Title: ENZYMATIC DEGRADATION OF PLUTONIUM-CONTAMINATED CELLULOSE PRODUCTS

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Enzymatic Degradation of Plutonium-Contaminated Cellulose Products
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ABSTRACT

Enzyme solutions produced for commercial purposes unrelated to waste management have the potential for reducing the volume of wastes in streams containing cellulose, lipid and protein materials. For example, we have shown that cellulases used in denim production and in detergent formulations are able to digest cellulose-containing sorbents and other cellulose-based wastes contaminated either with crude oil or with radionuclides. This presentation describes the use of one such enzyme preparation (Rapidasem™) for the degradation of cotton sorbents intentionally contaminated with low levels of plutonium. This is part of a feasibility study to determine if such treatments have a role in reducing the volume of low level and transuranic wastes to minimize the amount of radionuclide-contaminated waste that must be disposed of in secured storage areas.

INTRODUCTION

Waste streams from National Laboratories with historic and current missions involving radionuclides used in the defense industry present disposal problems. Plutonium, americium, neptunium and uranium, among other radioactive elements, contaminate these wastes. The problems they present include the potential for radiation poisoning since most have extremely long half lives, and the potential use for terrorist activities. Not only must weapons-grade plutonium be guarded from improper release from these facilities, but low level (LLW) and transuranic (TRU) wastes must be disposed of in ways that prevent improper contact with living organisms and the environment. Such low level and transuranic wastes include items resulting from the production, testing, and storage (and now disassembly) of nuclear weapons. At Los Alamos National Laboratory (LANL) and other Department of Energy (DOE) facilities, work regularly is performed in glove boxes where plutonium (Pu) and other radionuclide residues can contaminate items within the box or on the boxes internal surfaces. Normal maintenance of the glove boxes is done by cleaning with cheesecloth and other laboratory wipes. Now classified as suspect wastes, these materials must be handled as transuranic (TRU) or low-level radioactive wastes and disposed in special landfills or other repositories. In addition, kilogram amounts of other cellulose-based materials such as
contaminated waste paper are generated every year at Los Alamos. The variety of other materials of contaminated or suspect waste includes rubber, lead, laboratory clothing and supplies, laboratory and other equipment and anything else that might have come in contact with radioactive materials. Historic or legacy waste of such materials is currently stored on DOD or DOE facilities. It has been estimated that 275,000 55-gallon drums of such waste currently is awaiting disposal. From Los Alamos National Laboratory’s facility TA-55 alone, 55 kg of cellulose waste is currently generated every year.

Currently the TRU waste products are slated for disposal at the WIPP (Waste Isolation Pilot Plant) facility located in New Mexico, but opposition from various groups has prevented the use of this completed facility, so the materials remain on the governmental defense reservations. Even when (if) this or a similar facility can begin accepting TRU waste, it will be expensive to store such items in perpetuity. The same argument can be made for the disposal of LLW in appropriate facilities. Therefore, any technology that can reduce the volume of such wastes would greatly reduce the cost of disposal and the associated risk of potential human and environmental contamination.

The use of industrial-strength enzyme solutions developed and marketed for a variety of other applications may be useful in the reduction in volume of radionuclide-contaminated wastes. Unlike many enzymes used in research projects, most industrial enzyme preparations are able to withstand (and function under) harsh conditions of use such as low or high pHs and in the presence of detergents or heavy metals. A technology based on the use of industrial cellulases developed to treat oil-saturated cotton sorbents in the presence of seawater previously was found to be useful for degrading uranium-contaminated cotton fabrics, releasing the uranium into the enzyme solution (U.S. Pat. No. 5,597,728).

The present project was intended to demonstrate the feasibility of this process for treatment of cellulose-containing materials in LANL’s laboratory waste stream. Dr. Jim Brainard and associates at LANL who used a microbial source of enzymes rather than the enzymes alone have proved this technology with Pu-treated cotton. The current report describes the use of Rapidase™, an industrially-available cellulase, to degrade contaminated cellulose substrates, releasing a portion of the radioactivity into the enzyme solution from which it can be recovered more readily than from a solid matrix. The residue from the enzyme treatment, which still is contaminated with radionuclides, was greatly reduced in weight and volume after 6 days of treatment. Further reduction is possible with extended or repeated treatment.

Degradation of raw cotton intentionally contaminated with known levels of plutonium oxides or non-radioactive lanthanide surrogates including cerium oxide, cerium carbonate and praseodymium oxide was evaluated. Initial experiments were performed with various forms of cotton (raw, cleaned, scoured and bleached and manufactured items) to define the conditions needed by the enzyme, as supplied by the manufacturer, to degrade both the non-contaminated and contaminated cellulose-containing items. Next, cotton contaminated with lanthanides, then with plutonium was
tested to determine if actinides inhibited the ability of the enzyme to degrade raw cotton. The distribution of the plutonium between the residual substrate and enzyme solution following degradation of the cotton was determined. All experiments involving plutonium were conducted in a secured area at LANL by LANL personnel including Dr. Laura Worl and Mr. Daniel Padilla. Simultaneously, the ability of the enzyme solution to degrade unused materials such as paper and cheesecloth (of the type that is used as sorbing wipes in the glovebox line) that eventually contribute to the LANL waste stream was tested. Other experiments included determining the effect of various concentrations of sugars (glucose and sucrose) on the degradation process since the activity of one of the cellulase enzymes (cellobiase) is known to be inhibited by increasing concentrations of glucose. Further, the effect of added nitrate salts was tested since Pu is soluble in HNO3 and nitrates can be found in Pu-containing waste streams and thus potentially present on wipes used to clean gloveboxes. Funding for this project was provided by the Amarillo National Resource Center for Plutonium via a grant from DOE to the state of Texas. All participants are gratefully acknowledged.

MATERIALS AND METHODS

The commercial enzyme used for these studies was Rapidas™ (Genencor, Rochester, NY). A five percent solution of the commercial liquid was prepared in McIlvaine's buffer (McIlvaine 1921) to produce a final solution with a pH of 4.5. McIlvaine's is a citrate-phosphate buffer. Laboratory-scale experiments involved the use of 125 ml Erlenmeyer flasks which contained a total volume of 75mL liquid plus cotton substrate. Cotton substrates (0.5g/flask) consisted of raw cotton (not treated by scouring and bleaching) that had been cleaned of stem and leaf trash by treatment in a Shirley analyzer (British Cotton Industry Research Association, Patent No. 404888) and afterward referred to as Shirleyed cotton, or 0.5g of the other cotton substrates being tested (including cheesecloth and paper that had been powdered in a Wiley cutting mill to pass a 20-mesh screen). The results of treating each substrate are presented. The cellulose substrate was added to freshly prepared enzyme solution at the beginning of each experiment. Experimental and control (minus enzyme) flasks were incubated on a gyrotrary shaker (New Brunswick Scientific) at 45C; alternately, incubation was conducted on a stirring hotplate where the temperature was maintained at 45C. For comparative purposes, a standard incubation period of 6 days was used; experience indicated that approximately 50% or more of the raw cotton substrate was digested within a week under these conditions.

Substrate digestion due to enzyme treatment was evaluated by determining the dry weight of each substrate prior to and following enzyme treatment. Residual substrate collected by filtration was dried to a constant weight at 100C. Percent residual substrate is reported as both the range of values from three replicates and the mean value, unless otherwise noted in the tables.

Residual glucose levels were determined by high-pressure liquid chromatography (HPLC) using a Dionex DX 500 HPLC System with an AS 5300 autosampler. A
gradient eluent of 500mM sodium acetate/200mM sodium hydroxide was used. Five mL of spent enzyme solution recovered from each flask following filtration to remove the residual cellulosic substrate was adjusted to pH 6-7 with 0.3g (Na2)3(PO4)2 and heated for approximately 2hr in a 70°C waterbath to coagulate the protein. A 1mL sample of the heated and cooled supernatant fluid was diluted volumetrically 1:100 with 18.2 megaohm water. A portion of this solution was filtered through a 0.2μM filter into an autosampler vial from which 25μL was drawn for HPLC analysis. Glucose concentrations were calculated from the results as mg/mL.

The effect of the following were evaluated to determine if they inhibited or slowed the digestion process: glucose, sucrose and nitrate concentrations (0.1 to 1.0M), and the presence of cerium oxide, cerium carbonate, praseodymium oxide or plutonium oxide. Residual Pu or surrogates were determined by liquid scintillation counting and alpha spectroscopy.

The following section presents the details and results of individual experiments.

RESULTS

**Experiment 1:** Determine the loss in dry weight of various cellulose or cellulose-containing materials following treatment in enzyme solution under standard conditions and evaluate the potential for degrading cellulose-based materials used in the LANL plutonium facilities.

Control substrate: Standard raw cotton
Experimental substrates:
  - Shirleyed cotton
  - Scoured and bleached cotton balls
  - Paper, cut into strips
  - Paper powdered in a Wiley cutting mill to pass a 20 mesh screen
  - Cheesecloth used to wipe the insides of gloveboxes in which Pu is handled
  - Cotton blend fabric, cut into strips
Conditions: 5% enzyme solution, 0.5 g substrate, pH 4.5, 45°C, 200 rpm, 6 days
Residual glucose concentration determined by HPLC

Results:

<table>
<thead>
<tr>
<th>Cellulose-Containing Substrate (0.5g)</th>
<th>Percent Residual Substrate Range, n=3</th>
<th>(Mean)</th>
<th>mg/mL Residual Glucose Range, n=3</th>
<th>(Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw cotton standard</td>
<td>43-48</td>
<td>(45)</td>
<td>3.6-4.0</td>
<td>(3.9)</td>
</tr>
<tr>
<td>Shirleyed raw cotton</td>
<td>43-47</td>
<td>(46)</td>
<td>4.0-4.8</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Cotton balls (scoured/bleached)</td>
<td>33-36</td>
<td>(34)</td>
<td>4.0-4.4</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Paper*†</td>
<td>1-1</td>
<td>(1)</td>
<td>3.6-4.4</td>
<td>(3.9)</td>
</tr>
<tr>
<td>Material</td>
<td>3-4 n=2 (4)</td>
<td>3.6-4.4 (4.0)</td>
<td>2-3 (2)</td>
<td>not determined</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Kimwipes™</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milled paper”</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheesecloth*</td>
<td>39-41 (40)</td>
<td>4.4-4.4 (4.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Cotton fabric</td>
<td>42-44 (43)</td>
<td>2.8-3.2 (2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton blend fabric</td>
<td>59-60 (60)</td>
<td>2.0-2.0 (2.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LANL wastes

**Experiment 2:** Determine the effect of added glucose and sucrose on the amount of degradation of Shirleyed cotton since glucose inhibits cellulase activity

Control: Shirleyed cotton with no added carbohydrates
Experimental: Various concentrations of glucose and sucrose added to the enzyme solution
Conditions: 5% enzyme solution, pH 4.5, 45C, 200 rpm, 6 days, 0.5 g Shirleyed cotton dried to constant weight

Results:

<table>
<thead>
<tr>
<th>Added sugars (molarity)</th>
<th>Residual Substrate, Grams</th>
<th>Residual Substrate, (%)</th>
<th>mg/mL Residual Glucose n=3 (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)”</td>
<td>0.23 (46)</td>
<td></td>
<td>7.68</td>
</tr>
<tr>
<td>Glucose (0.1M)</td>
<td>0.28 (57)</td>
<td></td>
<td>11.52</td>
</tr>
<tr>
<td>Glucose (0.5M)</td>
<td>0.39 (77)</td>
<td></td>
<td>30.26”</td>
</tr>
<tr>
<td>Glucose (1.0M)”</td>
<td>0.48 (96)”</td>
<td></td>
<td>45.95</td>
</tr>
<tr>
<td>Sucrose (0.1M)</td>
<td>0.22 (44)”</td>
<td></td>
<td>3.74”</td>
</tr>
<tr>
<td>Sucrose (0.5M)”</td>
<td>0.33 (66)”</td>
<td></td>
<td>6.00”</td>
</tr>
<tr>
<td>Sucrose (1.0M)”</td>
<td>0.45 (91)”</td>
<td></td>
<td>5.67”</td>
</tr>
</tbody>
</table>

**Experiment 3:** Evaluate the effect of nitrates on the degradation of raw cotton

Control: Standard raw cotton without added nitrates
Experimental: Added nitrates at the indicated concentrations

NaNO₃ (0.1M)  
NaNO₃ (0.5M)  
NaNO₃ (1.0M)  
KNO₃ (0.1M)  
KNO₃ (1.0M)  
HNO₃ (0.1%)

Conditions: 5% enzyme solution, 0.5 g substrate, pH 4.5, 45C, 200 rpm, 6 days

Results:
<table>
<thead>
<tr>
<th>Added nitrates (molarity)</th>
<th>% Residual Substrate Results, n=3 (Mean)</th>
<th>mg/mL Residual Glucose n=3 (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40-42 (41)</td>
<td>70-83, n=2 (77)</td>
</tr>
<tr>
<td>NaNO₃ (0.1)</td>
<td>48-60 (53)</td>
<td>70-149 (97)</td>
</tr>
<tr>
<td>NaNO₃ (0.5)</td>
<td>58-70 (61)</td>
<td>44-59 (50)</td>
</tr>
<tr>
<td>NaNO₃ (1.0)</td>
<td>84-90 (87)</td>
<td>7-38 (28)</td>
</tr>
<tr>
<td>KNO₃ (0.1)</td>
<td>46-54 (51)</td>
<td>63-73 (69)</td>
</tr>
<tr>
<td>KNO₃ (1.0)</td>
<td>94-100 (97)</td>
<td>10-13 (15)</td>
</tr>
<tr>
<td>HNO₃ at 0.1%</td>
<td>44-68 (56)</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Experiment 4: Evaluate the effect of various types of agitation on the degradation of LANL cheesecloth of the type used in cleaning the gloveboxes

Control: Standard raw cotton
Experimental:
- Cheesecloth - agitation using the shaker
- Cheesecloth - agitation by stirring on hotplate
Conditions: 5% enzyme solution, 0.5 g substrate, pH 4.5, 45°C, 200 rpm, 6 days
Results:

<table>
<thead>
<tr>
<th>Substrate (0.5g)</th>
<th>% Residual Substrate Results, n=3 (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw cotton - shaken</td>
<td>36, 36, 35 (36)</td>
</tr>
<tr>
<td>Cheesecloth - shaken</td>
<td>31, 31, 33 (31)</td>
</tr>
<tr>
<td>Cheesecloth - stirred</td>
<td>9, 11, 13 (11)</td>
</tr>
</tbody>
</table>

Experiment 5: Determine the effect of lanthanide surrogates on the digestion of LANL cheesecloth

Control: Cheesecloth, no lanthanides
Experimental: plus added lanthanide salts at various concentrations (0.05, 0.1, 0.5 and 1.0mM), including:
- Cereum carbonate Ce(CO₂)₃,
- Cerium oxide CeO₂
- Praseodymium oxide PrO₃
Conditions: 5% enzyme solution, pH 4.5, 45°C, 200 rpm, 6 days, 0.5g cheesecloth
Results:
**Experiment 6:** Determine the effect of plutonium oxide (PuO2) on the digestion of LANL cheesecloth

**Controls:**
- Cheesecloth, enzyme, no PuO2
- Cheesecloth, no enzyme, PuO2

**Experimental:**
- Cheesecloth plus 0.0025% added PuO2

**Conditions:** 5% enzyme solution, pH 4.5, 45°C, 200 rpm, 6 days, 0.5g cheesecloth

**Results:**

<table>
<thead>
<tr>
<th>Substrate (measured Pu, ng/mL)</th>
<th>% Residual Substrate</th>
<th>Pu (μCi/L) (spent enzyme)</th>
<th>Pu (μCi/L) (residue by TA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheesecloth control</td>
<td>27, 37 (32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, no Pu, no enzyme</td>
<td>106*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pu control, no enzyme (211)</td>
<td>108*</td>
<td>15.4 LS/16.1 TA*</td>
<td>2830</td>
</tr>
<tr>
<td>Pu experimental (755)</td>
<td>31</td>
<td>55.2 LS/34.5 TA*</td>
<td>2470</td>
</tr>
<tr>
<td>Pu experimental (405)*</td>
<td>30</td>
<td>29.6 LS/20.7 TA*</td>
<td>2120</td>
</tr>
<tr>
<td>Pu experimental (370)</td>
<td>38</td>
<td>30.0 LS/25.8 TA</td>
<td>2090</td>
</tr>
</tbody>
</table>

LS: Liquid Scintillation analysis
TA: Total Alpha analysis
* no digestion

**DISCUSSION**
Industrial enzyme preparations are able to dissolve natural organic polymers such as proteins (proteases), lipids (lipases) and cellulose (cellulases). Some uses for proteases include leather tanning, the production of soy sauce and use in contact lens cleaning solutions. Lipases are used in drain cleaners to dissolve the grease, which entraps materials to form clogs. Cellulases are added to detergent formulations to digest grass and other stains, they are used in the finishing of denim (to provide and improved “hand” or soft feeling to the material), and for the ex situ disposal of sorbents used to clean up oil spills.

Cellulases are composed of a series of enzymes each with a specific affinity for a specific part of the cellulose molecule. Endoglucanases break internal glucose-glucose bonds within the cellulose polymer. Exoglucanases remove individual glucose molecules from the ends of the cellulose chain and celllobiases cleave di-glucose units (cellobiase) into individual glucose molecules. With the exception of the pectins and waxes on raw cotton, the entire cellulose molecule theoretically can be degraded into individual glucose units. In practice, as the glucose concentration increases, the cellobiase enzyme activity is reduced. However, the structure of the cellulose fiber can be entirely destroyed by enzyme treatment, if carried out for a sufficient period of time. In practice, treatment usually is terminated after 48 hr when the structure of the mat of fibers has been reduced to tiny individual filaments unable to hold the pollutant any longer. For crude oils, the major means whereby cotton acts as a sorbent is by entrainment rather than adsorption onto the surface of the fiber or absorption into its lumen. For actinides, the mechanism of sorption is not known, but we have shown in this study, and in a previous one (REF), that radioactive particles no longer are associated totally with the fiber residue.

In previous studies testing the ability of biodegradable oil sorbents to be degraded by cellulases, a 5% solution of Rapidase™ in pH 4.5 McIlvaine's buffer was found to be an effective way to digest raw cotton contaminated with either diesel fuel or crude oil. The optimum temperature for digesting 0.5g cotton in 100mL solution was 45C, although degradation occurred between 25 and 60C. Agitation resulted in faster degradation than static treatment.

Since enzymes are protein catalysts and subject to inhibition by heavy metals, salts and other agents, the effect of monovalent and divalent cations on the process was evaluated. In addition, since one of the enzyme components of the commercial cellulase (cellobiase) is inhibited by increasing concentrations of glucose, this also was tested on the degradation process. The intent of this study was to determine the feasibility of using cellulases to reduce the volume of cellulose-containing actinide-contaminated waste materials such as those that might be encountered at a facility with a military mission. Consideration of the following list of items led to the optimization of treatment parameters at a laboratory scale, in preparation for future scale-up experiments. The following were found to be of significance to the enzyme digestion process: physical state of the substrate (the greater the surface area, the more rapid and complete the digestion and the more non-cellulosic components, the greater the residue following enzyme treatment), a temperature of 45C, pH 4.5, agitation (by stirring the contents of the
flasks using either a magnetic stirrer or a gyrotory shaker) and time of treatment (a standard treatment time of 6 days was used for these experiments).

In the current study, the dry weight of residual substrate was used to determine the effectiveness of the enzyme treatment (% reduction in dry weight). Residual substrate was collected by filtration followed by drying to a constant weight at (100C). Residual reducing glucose was determined by HPLC analyses. Residual PuO₂ was determined by liquid scintillation counting and alpha spectroscopy.

The first set of experiments compared the ability of the enzyme solution to degrade hand-ginned raw cotton and raw cotton that had been mechanically cleaned of stem and leaf trash (Shirleyed cotton) with scoured and bleached cotton balls (100% cotton), 100% cotton fabric, 50/50 cotton/polyester blend fabric and typical cellulose-containing items that wind up as Pu-contaminated wastes such as cheesecloth wipes and paper. The latter items ordinarily would be slated for disposal in the WIPP site. In these experiments, it was found that, based on residual dry weights of the substrates, Shirleyed raw cotton and the raw cotton standard were approximately equal with regard to the amount degraded by the enzyme treatment after 6 days of treatment. Scoured and bleached cotton balls were degraded more completely than the raw cottons. Since residual waxes or other non-cellulose materials found on raw cottons are not present on scoured and bleached cotton, the additional residual weight in the raw cotton samples likely is due to the materials removed by scouring and bleaching. Fabric prepared from 100% cotton was degraded more completely than a 50/50 blend of cotton/polyester. Cheesecloth and 100% cotton fabric degradation was nearly equivalent to that of scoured and bleached cotton balls (100% cotton), as would be anticipated from the composition of these materials.

Regarding LANL wastes, the amount of cheesecloth degraded was approximately the same as that of raw cotton and 100% cotton fabric; shredded and milled paper were approximately equivalent in digestibility; therefore extensive pre-treatment of paper waste to increase its surface area should be unnecessary prior to enzyme treatment. Other LANL waste items such as HEPA filters, Tyvek sleeves and Tyvek labcoats were not degraded (results not reported). Since these items are made from plastics, it was not expected that they would be degraded by cellulase.

Residual glucose, as determined by HPLC analyses for each of these substrates was approximately 10-fold less than the amount known to cause inhibition of digestion, as determined in the experiment to which varying concentrations of glucose and sucrose were added to the enzyme solution at the beginning of the experiment. Inhibition of the degradation process increased as the concentrations of both glucose and sucrose in the solutions increased. Added glucose or sucrose at concentrations of 0.1M resulted in a slight increase in the amount of residual substrate as compared to the control. Inhibition of enzyme activity was greater at 0.5M than 0.1M glucose and sucrose, whereas 1.0M concentrations of both glucose and sucrose nearly completely inhibited enzymatic digestion of the cotton substrate. It is likely that inhibition of digestion in the presence of
sucrose is due to factors other than glucose accumulation in the enzyme solution since all sucrose solutions had residual glucose levels less than controls.

When the effects of added nitrate salts at concentrations from 0.1 to 1.0M and nitric acid at 0.1% on substrate digestion were evaluated, it was found that increasing nitrate salt concentrations also inhibited enzyme activity and substrate degradation. Roughly three-fold decreases in degradation were observed at a concentration of 0.1M nitrate salt, regardless of whether KNO₃ or NaNO₃ was used. At 1.0M concentrations of both salts, inhibition was nearly complete. These results indicate nitrates in high concentrations likely will be a problem in degradation, but it is not known whether this is due to salt effects or specifically to the nitrate ion. HNO₃ at 0.1% had approximately the same effect as the nitrate salts at 0.1M, i.e., there was a slight decrease in the amount of cotton degraded, as compared to the control values.

In anticipation of scale-up experiments, a comparison was made between the type of agitation that was used in these laboratory-scale experiments (gyrotory shaker) and stirring which is a more likely type of agitation that will be used in the testing of larger volumes. There was no substantial difference between the amount of residual substrate degraded between raw cotton and LANL cheesecloth when agitation was on the shaker, but more cheesecloth was degraded under the same conditions if the mixture was stirred and not shaken.

When lanthanide surrogates including cerium carbonate Ce(CO₂)₃, cerium oxide CeO₂, and praseodymium oxide PrO₃ at various concentrations (0.05, 0.1, 0.5 and 1.0mM), were added to the enzyme solution, enzymatic digestion of the cheesecloth was not inhibited by concentrations of up to 1.0mM.

In the experiments that were conducted inside the Pu gloveboxes, it was found that enzymatic digestion of cheesecloth occurred in the presence of 0.0025% PuO₂ at 45°C. The radioactivity was compared between the solid residue (residual cellulose and filter) and the spent enzyme solution after removal of the residual substrate. Variations between the liquid scintillation (LS) and alpha spectoscopy (TA) results for the same enzyme sample are due to high counts in the liquids (approaching the limits of the LS detector).

SUMMARY AND CONCLUSIONS

Overall, we have been able to show that a commercially-available cellulase was able to reduce the volume of cellulose substrates contaminated with lanthanide surrogates and PuO₂. Residual radioactivity was distributed between the liquid (spent enzyme) and solid (cellulose residue plus filter). This indicates a promising mechanism for volume reduction of low-level or TRU wastes by way of enzyme digestion. Other advantages to using enzymes for waste treatment include the following:

1. they are protein solutions which are readily degraded and the enzyme solutions do not themselves cause any significant disposal problems (after removal of radionuclides, non-toxic, non-polluting residual materials remain)
2. industrial enzyme solutions are relatively inexpensive to purchase from the manufacturer. And, since they are used in large quantities for other industries, they are readily available
3. in the case of cellulases, there is more than one manufacturer so that availability is not a problem
4. cellulases act by converting cellulose to short fibers and the various length glucose polymers and glucose, no intermediates that provide a disposal problem are created during the digestion process from the cellulose substrate
5. live organisms are not required, therefore attendant problems of keeping them alive and providing growth conditions for them to produce enzymes is not necessary
6. volume reduction of the waste items means less material to be disposed of; in the case of radionuclide contaminated materials, this can result in a substantial savings in both disposal costs and the space required for such waste
7. cost effective
8. works in aqueous solutions
9. low temperature process (well below 100C)
10. non-corrosive
11. continuous process is possible with waste materials being prepared robotically, if necessary
12. scaleable to gloveboxes or larger sizes, as necessary and no problems are foreseen with scale-up
13. glovebox-sized enzyme reactors may be the solution to disposal of future contaminated cheesecloth

Some of the disadvantages of using enzymes for waste treatment include:

1. sorting is likely to be necessary for some of the waste streams that can be degraded by the enzyme to separate the cellulosic materials for cellulase treatment
2. enzymes are protein solutions which require appropriate handling and have a finite time during which their activity is optimal.

Future experiments will address parameters associated with scale-up of the process to sizes compatible with the needs of the defense industry, including questions of substrate volumes and pre-treatment effects (to determine optimum physical size and state), batch vs. continuous feed additions of substrates, substrate retention time in the enzyme solution, re-use of enzyme solutions, possible losses of enzyme activity over time, appropriate configurations of the reaction vessels and issues regarding the disposal of spent enzyme solutions and residual substrate. Whether enzymes can contribute to reducing the disposal volume of contaminated items not normally of biological origin, such as plastics, rubber and other common organic components of TRU waste also is of interest. Although current industrial enzyme preparations which degrade proteins, carbohydrates and lipids probably would not be effective, it is possible that enzymes with different specificities, such as polyester depolymerases, would be an effective way to degrade radioactive wastes of organic, but non-biological, origins.
REFERENCES

Enzyme Compositions and methods for biodegradation separation of natural fibers and adsorbed petroleum products. Wyatt, Caryl Heintz, B.G. Wyatt and D.L. Carr

McIlvaine, JBC 49, 183 (1921)