of fungal growth, thus showing that neither the NS agar nor the spore suspension contained usable carbon. After a 30-day period of incubation of VES components in liquid NS media, the contents of the flasks were examined for the presence of fungal growth. Growth was noted visually (appearance of hyphal strands) in all cases, with the most apparent growth in the NS/catalyst mixture and the least in the NS/promoter mixture. Even after 18 months, growth associated with the catalyst and binder remains viable. This was shown when samples of fungal hyphae from these mixtures produced abundant growth on nutrient agar plates. Within 30 days of incubation, sporadic heavy growth could be seen on both duplicate inoculated plates hosting powdered VES (Figure 1) but not on the control. An enlargement (10x) of one of the areas of growth (Figure 2) shows that the fungi were growing on the VES. Growth of fungi at the NS agar interface with both wafers with and without exchange resins was noted within 30 days. Close examination after 60 days showed that fungi were growing on the edges of both types of wafers (Figures 3 and 4). After 18 months, more extensive growth (ASTM rating of three) was seen on the edge and surface of the wafer with resins (Figures 5 and 6).

During the development of a method to implement the ASTM testing of full-sized waste forms, an unidentified fungus (later given the designation of ILF-1) was found growing on the agar surface directly above a buried, unirradiated VES waste form. The source of the fungus was apparently the waste form, since the agar was not inoculated. Within 30 days, the fungus covered the exposed NS agar surface (Figure 7). The supposed carbon source for the fungus was some soluble component of the waste form. This fungus was cultured on...
Figure 1. Fungal growth on a powdered unirradiated VES waste form.
Figure 2. Close-up view (10x) of fungal growth on a powdered unirradiated VES waste form.
Figure 3. Close-up view (10x) of fungal growth on wafer cut from VES waste form (after 60 days).
Figure 4. Close-up view (10x) of fungal growth on wafer cut from unirradiated VES waste form (after 60 days).
Figure 5. Fungal growth on wafer cut from unirradiated VES waste form (after 18 months).
Figure 6. Close-up view (10x) of fungal growth on wafer cut from unirradiated VES waste form (after 18 months).
Figure 7. Fungal growth on NS agar covering unirradiated VES waste form.
PDA and, as allowed by the ASTM G21 protocol, was included as a stock culture for that test.

After the initial work using the unirradiated VES was complete, ASTM G21 testing of the irradiated EPICOR-II waste forms began. Cement waste forms in NS agar failed to support fungal growth. The cement waste form which was used as a control in the carbon nutrient PDA did allow for the growth of fungi (Figure 8). Growth at the top of the horizontal specimen appears to be touching the waste form. So, while the waste form itself was not able to support the fungi, growth was supported by a supplemental carbon source.

Results of VES testing showed that these waste forms could support the growth of fungi (Figures 9 and 10). Growth can clearly be seen at the agar waste form interface. Whether or not the waste form surface is supporting fungal growth could not be determined, because its mottled appearance could not be closely examined. No growth rating based on the ASTM scale was assigned. The PDA surface containing a VES waste form had a heavy covering of fungal growth (Figure 11). The NS agar control with no carbon source had no fungal growth, while NS agar with paper strips had good growth on the paper.

Following the NRC TP procedures, those waste forms that had supported fungal growth (VES) were prepared for retesting while the others (cement) were used for compression testing. First, the specimens were rinsed in methanol, and then they were allowed to air dry for 30 days. The waste forms which had supported fungal growth initially were again subjected to the G21 procedure. After the 20-day period of incubation, fungi were again seen to be growing at the agar waste form interface of each specimen (Figures 12, 13, 14, and 15). The control of NS agar remained free from fungal growth.

**Results of Compression Testing**

Three of the cement waste forms (C1A-28, C1A-29, C1A-31) were selected as representative samples (two incubated with fungi, one with bacteria) for compression testing. As shown in Table 3, all of these specimens had compressive strengths far in excess of 350 kPa as required by the TP (average of 15,600 kPa). Based on results which showed that other VES waste forms also had excess compressive strength and lack of observable degradation of the VES waste forms, no effort was made to test the VES biodegradation specimens.

**Discussion of ASTM G21 Results**

As discussed earlier, work with cement waste forms should have been conducted with microorganisms known to induce degradation in cement. While fungal growth was not seen on the NS agar, growth was noted in the cement PDA control (Figure 8). Piciulo et al. noted that fungi did not grow during the ASTM G21 test with cement and attributed this to pH or ionic strength changes created by the cement. While cement probably does cause such changes, it still appears that an available carbon source will promote growth near the waste form. Using the results from Piciulo et al., Bowerman et al. suggested that the cement waste forms might cause a biocidal or inhibitory effect in a burial environment. It now appears that this effect may not be as pronounced as first reported.

Work with VES has demonstrated that it can promote fungal growth, although the carbon source has not been determined. Evidence from this study indicates that the polymer or components (particularly the catalyst and binder) could be contributors. In addition, the study shows that once organisms have established themselves with these materials they can remain viable for several months. The growth of fungi away from a VES waste form suggests that some carbon source is being leached from the VES. Since this pattern of growth continued in a second testing even after the waste form surface was rinsed, it can be assumed that the source of the soluble carbon was internal. The application of the Bartha-Pramer test method would provide information which could be used to determine the extent of the carbon source.

Because it was not possible to closely examine the irradiated VES waste forms, it was not clear whether fungi were actually growing on the VES surface. However, results from two of the initial studies with unirradiated material suggest that fungi could indeed have been growing on the surface. It was shown that fungi would grow on a powdered VES waste form (Figure 2) as well as wafers cut from waste forms with and without ion exchange resins (Figures 3 and 4). With time, this growth can become heavy (Figure 6). No data are available on whether the fungi are penetrating the waste form surface.

This work was not intended to determine if VES polymer bonds were being biocatalyzed. It is a commonly held theory that chain branching in polymers has an inhibitory effect on the biodegradation of VES. However, when VES samples...
Figure 8. Fungal growth on PDA containing irradiated cement waste form CIA-30 (control).
Figure 9. Fungal growth associated with irradiated VES waste form DI-8 (first ASTM test).
Figure 10. Fungal growth associated with irradiated VES waste form D1-9 (first ASTM test).
Figure 11. Fungal growth on PDA containing irradiated VES waste form D2A-7 (control).
Figure 12. Fungal growth associated with irradiated VES waste form D1-8 (second ASTM test).
Figure 13. Fungal growth associated with irradiated VES waste form D1-9 (second ASTM test).
Figure 14. Fungal growth associated with irradiated VES waste form D2A-3 (second ASTM test).
Figure 15. Fungal growth associated with irradiated VES waste form D2A-4 (second ASTM test).
Table 3. Compressive strengths of some EPICOR-II cement waste forms used for biodegradation testing

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Compressive Strength (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1A-28</td>
<td>12,630</td>
</tr>
<tr>
<td>C1A-29</td>
<td>12,660</td>
</tr>
<tr>
<td>C1A-31</td>
<td>21,400</td>
</tr>
</tbody>
</table>

(mixed with distilled water) were evaluated in a Bartha-Pramer test, CO₂ production was maintained at a continuous rate during a 6-month test.¹⁴ Those data suggest that a carbon source in excess of any free monomer, promoter, or catalyst was being utilized.

A study of the data from compression testing of those cement waste forms exposed to biodegradation testing reveals that compression testing is unlikely to detect any biologically induced physical changes in a waste form. This is because of the insensitivity of compression testing to detect those slight structural variations which could occur over the brief period (21 days) of the biotests with relatively bulky samples.

Finally, there have been some suggestions that the biodegradation of waste forms is moot, since the radiation associated with this material would kill any microorganisms. This does not appear to be the case as was evidenced by the results from waste forms tested in this study which contained high radionuclide loadings and exhibited radiation doses of 2 to 16 R/h at contact (Table 1). In those cases where a readily available carbon source was supplied (PDA), the VES as well as the cement waste forms had copious quantities of fungal growth. It is expected that microbes can exist in an exposure range of 2 to 100,000 rads.¹⁶ This would appear to be well within the range produced by commercial-sized waste forms.
CONCLUSIONS AND RECOMMENDATIONS

As a result of this study, the following conclusions can be drawn:

1. VES with or without irradiated ion exchange resins promotes microbial growth.
2. The carbon source for growth could be any or all unreacted components used to produce VES or it could be the VES itself.
3. Cement waste forms containing irradiated ion exchange resins do not support microbial growth of the types specified by ASTM G21 and G22; neither do they completely inhibit growth when a carbon source is provided.
4. Neither VES or cement waste forms promote the growth of the bacterium *P. aeruginosa*.
5. Radiation at the levels found in the EPICOR-II irradiated waste forms used in this study does not inhibit microbial growth.
6. The ASTM G21 and G22 tests are not suitable for determining the extent of waste form biodegradability or for establishing physical affects due to biodegradation.

While this study and others have provided evidence that VES and cement can support the growth of microbes, it was concluded that the fundamental requirements of the TP have not been satisfied because the extent of degradation and any effect it could have on the physical characteristics of the waste form were not and could not be determined without employing an inherent biodegradability test. In light of the lack of visible degradation, the compression testing of specimens after exposure to ASTM G21 and G22 should be deleted from the protocol.

Because of the unlikelihood that the present methodology can be modified in a way that will completely satisfy the demands for an accelerated biodegradation test, it is recommended that the emphasis of the test be changed from the evaluation of waste forms to an evaluation of materials that will be used as solidification agents. By doing this, the question of biodegradation of a solidification agent and its consequences could be answered by one in-depth investigation conducted by personnel familiar with biodegradation testing rather than recurring limited tests conducted at numerous waste generation sites using individuals with varying training, experience, and equipment to conduct the testing. This concept is based on the conclusion that the greatest possibility for structural failure of a waste form rests with the solidifying agent. If the solidified matrix retains its integrity, then there is reason to assume that there will be no structural failure even if the waste material is biodegradable. However, some testing with standard waste forms containing various known wastes would be required to substantiate this position. If this suggestion were to be adopted, then the NRC could maintain complete control of verifying the integrity of waste forms simply by certifying the solidification agent. Such certification would come from a source other than the manufacturer and would involve a very conservative (i.e., rigorous) test protocol. The justification for such rigorous testing is that it needs to occur only once for each solidification agent.

The proposed testing would be of the type which Bowerman et al. summarized as inherent biodegradability testing. Such a method would include: exposure to a wide range of microbial species (especially to those known to affect the solidification agent); appropriately sized specimens for testing; possible use of alternative carbon (energy) sources to provide opportunity for co-metabolism of a specimen; and extension of the time period allowed for testing (up to 6 months). Degradative effects would be determined directly by measuring metabolic activity, such as *CO₂* production or oxygen consumption, or some physical parameters like specimen weight loss and visual deterioration.
REFERENCES


Waste-form testing is being conducted by the Three Mile Island Unit 2 (TMI-2) EPICOR-II Resin/Liner Investigation; Low-Level Waste Data Base Development Program of the U.S. Nuclear Regulatory Commission (NRC) in accordance with the NRC Branch Technical Position on Waste Form. Waste forms which were tested contain ion exchange resins solidified with vinyl ester-styrene and Portland Type I-II cement. This report describes the biodegradation testing of those waste forms, presents the test results, and provides recommendations for alternate test methodology.