Progress Report
Beneficial Uses Program
Period Ending March 31, 1977

Waste Management and Environmental Programs Department

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Sandia Laboratories
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PROGRESS REPORT
BENEFICIAL USES PROGRAM
PERIOD ENDING MARCH 31, 1977

Waste Management and Environmental
Programs Department 5440
Sandia Laboratories
Albuquerque, New Mexico 87115

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PROGRESS REPORT
BENEFICIAL USES PROGRAM

I. Introduction

This progress report on the Beneficial Uses Program covers the quarter ending March 31, 1977. The Beneficial Uses Program is a comprehensive program aimed at developing necessary technologies for cost/beneficial uses of existing and future surplus radioactive materials. The major portion of the work at Sandia has been concentrated in two subprograms: the Waste Resources Utilization Program and the Separation Technology and Source Development Program.

Highlights of the work reported within these subprograms are as follows:

Cost estimates for the proposed Sandia Irradiator for Dried Sewage Sludge (SIDSS) have been submitted and tentatively approved. This irradiator will be located at Sandia (Area III) and will serve as a test facility capable of treating several tons of dry sludge daily. If successful, it will serve as a model system for the suggested compost irradiator to be located in the Washington, D. C., area.

In the continuing studies at New Mexico State University, analyses of blood, livers and kidneys of lambs fed diets with and without sewage solids suggest that there is little, if any, hazard to meat products from accumulation of the heavy metals and trace elements.

The discovery of an agent in sludge that causes heat inactivation of reovirus to occur at reduced temperatures was analyzed. It was determined to be pH dependent and extremely heat stable. The activity of this agent causes the rate of inactivation of reovirus to be greatly accelerated even at 35° C and occurs in a bimodal manner.
During the past quarter, source efficiency calculations on cesium aluminosilicate compositions have been completed and compared with CsCl for source radii up to 3 cm. In addition, source efficiency for calcined fuel waste has been determined. It appears that direct use of fuel rods or calcine may not be feasible from the source efficiency standpoint.

II. Waste Resources Utilization Program

Microbiology

Bacteriology -

Heat Inactivation of Coliforms and Fecal Streptococcal Bacteria in Composted Sewage Sludge--One of the primary reasons for composting is reduction of pathogens, including bacteria. Thus, it is of interest to measure the effects of heat with time on bacterial populations. Figures 1 and 2 show rates of inactivation for coliforms and for fecal strep bacteria, respectively, at different temperatures in composted sludge. It is clear that efficiency of composting can drastically affect bacterial inactivation, as was shown last quarter for Salmonella enteritidis ser. Montevideo.

Radiation Inactivation of Mixed Salmonella Bacteria in Composted Sludge--Prior to this quarter, studies of the inactivation of salmonellas in compost were confined to S. enteritidis ser. Montevideo seeded at the ARS lab in Beltsville. In order to expand the information on the radiation sensitivity of salmonellas in compost, a mixed culture was isolated from sludge and a standard population grown in the laboratory. Tubes of this culture (10^8/ml) are stored frozen in a nutrient broth/glycerol mixture.

Sterilized compost was seeded with this mixed population by adding it dropwise into a blender and mixing for one minute to evenly disperse the bacteria.

Figure 3 shows the inactivation rate for this mixed culture of salmonellas at 23° C. The radiation sensitivity of this culture appears to
Figure 1. Heat inactivation of coliform bacteria in composted sludge at 50° C (Δ), 52° C (■), 55° C (○), 61° C (●), and 64° C (□).
Figure 2. Heat inactivation of fecal streptococcus bacteria in composted sludge at 52°C (O), 55°C (■), 58°C (▲), 61°C (●), 64°C (□), and 67°C (▲).
Figure 3. Radiation inactivation of mixed salmonellas in composted sludge. Dose rate was ~9 krads/min.
be slightly greater than that previously reported for *S. enteritidis* ser. Montevideo (~15 krad/log vs ~20 krad/log) grown in compost.

**Effects of Irradiation and Oxygenation on Bacteria in Raw Liquid Sludge**--It had been previously determined that fecal streptococcal bacteria exhibit the same sensitivity to ionizing radiation (but not to heat) in raw sludge as in digested sludge. Current results indicate (Figure 4) that the effect of oxygenation during irradiation at 23°C is the same in raw and digested sludge for fecal strep (D ≈ 90 krad/log). It has also been found that irradiation, with and without oxygenation, has essentially the same effect on coliforms in raw sludge as in digested sludge at 23°C (Figure 5). No data have been obtained on salmonellas.

**Nitrate Reduction**

**Effects of Ammonia**

Nitrate Reduction—Wild-type Chromobacterium violaceum was previously shown to rapidly reduce nitrate when grown anaerobically in a medium supplemented with 20 mM KNO₃. The apparent rate of nitrate reduction slows by late-log phase, presumably due to repression of the nitrate-reducing enzymes by their ultimate end-product, ammonia. The minimum concentration of ammonia necessary to fully repress nitrate reduction was unknown, however. This information will be required for the selection of derepressed mutants. The minimum concentration of ammonia that affects growth, i.e., cytotoxic, was also unknown. This parameter will be needed to define the range of ammonia concentrations in wastewater for which denitrification by *C. violaceum* could be effective.

Accordingly, experimentation was done to define the kinetics of growth and nitrate reduction in ammonium-supplemented media. Culture vessels of nutrient broth (NB) containing 20 mM glucose and 20 mM KNO₃ were supplemented with sterile 1 M NH₄Cl to give final concentrations of 0, 10, 20, 35, 50, and 100 mM. They were sparged with N₂ - CO₂ (95:5), inoculated with an overnight culture of cells grown in unsupplemented NB (initial OD₅₅₀ ≈ 0.05), and incubated at 37°C. Aliquots were removed at intervals. The presence of nitrite and residual nitrate was determined as follows: Nitrite is determined qualitatively by a
Figure 4. Effect of oxygenation on the radiation inactivation of fecal streptococcus bacteria in raw sludge at 23° C.
Figure 5. Effect of oxygenation on the radiation inactivation of coliform bacteria in raw sludge at 23° C.
simple spot test. To 1.0 ml of culture fluid are added 0.1 ml each of 1 percent sulfanilamide in concentrated HCl and 0.02 percent N-1-naphthylethylenediamine. The development of a pink color indicates nitrite. Detectable amounts of the first intermediate product of nitrate reduction, nitrite, are present only during active denitrification. Residual nitrate may be assessed in samples negative for nitrite by the subsequent addition of zinc dust to the tube. The development of a red color indicates nitrate. Its presence in samples negative for nitrite implies that no denitrification occurred; its absence implies that denitrification is complete.

All concentrations of ammonium used inhibited nitrate reduction for at least 48 hours of growth. A positive nitrite reaction was observed in one replicate of the 10 mM culture at 48 hours.

Nitrate reduction in Aspergillus nidulans is partially inhibited by as low as 5 mM ammonium. Concentrations in the 1 to 10 mM range will be tested in the future for their ability to repress this function in C. violaceum.

Cytotoxicity—Ammonium has no detectable effect on growth of C. violaceum until the concentration reaches 50 mM. In a detailed study, several parameters were evaluated during anaerobic growth in NB (20 mM glucose; 20 mM KNO₃) which had been made 20, 35, and 50 mM in ammonium (NH₄Cl). Viable cell count, cellular protein, and absorbance at 550 nm were monitored. The culture vessels were inoculated with an overnight culture grown in unsupplemented NB, and incubated at 37° C. Aliquots were removed at intervals.

A triple pour-plating technique was developed to obtain viable cell counts. The sample was appropriately diluted in sterile saline; aliquots were pipetted into molten agar (1.5 percent nutrient agar) equilibrated at 45° C in a water bath, swirled and immediately poured into plates containing a thin underlay of the same medium. To maintain anaerobiosis, a thin overlay of nutrient agar (45° C) was poured over the plates when the second layer had set. Plates were inverted and incubated at 37° C. Colonies were counted after a two-day incubation period. This technique ensures reliable counts because simple spreading techniques in which growth is aerobic or semi-aerobic results in artifactually low counts due to the catalase sensitivity of C. violaceum.
grown aerobically. Protein was determined by the method of Lowry et al., with bovine serum albumin as standard, on cells washed twice by centrifugation in 0.2 M phosphate buffer, pH 7.

Viable cell counts and absorbance at 550 mM were detectably lowered in the 50 mM culture by late-log phase (Figure 6). The 20 and 35 mM cultures behaved almost identically and did not vary significantly from control cultures (20 mM KNO₃, no added ammonium) with one exception noted below. In addition to this general depression of growth, it was also noted that the specific protein content of the cells varied from an average of around 6 μg protein/mg dry weight in the 20 and 35 mM cultures to 18 μg/mg for the 50 mM culture.

The former values are comparable to those obtained for control cultures grown without ammonium, an average of 5.3 μg/mg. The increase in the 50 mM culture is likely attributable to an increase in protein synthesis which is sometimes observed in organisms subjected to intense ammonium nutrition. The overall behavior of ammonium-grown cells differs from that of cells grown in nitrate alone. A growth curve typical of catabolite repression is observed for all cultures grown in the presence of ammonium but not for those grown in nitrate. Catabolite repression is a phenomenon in which a preferred substrate, e.g., glucose, suppresses utilization of other substrates until the preferred substrate is exhausted. At this point, a short pause in growth occurs which is followed by resumption of growth, usually at a lower rate, on the second substrate. Nitrogen sources as well as carbon sources can cause catabolite repression. Because nutrient broth is a complex organic medium, however, it is not possible to distinguish between nitrogen- and carbon-catabolite repression using NB as a basal medium.

**Minimal Medium**

For the reason discussed above, and because a chemically-defined medium that supports growth of C. violaceum will also be required for genetic studies, an effort to define this organism's minimal nutritional requirements has been initiated.

Minimal L Broth which is commonly used to cultivate E. coli contains per liter: 13.6 g KH₂PO₄, 2.0 g (NH₄)₂SO₄, 0.5 mg FeSO₄ · 7H₂O,
Figure 6. Growth of C. violaceum in the presence of nitrate and varying concentrations of ammonium.
1 mM MgSO$_4$ · 7H$_2$O, and glucose (0.2 percent). This medium, solidified with Difco purified agar (1.5 percent), was used alone and with added mixtures of growth factors. Heavy anaerobic inocula (approximately $10^8$ cells) were plated by the triple pour-plate technique. A mixture of twelve amino acids was added to four plates; a mixture of nine vitamins was added to four separate plates. Two additional sets of plates containing both supplement mixtures and neither, respectively, were also prepared. The plates were examined after incubation at 37° C for two days. No growth occurred on the unsupplemented medium. Plates containing the amino acids had very dense, small, uniform colonies. Much more limited growth occurred in the vitamin-supplemented medium, but no particular enhancement of growth by vitamins was observed on the plates containing amino acids plus vitamins.

*C. violaceum* appears to require one or more of the twelve amino acids for growth. The amino acids used were: arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine. The vitamins used were: biotin, calcium pantothenate, choline, folic acid, inositol, nicotinamide, pyridoxal, riboflavin, and thiamine-HCl. Both mixtures are commercial preparations (GIBCO) and were used at the recommended concentrations. The amino acids are being tested individually at this time.

**Virology -**

*Discovery and Analysis of an Agent in Sludge that Causes Heat Inactivation of Reovirus to Occur at Reduced Temperatures*—One of the major groups of enteric viruses excreted in the feces of infected persons belongs to the family Reoviridae. The members of this family most commonly isolated in raw sewage belong to the genus Reovirus. Although reoviruses are frequently recovered from patients with respiratory infections, gastroenteritis, or rashes, these viruses have not been proven to cause human disease.$^3$

Another member of the Reoviridae family also found in human feces is rotavirus. This group of viruses is thought to be responsible for at least 50 percent of the world's cases of enteritis among infants,$^3$ thus making rotaviruses one of the most important pathogens in sewage.
Until these viruses can be studied in the laboratory by less sophisticated techniques, however, it will probably be feasible to develop methods for inactivating rotaviruses only through the use of model viruses which can be readily grown in cell culture.

The model virus that has been chosen here for this purpose is reovirus. Although reoviruses are antigenically and morphologically distinct from rotaviruses, their physicochemical characteristics are quite similar. It is, therefore, hoped that the responses of rotavirus and reoviruses will be comparable under given sets of conditions.

Enteric viruses found in wastewater have a strong tendency to become associated with solid matter and, as a result, ultimately become components of wastewater sludge. A possible method of inactivating viruses in sludge is with heat. Because heating sludge can be a costly procedure, it is important to define conditions for effective inactivation of enteric viruses in sludge by heat which requires a minimal input of energy. For example, temperatures in excess of 60°C are maintained for a period of several days during composting of sludge, but it is not known whether these conditions are sufficient to reduce the virus load to a safe level or if additional heat is required. The complexity of this question is compounded by the finding that sludge solids are highly protective of at least one type of virus (poliovirus) against inactivation by heat, and are probably protective of viruses of other groups as well.

The virus used in the present study is the reovirus type 3 strain Dearing. Although the heat resistance of this virus was not known, it had been found to be insensitive to disinfection by ammonia at concentrations that rapidly inactivate poliovirus. If this strain of reovirus is also significantly more heat stable than poliovirus and protected by sludge solids against heat to an extent comparable to that of poliovirus, it is possible that reovirus may remain viable in sludge after extended times at quite high temperatures. Therefore, a study on the effect of wastewater sludge on the rate of heat inactivation of reovirus was carried out.
Discovery of an Agent in Sludge that Accelerates the Rate of Loss of Plaque-forming Ability of Reovirus by Heat--The initial experiment in this study was to compare the effects of phosphate buffered saline (PBS), raw sludge and anaerobically digested sludge on the rate of heat inactivation of reovirus. Buffer or sludge seeded with virus was heated at temperatures from 45° C to 60° C for 20 min and the recovery of plaque-forming units was measured after sonication of the heated samples with 0.2 percent sodium dodecyl sulfate (SDS). This procedure allowed full recovery of infectious virus from unheated samples of either PBS or sludge.

The strain of reovirus used for this experiment is quite resistant to heat inactivation in PBS because a temperature of nearly 60° C is required to reduce the plaque-forming ability of this virus by more than 3 logs during 20 min of heat treatment (Figure 7). A comparable reduction in the titer of poliovirus type-1 strain CHAT was obtained after 20 min at just 43° C. However, contrary to the results found with poliovirus, both raw and digested sludge accelerate the loss of reovirus plaque-forming units by heat. This result suggests that wastewater sludge contains a virucidal agent against reovirus that is activated by heat.

It was previously shown that the ammonia contained in sludge is virucidal against enteroviruses and its activity increases with temperature. Although it was also shown that ammonia has little or no virucidal activity against reovirus at ambient temperature (21° C), the effect of ammonia on this virus at higher temperatures had not been determined. Therefore, the recovery of reovirus after heating at 47° C in 0.5 m NH₄Cl was measured. Because the amount of reovirus inactivation under these conditions was unaffected by its presence (Table I), the agent in sludge causing loss of reovirus plaque-forming units by heat to be accelerated is not solely ammonia.

Solubilization of the Virucidal Agent--Having eliminated ammonia as this virucidal agent in sludge, it was of immediate interest to study the properties of this unidentified material. The initial concern in examining the agent was to free it from the majority of the sludge solids. The solids and liquid fractions of anaerobically digested sludge were, therefore, separated by centrifugation and individually analyzed for virucidal activity. Following the initial
Figure 7. Effect of sludge on recovery of reovirus plaque-forming units after heat treatment. Reovirus lysate was diluted 10-fold with either PBS (●), raw sludge (■) or anaerobically digested sludge (▲), heated for 20 min at the specified temperature, and assayed for plaque-forming units.
centrifugation of sludge, essentially all of the virucidal activity was retained in the solids (Table II). However, a second centrifugation of these resuspended solids caused the release of a large portion of the activity into the supernatant fraction. When the solids were again resuspended and recentrifuged, the supernatant from the second wash of these solids also contained much virucidal activity, but the activity in the solids following this third centrifugation was greatly diminished. Thus, the virucidal agent appeared to be initially associated with sludge solids but could be worked from these solids by blending with water.

**Heat Treatment in the Presence of the Virucidal Agent Causes Reovirus Particles to Break Down**—Once the new agent of sludge was freed of most solids, it became feasible to make a more detailed study of its properties. It had been shown that the agent can greatly reduce the heat required to prevent plaque formation by reovirus. However, it had not been established that reovirus is irreversibly inactivated under these conditions. It is possible that a component of sludge only temporarily blocked some steps of viral replication. In order to test this possibility, the effect of heat treatment on purified, radioactively-labeled reovirus particles was examined. For this study, labeled particles were heated for 20 min at 45°C in the combined sludge washes (see Table II), and analyzed by density gradient centrifugation. Significant alterations of the sedimentation coefficients of viral particles typically indicate that the particles have been inactivated.

The sedimentation coefficients of most 630S reovirus particles were found to be less than 200S after heat treatment with the agent (Figure 8). The same treatment without the virucidal agent or without heat caused no detectable change in the S-values of labeled particles (results not shown). From these results it appears that reovirus is irreversibly inactivated during heat treatment in the presence of the virucidal agent.

**The Activity of the Agent is Dependent on pH**—Having satisfactorily shown that the new virucidal agent of sludge causes the breakdown of reovirus at reduced temperatures, other properties of the agent were studied. Previous studies showing that ammonia is virucidal for enteroviruses established that the activity of this compound is extremely sensitive to pH because only the uncharged form of ammonia
Table I

Effect of ammonia on heat inactivation of reovirus

<table>
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<tr>
<th>Buffer Solution</th>
<th>Heat Treatment</th>
<th>Recovery of pfu</th>
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<tbody>
<tr>
<td>PBS</td>
<td>21° C, 15 min</td>
<td>5.8 x 10⁷</td>
</tr>
<tr>
<td>PBS</td>
<td>47° C, 10 min</td>
<td>5.9 x 10⁷</td>
</tr>
<tr>
<td>0.1 M Tris, pH 8.5</td>
<td>47° C, 10 min</td>
<td>2.1 x 10⁷</td>
</tr>
<tr>
<td>0.1 M Tris, pH 8.5 + 0.5 M NH₄Cl</td>
<td>47° C, 10 min</td>
<td>4.7 x 10⁷</td>
</tr>
</tbody>
</table>

Table II

Separation of virucidal agent from sludge solids

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>Recovery of Reovirus pfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>21° C, 15 min</td>
<td>2.2 x 10⁷</td>
</tr>
<tr>
<td>Digested sludge</td>
<td>&quot;</td>
<td>2.3 x 10⁷</td>
</tr>
<tr>
<td>PBS</td>
<td>45° C, 20 min</td>
<td>2.2 x 10⁷</td>
</tr>
<tr>
<td>Digested sludge</td>
<td>&quot;</td>
<td>8.0 x 10²</td>
</tr>
<tr>
<td>Sludge supernatant #1</td>
<td>&quot;</td>
<td>2.3 x 10⁶</td>
</tr>
<tr>
<td>Sludge pellet #1</td>
<td>&quot;</td>
<td>5.0 x 10²</td>
</tr>
<tr>
<td>Sludge supernatant #2</td>
<td>&quot;</td>
<td>2.0 x 10²</td>
</tr>
<tr>
<td>Sludge pellet #2</td>
<td>&quot;</td>
<td>3.2 x 10⁴</td>
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<tr>
<td>Sludge supernatant #3</td>
<td>&quot;</td>
<td>6.0 x 10²</td>
</tr>
<tr>
<td>Sludge pellet #3</td>
<td>&quot;</td>
<td>6.5 x 10⁵</td>
</tr>
</tbody>
</table>

aAnaerobically digested sludge was centrifuged at 18,000 x g for 20 min, and the pellet was resuspended by blending in a volume of water equal to that recovered in the supernatant. The resuspended pellet was again centrifuged, the pellet was resuspended as before, and the process was repeated a third time. The virucidal activity in samples of each fraction was then measured.
Figure 8. Sedimentation analysis of reovirus after heat treatment with the virugidal agent. Purified reovirus radioactively-labeled with $^3$H-uridine was diluted 10-fold with either PBS (●) or the combined #2 and #3 sludge supernatant fractions shown in Table II (■). The sample in sludge supernatant was heated at 45°C for 20 min and both samples were analyzed by centrifugation in glycerol gradients (20-40 percent glycerol in PBS, 24,000 rpm, SW 27.1 rotor, 1 hr, 4°C). Purified reovirus labeled with $^{14}$C-protein hydrolysate was added to the samples just prior to centrifugation to mark the 630S position. The arrow shows the direction of centrifugation.
is virucidal. A similar dependence on pH has been reported for other virucides, such as hypochlorous acid, a disinfectant commonly used in wastewater treatment. The antiviral activity of this new agent in sludge was, therefore, measured as a function of pH.

The material used to test the pH dependence of the agent was the combined aqueous washes of digested sludge (supernatant #2 and #3) which were shown to contain most of the virucidal activity (Table II). Aliquots of this material were buffered with 0.1 m phosphate, Tris or borate solutions and the pH adjusted to 6, 8, or 10, respectively. The virucidal activity of each sample was then determined.

The activity of the agent against reovirus is very dependent upon pH (Figure 9). The agent has very little activity at pH 6 but much greater activity at pH 8 and 10. This result suggests that the more active form of the agent exists at higher pH values.

At least one other explanation for this result must be considered, however, which is that the agent may be insoluble at low pH values and becomes inactive, not because it is intrinsically less active at pH 6, but because it precipitates out of solution at this pH. This explanation was supported by the observation that a cloudy precipitate developed in the sludge washes containing the agent when the pH was lowered to 5.4. This cloudy material could easily be removed by centrifugation, but when the pH of the supernatant was readjusted to pH 8.5, it had very little antiviral activity (Table III). Resuspension of the pellet in pH 8.5 buffer produced a relatively clean solution whose antiviral activity was comparable to that of the sludge washes before the pH was lowered. This result indicates that the agent precipitates at a pH of about 6, but resolubilizes when the pH is raised to 8.5. Thus, the loss of antiviral activity at lower pH values appears to be at least partially due to the insolubility of the agent. The pH of the sludge material was carefully controlled during all further studies on the antiviral agent.

Other Properties of the Virucidal Agent--The heat stability and volatility of the agent were next determined. Because it survives autoclaving, the agent cannot be easily inactivated with heat (Table IV). Furthermore, when the solution containing the agent is boiled dry
Figure 9. Effect of pH on the activity of the viral agent. The combined #2 and #3 sludge supernatants shown in Table II were mixed with one-twentieth volume of 2 M phosphate, Tris or borate buffer and the pH was adjusted to 6, 8, and 10, respectively. Buffered solutions containing PBS were made up in the same fashion. Reovirus lysate was diluted 10-fold into all solutions, the samples were heated for 20 min at 45°C, and each sample was assayed for recoverable plaque-forming units. Symbols: PBS (●); sludge supernatant (■).
Table III

Precipitation of virucidal agent by lowering pH to 5.4

<table>
<thead>
<tr>
<th>Sample (Treatment)</th>
<th>Recovery of Reovirus pfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (no heat)</td>
<td>$4.6 \times 10^7$</td>
</tr>
<tr>
<td>Clarified sludge washes (45° C, 20 min)</td>
<td>$4.6 \times 10^4$</td>
</tr>
<tr>
<td>pH 5.4 supernatant (45° C, 20 min)</td>
<td>$1.8 \times 10^7$</td>
</tr>
<tr>
<td>pH 5.4 pellet (45° C, 20 min)</td>
<td>$3.0 \times 10^4$</td>
</tr>
</tbody>
</table>

*The combined washes of anaerobically digested sludge were clarified by centrifugation (80,000 x g, 2 hrs) at pH 9.2 and brought to a pH of 5.4. The precipitation that formed was pelleted at 18,000 x g, 20 min and resuspended in saline solution containing 10 mM Tris. The virucidal activity of each sample was then determined at pH 8.5.

Table IV

Recovery of virucidal activity in autoclaved or boiled-dry samples

<table>
<thead>
<tr>
<th>Sample (Treatment)</th>
<th>Recovery of Reovirus pfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS (no heat)</td>
<td>$5.4 \times 10^7$</td>
</tr>
<tr>
<td>Clarified sludge washes (45° C, 20 min)</td>
<td>$2.8 \times 10^4$</td>
</tr>
<tr>
<td>Autoclaved sludge washes (45° C, 20 min)</td>
<td>$2.7 \times 10^4$</td>
</tr>
<tr>
<td>Boiled dry sludge washes (45° C, 20 min)</td>
<td>$2.9 \times 10^4$</td>
</tr>
</tbody>
</table>

*The combined washes of digested sludge were clarified by centrifugation (54,000 x g, 1.5 hrs) at pH 10.1 and either autoclaved (121° C, 20 min) or boiled dry and resuspended in 0.01 M Tris. The virucidal activity of each sample was determined at pH 8.5.
leaving the residue badly charred, the agent is still recoverable and active. Therefore, it is not volatile but extremely heat stable.

Studies on the Heat Inactivation Rates of Reovirus in the Presence of the Virucidal Agent--It appears that the new antiviral agent of sludge functions by causing the heat resistance of reovirus to be reduced. However, because no studies had been performed which showed the amount of inactivation occurring as a function of time, it was unclear to what extent the agent modified the inactivation rate of reovirus at any temperature. Likewise, it was not known whether the agent causes reovirus to be sensitive to heat at temperatures below 45° C or, on the other hand, whether a threshold temperature is reached below which the agent does not accelerate the rate of heat inactivation of this virus.

At 45° C, the agent caused heat inactivation of reovirus to occur rapidly during the first 10 min and then more slowly during at least the next 110 min (Figure 10). A similar observation was made at 40° C except that the time range was expanded. At 35° C the rate of heat inactivation of reovirus in the presence of the agent occurred much more slowly. It should be noted that simply mixing reovirus with Tris buffer at pH 8.5 caused a reduction in titer of 20 to 50 percent (results not shown), while no loss was observed under these same conditions in the presence of the virucidal agent. After the initial loss of titer in Tris buffer, however, little or no additional loss occurred during 2 hrs at 45° C, 3 days at 40° C, or 24 hrs at 35° C (the maximal times studied). This result indicates that even at 35° C, the agent greatly accelerated the rate of heat inactivation of reovirus. Therefore, it appears that the agent causes the rate of heat inactivation of reovirus to increase at quite low temperatures and to occur in a bimodal fashion. Further studies on the properties and the nature of the agent are in progress.

Summary--In conclusion, an agent has been found in sludge that causes heat inactivation of reovirus to occur at much lower temperatures than is normally required. This agent is originally associated with sludge solids but can be washed from these solids by blending with water. The activity of the agent is greatly reduced at low pH, probably because it precipitates out of solution. The agent is both
Figure 10. Inactivation of reovirus by the virucidal agent as a function of time and temperature. Reovirus was diluted 10-fold into the combined #2 and #3 sludge supernatants (Table II) which had been adjusted to a pH of 8.5 in 10 mM Tris. The samples were heated at the temperature and for the time specified along with control samples not containing sludge supernatant. All samples were then assayed for plaque-forming units. Symbols: $45^\circ$ C (■); $40^\circ$ C (▲); $35^\circ$ C (●).
heat stable and nonvolatile. Finally, it was shown that the rate of heat inactivation of reovirus by the agent occurs in a bimodal manner and is relatively rapid, even at 35° C.

Physical-Chemical Properties of Sewage Sludge

**Sludge Filterability Studies**

A large fraction of sewage sludge dewatering costs (approximately 40 percent) is attributed to chemical additives (ferric chloride, lime, alum, or polyelectrolytes) which are used to facilitate solids separation. Recent studies have shown that gamma radiation treatment (1 Mrad) caused half a log reduction in the specific resistance of sewage sludge. The combined effects of gamma radiation and a polyacrylamide polymer were studied as a possible means of defraying some of the chemical costs in the dewatering process.

The results of this study are shown in Figure 11. The polymer concentration providing the optimum decrease in specific resistance is 0.03 percent. To obtain a similar reduction with irradiated sludge (1 Mrad), the polymer concentration must be 0.06 percent. Even though the polymer concentration is doubled, the time to dewater the irradiated sludge is halved. Therefore, the combination of polymer additive plus gamma radiation is a more efficient method of conditioning the sludge prior to dewatering. There is some interaction between the polymer and the radiation, however, it shows no useful synergistic effects, does not defray any of the chemical costs in the dewatering process, and is not great enough to be of any economic importance.

Other Activities

**Facilities Considerations**

Sandia Irradiator for Dried Sewage Sludge--The proposed Sandia Irradiator for Dried Sewage Sludge (SIDSS) has been designed at the conceptual level. The irradiator will irradiate up to 20 tons per day of dry sewage sludge. The two major mechanical components of the irradiator are the bracket conveyor system for transporting the sludge past the gamma source plaque, and the mechanism to retract the source.
Figure 11. Specific resistance curves with polymer conditioning and radiation + polymer conditioning.
plaque back into a shielded compartment so that the conveyor could be safely examined. The bucket conveyor system has been shipped from England and should arrive by May 15. Both NUS Corporation and Molzen-Corbin and Associates have provided construction cost estimates for the irradiator, exclusive of gamma source and conveyor. The costs agreed within 10 percent and were about 340 K$.

These cost estimates have been submitted to ERDA/DNRA for funding of the project. The funding has tentatively been approved by DNRA, and specifications for the architectural and engineering design are being prepared for an A&E design contract.

This irradiator will be a test bed for the mechanical components as well as other design concepts. Source use efficiencies will be measured and compared with theoretical values.

This irradiator is a pilot for a 1.5 M$ compost irradiator suggested for the Washington, DC, area. Design of the Washington, DC, facility should begin in January 1978, shortly before full scale operation of the Sandia irradiator.

**Sludge Drying System**—Sludge drying beds that utilize solar heating have been constructed in Area III to dry sludge for animal feeding and fertilizer studies at New Mexico State University. During this quarter, two loads of liquid raw sludge (5 to 8 percent solids) have been dried in the covered drying beds. Approximately 500 pounds of dried raw sewage sludge have been irradiated to 1 Mrad and shipped to NMSU. During the spring, approximately six weeks were required to dry a full load of sludge.

A centrifuge capable of dewatering up to 10 gallons per minute of sludge from 5 percent liquid into 20 percent solids sludge cake has been on order for over seven months. The scheduled delivery date was the end of March, but has slipped several weeks. The centrifuge will be used to produce sludge cake which can be final dried to greater than 80 percent solids in the drying beds. In this manner, ton quantities of dried raw sewage sludge can be produced over a time period of several weeks. More than 20 weeks would be required to dry this quantity of sludge without the centrifuge.
Other Facilities--The 12 pins of cobalt-60 stored in the SERF reactor have been reconfigured into a cylindrical array. Preliminary dosimetry indicates that the 1.1 MCi gamma source in this configuration provides a dose rate of approximately 6 Krad/sec over a cylindrical source volume 16 inches high with a diameter of 12 inches. The dose rate is quite uniform over most of the volume.

Cost/Benefit Analysis -

The purpose of the cost/benefit document that is being prepared for Sandia's proposed sludge irradiation processes has been changed at ERDA's request. The study as originally proposed was to document the unit process costs of wet sludge irradiation, thermoradiation, and heat pasteurization, and dry sludge irradiation. The scope of the document has been enlarged to include cesium-137 availability, general marketing strategy, market penetration studies, and other items designed to make the document a major policy study.

Because of the different directions of the study, Richard Stone of Arthur D. Little, Inc., has been contacted to provide guidance with the policy sections of the document. PNL is still providing the other unit process costs.

Food Irradiation Program -

The major activity in food irradiation was in support of the Intergovernmental Committee's effort to bring the papaya petition to the point where it could be presented formally to the Food and Drug Administration for approval. This subcommittee was chaired by Ari Brynjolfsson of the U. S. Army Natick Labs and was supported by DNRA and DBER, ERDA; Animal and Plant Health Inspection Service/USDA, Dept. of Commerce, Bureau of Standards; and experts from the Universities of Florida and Hawaii. The animal feeding studies results from Industrial Biotest were evaluated for deficiencies and research required was identified. A preliminary meeting with FDA is planned for May 1977.

The Red Meats Panel and the Poultry Panel to determine the problems and needs for radiation processing were also supported. The first major working sessions by the panel members are scheduled for May 1977.
Animal Experimentation -

The digestibility trials with sheep and cattle fed pelleted, fibrous diets that were described in the last report have been completed and the data are summarized in Tables V and VI. The results confirm earlier assessments of nutritive energy and nitrogen in irradiated undigested (raw) sewage solids from Albuquerque, New Mexico, which have indicated nutritive value in sewage solids worth at least half the value of cottonseed meal, a typical supplemental feed for ruminants which currently is priced at $180-225 per ton at major markets in the USA.

Chemical analysis of blood, livers and kidneys from lambs fed diets with and without sewage solids (TRUSC) for 29 days or 69 days and subsequently finished for slaughter with a conventional diet (for 104 days or 61 days) have been completed. The data are summarized in Tables VII, VIII, and IX. The results show that feeding of sewage solids at levels up to 30 percent of the diet did not significantly affect the levels of Ca, P, Na, K, Mg, Ag, Cd, Co, Cr, Cu, Hg, Mn, Pb, or Zn in whole blood sampled after 29 or 69 days on diets containing sewage solids. Samples of livers and kidneys taken at slaughter after 61 or 104 days on the conventional diet following 69 or 29 days on 30 percent sewage solids (TRUSC) showed no measurable increase (over samples from experimental controls) in contents of Ca, P, Na, K, Fe, Cu, Mg, Mn, Zn, Co, or Cr. The content of Ag in livers was slightly greater for lambs previously fed 30 percent TRUSC (1182 ± 146 ppb) than for the "controls" (950 ± 119 ppb), and liver contents of Pb were likewise slightly increased (285 ± 66 ppb) for "experimentals" vs 226 ± 44 ppb for "controls"; but these differences were not statistically significant (P > .05). Hg in livers of "experimentals" (31 ± 10 ppb) was greater (P < .05) than for "controls" (< 14 ppb), and likewise Pb in kidneys of "experimentals" (281 ± 24 and 226 ± 35 ppb) was greater (P < .05) than for "controls" (148 ± 13 ppb). Although these differences in levels of Hg in livers and Pb in kidneys are statistically significant, they reflect extremely small amounts of these elements, and thus are regarded as having questionable biological importance.
Table V

Digestibility of energy and nitrogen in fibrous diets for lambs as affected by cottonseed meal (CSM) or irradiated undigested sewage solids (TRUSC)\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Items</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASAL</td>
</tr>
<tr>
<td>Lambs per group</td>
<td>4</td>
</tr>
<tr>
<td>Energy intake, Kcal/(Kg BW)\textsuperscript{.75}</td>
<td>$375 \pm 76^{\text{c}}$</td>
</tr>
<tr>
<td>Energy digested, Kcal/(Kg BW)\textsuperscript{.75}</td>
<td>$191 \pm 57$</td>
</tr>
<tr>
<td>Energy digestibility, percent</td>
<td>$50 \pm 6$</td>
</tr>
<tr>
<td>Nitrogen intake, g/(Kg BW)\textsuperscript{.75}</td>
<td>$1.69 \pm .34$</td>
</tr>
<tr>
<td>Nitrogen digested, g/(Kg BW)\textsuperscript{.75}</td>
<td>$.68 \pm .24$</td>
</tr>
<tr>
<td>Nitrogen digestibility, percent</td>
<td>$39 \pm 7$</td>
</tr>
</tbody>
</table>

\textsuperscript{a}NMSU experiments, summer 1976.

\textsuperscript{b}TRUSC represents undigested sewage solids from Albuquerque, NM, treated by irradiation at Sandia Laboratories, Albuquerque, NM.

\textsuperscript{c}Values shown are means ± standard deviations.
Table VI

Digestibility of energy and nitrogen in fibrous diets for cattle as affected by cottonseed meal (CSM) or irradiated undigested sewage solids (TRUSC)\(^a,b\)

<table>
<thead>
<tr>
<th>Items</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASAL</td>
</tr>
<tr>
<td>Steers per group</td>
<td>4</td>
</tr>
<tr>
<td>Energy intake, Kcal/(Kg BW)(^{75})</td>
<td>280 ± 9(^c)</td>
</tr>
<tr>
<td>Energy digested, Kcal/(Kg BW)(^{75})</td>
<td>127 ± 30</td>
</tr>
<tr>
<td>Energy digestibility, percent</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Nitrogen intake, g/(Kg BW)(^{75})</td>
<td>1.31 ± .05</td>
</tr>
<tr>
<td>Nitrogen digested, g/(Kg BW)(^{75})</td>
<td>.46 ± .20</td>
</tr>
<tr>
<td>Nitrogen digestibility, percent</td>
<td>35 ± 15</td>
</tr>
</tbody>
</table>

\(^a\) NMSU experiments, summer 1976.

\(^b\) TRUSC represents undigested sewage solids from Albuquerque, NM, treated by irradiation at Sandia Laboratories, Albuquerque, NM.

\(^c\) Values shown are means ± standard deviations.
Table VII

Elemental composition of whole blood in lambs as affected by diets with and without sewage solids\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Elements</th>
<th>BASAL</th>
<th>5% CSM\textsuperscript{c}</th>
<th>10% CSM</th>
<th>15% CSM</th>
<th>10% TRUSC\textsuperscript{c}</th>
<th>20% TRUSC</th>
<th>30% TRUSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/100 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>6.2 ± 2.7</td>
<td>4.3 ± 0.6</td>
<td>4.1 ± 0.6</td>
<td>3.7 ± 1.1</td>
<td>4.3 ± 0.9</td>
<td>4.8 ± 0.8</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>P</td>
<td>17.9 ± 1.5</td>
<td>18.4 ± 1.6</td>
<td>19.4 ± 1.7</td>
<td>22.1 ± 1.8</td>
<td>20.1 ± 1.3</td>
<td>19.2 ± 2.2</td>
<td>18.4 ± 1.0</td>
</tr>
<tr>
<td>Na</td>
<td>201 ± 6</td>
<td>203 ± 2</td>
<td>203 ± 2</td>
<td>207 ± 1</td>
<td>204 ± 2</td>
<td>206 ± 5</td>
<td>208 ± 5</td>
</tr>
<tr>
<td>K</td>
<td>44 ± 11</td>
<td>31 ± 4</td>
<td>35 ± 2</td>
<td>37 ± 6</td>
<td>31 ± 3</td>
<td>36 ± 3</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Fe</td>
<td>38 ± 4</td>
<td>38 ± 5</td>
<td>38 ± 2</td>
<td>34 ± 5</td>
<td>45 ± 12</td>
<td>38 ± 5</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Mg</td>
<td>2.5 ± 3</td>
<td>2.4 ± 3</td>
<td>2.5 ± 1</td>
<td>2.5 ± 2</td>
<td>2.3 ± 2</td>
<td>2.6 ± 1</td>
<td>2.5 ± 1</td>
</tr>
</tbody>
</table>

\[ \mu g/100 \text{ml} \]

<table>
<thead>
<tr>
<th>Elements</th>
<th>&lt;3.6*</th>
<th>&lt;2*</th>
<th>&lt;1.4*</th>
<th>&lt;7.8*</th>
<th>&lt;4*</th>
<th>&lt;4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Cr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>93 ± 9</td>
<td>102 ± 8</td>
<td>94 ± 16</td>
<td>102 ± 6</td>
<td>98 ± 0</td>
<td>115 ± 13</td>
</tr>
<tr>
<td>Hg</td>
<td>65 ± 13</td>
<td>54 ± 18</td>
<td>70 ± 16</td>
<td>45 ± 7</td>
<td>75 ± 13</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>Mn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>24 ± 2</td>
<td>23 ± 5</td>
<td>22 ± 1</td>
<td>23 ± 0</td>
<td>29 ± 3</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Zn</td>
<td>572 ± 183</td>
<td>522 ± 143</td>
<td>531 ± 135</td>
<td>480 ± 122</td>
<td>535 ± 155</td>
<td>645 ± 75</td>
</tr>
</tbody>
</table>

\textsuperscript{a}NMSU experiments, summer 1976; diets had been fed for 29 days; samples were wet-ashed and analyzed by atomic absorption spectrophotometry.

\textsuperscript{b}Values shown are means ± standard deviations representing 4 lambs for each group.

\textsuperscript{c}CSM is cottonseed meal; TRUSC is irradiated undigested sewage solids from Albuquerque, NM.

\*Values represent limits of detection under analytical conditions used.
Table VIII

Elemental composition of whole blood in lambs as affected by diets with cottonseed meal (CSM) or irradiated sewage solids (TRUSC)a,b

<table>
<thead>
<tr>
<th>Elements</th>
<th>15% CSM</th>
<th>30% TRUSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/100 ml</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>5.7 ± .6</td>
<td>5.6 ± 1.3</td>
</tr>
<tr>
<td>Na</td>
<td>260 ± 30</td>
<td>265 ± 10</td>
</tr>
<tr>
<td>K</td>
<td>37 ± 10</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>Fe</td>
<td>45 ± 9</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>Mg</td>
<td>1.2 ± .3</td>
<td>1.2 ± .4</td>
</tr>
<tr>
<td></td>
<td>μg/100 ml</td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>all &lt;3.6*</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>6.0 ± 1.4</td>
<td>6.2 ± .7</td>
</tr>
<tr>
<td>Co</td>
<td>all &lt;1.4*</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>all &lt;7.8*</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>164 ± 7</td>
<td>162 ± 8</td>
</tr>
<tr>
<td>Hg</td>
<td>48 ± 20</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>Mn</td>
<td>all &lt;4*</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>49 ± 4</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Zn</td>
<td>259 ± 17</td>
<td>245 ± 40</td>
</tr>
</tbody>
</table>

aNMSU experiments, summer 1976; diets had been fed for 69 days; samples were wet-ashed and analyzed by atomic absorption spectrophotometry.

bValues shown are means ± standard deviations representing 6 lambs per group.

*Values represent limits of detection under analytical conditions used.
Table IX

Elemental composition of livers and kidneys from lambs fed diets with and without irradiated undigested sewage solids (TRUSC)\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Dietary Regimen</th>
<th>Livers</th>
<th>Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASAL, 69 days</td>
<td>30% TRUSC, 69 days</td>
<td>BASAL, 69 days</td>
</tr>
<tr>
<td>Conventional diet, 61 days</td>
<td>Conventional diet, 61 days</td>
<td>Conventional diet, 104 days</td>
</tr>
<tr>
<td>Number of lambs</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Elements</td>
<td>mg/Kg (ppm), fresh tissue</td>
<td>mg/Kg (ppm), fresh tissue</td>
</tr>
<tr>
<td>Ca</td>
<td>52 ± 14</td>
<td>58 ± 4</td>
</tr>
<tr>
<td>P</td>
<td>3824 ± 236</td>
<td>3646 ± 128</td>
</tr>
<tr>
<td>Na</td>
<td>783 ± 42</td>
<td>748 ± 57</td>
</tr>
<tr>
<td>K</td>
<td>2819 ± 174</td>
<td>2679 ± 138</td>
</tr>
<tr>
<td>Fe</td>
<td>63 ± 10</td>
<td>87 ± 13</td>
</tr>
<tr>
<td>Cu</td>
<td>95 ± 18</td>
<td>93 ± 22</td>
</tr>
<tr>
<td>Mg</td>
<td>183 ± 9</td>
<td>177 ± 1</td>
</tr>
<tr>
<td>Mn</td>
<td>3.1 ± .4</td>
<td>3.0 ± .3</td>
</tr>
<tr>
<td>Zn</td>
<td>35 ± 2</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Ag</td>
<td>950 ± 119</td>
<td>1182 ± 146</td>
</tr>
<tr>
<td>Cd</td>
<td>78 ± 34</td>
<td>90 ± 13</td>
</tr>
<tr>
<td>Co</td>
<td>&lt;50*</td>
<td>&lt;50*</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;60*</td>
<td>&lt;60*</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt;14*</td>
<td>31 ± 10\textsuperscript{d}</td>
</tr>
<tr>
<td>Pb</td>
<td>226 ± 44</td>
<td>285 ± 66</td>
</tr>
</tbody>
</table>

\textsuperscript{a}NMSU experiments, summer 1976; samples were wet-ashed and analyzed by atomic absorption spectrophotometry.

\textsuperscript{b}Values shown are means ± standard deviations.

\textsuperscript{c,d}Values with different superscript letters are significantly different (P < .05).

*Values represent limits of detection under analytical conditions used.
These results suggest that feeding of sewage solids during an initial phase in the feeding program of growing-finishing ruminants poses little, if any, hazard to meat products from accumulation of the heavy metals and trace elements assayed to date in these studies.

A series of three experiments with sheep fed diets with cottonseed meal or sewage solids (TRUSC) was conducted to determine whether addition of sodium silicate to the drinking water would affect absorption and retention of heavy metals and trace elements. Data are complete for the first of these and the results show that silicate at about 500 ppm (calculated as SiO₂) failed to appreciably affect the apparent absorption or retention of heavy metals and trace elements, as measured in terms of intakes minus fecal and urinary excretions.

**Agronomic Experimentation**

Hydraulic probe (truck mounted) soil sample cores were taken at Clovis, New Mexico, from fields that have had 41 years of sewage effluent application as the only source of irrigation water and an adjacent soil where no sewage has been used. These soil samples are being prepared for chemical analysis for a broad range of parameters as well as heavy metals for comparison between treated and untreated.

Eight barrels of soil were collected from 0 - 6" soil depth (four barrels from 40-year sewage effluent treated field and four from untreated field) for use in greenhouse experimentation on a variety of crops. Greenhouse experiments are being set up at the present time.

A series of sewage effluent samples were collected over a 24-hr period from the effluent treated fields main irrigation ditch to measure inputs of nutrients, etc., at the field location. Chemical analysis is proceeding on these samples.

In addition, samples of winter wheat tissues were taken from treated and untreated fields for comparison of tissue contents of elements. These samples have been dried, ground, and are ready for chemical analysis.
The chemical analysis of the corn and sorghum tissue collected in the fall, 1975, from Clovis from treated and untreated soils is almost complete.

III. Separation Technology and Source Development Program

Source efficiency calculations on cesium aluminosilicate compositions have been completed and compared with CsCl for source radii up to 3 cm. In addition, source efficiency for calcined full waste has been determined. A multiple source concept has been explored to determine the number of ceramic sources required to give equivalent gamma flux. Although 2.5 times as many ceramic sources are required as CsCl sources, the total $^{137}$Cs required is 0.82 of that in the CsCl sources.

Hot-pressed pellets near the pollucite composition $(\text{Cs}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2)$ and with a composition $\text{Cs}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 6.6\text{SiO}_2$ show satisfactory performance in leach testing and vaporization studies. X-ray diffraction patterns for these materials index with the pollucite structure.

Source Development

Three samples, given in Table X, representing the range of candidate material compositions and preparation methods were selected for more detailed characterization.

The one-inch diameter samples were vacuum hot pressed at 1250°C and 6.9 MPa (1000 psi) for 10 minutes. Powders for samples 263 and 275 were prepared by the gel method with target cesium contents of 38.9 and 35.7 wt percent, respectively. Each sample varies from the design concentration; sample 263 considerably. Departure of the cesium content from the design values for all materials prepared by the gel method was observed. Except for 275, all the cesium contents were higher than intended. The reason for the variation is not known at present. Sample 263 is polycrystalline and opaque in appearance, while sample 275 appears glassy and is translucent in thin slices.
Sample 273 was prepared by the clay method using a montmorillonite (MCBP) clay. Assuming the formation of pollucite \((\text{Cs}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2)\), the cesium capacity of the montmorillonite is determined by the \text{SiO}_2 content, in this case, 39.6 wt percent. The theoretical and actual capacities are in good agreement. Note also that the clay derived material is considerably more dense than the gel prepared samples. The \text{Cs}_2\text{O}/MCBP is black with a green core due to reduction of the \text{Fe}^{+3} and \text{Ti}^{+4} impurities during hot pressing. The sample is opaque.

The characterization studies included microstructure examination, x-ray diffraction, vaporization behavior and leach testing (in progress).

**Microstructure.** The samples were mounted in epoxy and polished using standard techniques. The samples were etched for 20 s in HF vapors and examined in an optical microscope. Sample 263 showed a well-developed, polycrystalline structure with considerable porosity in the grain boundaries (~10 vol percent). The grain size varied from several microns to 300 - 400 microns in diameter.

Sample 275 contained much less porosity than 263. Pores were uniformly dispersed throughout the matrix. The microstructure was very poorly resolved; however, a very fine (1 - 5 \(\mu\text{m}\)) discontinuous phase appears to be dispersed in a featureless matrix.

Very little porosity was observed in 273 which is consistent with its high density. The microstructural features are similar to 275 but more coarse in appearance. At low magnifications the sample has a distinct mottled look which is apparently associated with the coalescence of the finely divided phase into a continuous network.

**X-ray diffraction.** Pieces of each material were finely ground and x-ray diffractograms obtained using the Debye-Scherrer method. The same phase was present in each material and indexes to the pollucite \((\text{Cs}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2)\) structure.

**Vaporization Studies.** Vaporization studies have been performed. The studies were intended to identify two parameters: (1) the temperature for first appearance of Cs, and (2) the weight loss
during extended heat treatment at elevated temperatures. Both series of experiments were performed on crushed samples heated in vacuum. A mass spectrometer was used to detect first appearance of Cs as temperature was increased stepwise. TGA apparatus was used to measure the weight loss. The results are shown in Table XI.

The Cs₂O/montmorillonite sample exhibited the poorest vaporization behavior. The appearance of Cs⁺ was at the lowest temperature, and the total mass lost was the highest of the three samples. Sample 263 was the next poorest performer, however, there is reason to believe that its long-term behavior would be significantly improved over these initial results. The cesium content of this sample is approximately 1 percent greater than the stoichiometric pollucite, i.e., 43.6 percent vs 42.6 percent, respectively. The vaporization studies suggest that the excess Cs₂O exists in an uncombined state and is easily vaporized. Once the excess Cs₂O is gone, virtually no additional weight loss occurs after several hours at 1100° C.

Sample 275 exhibited excellent vaporization behavior.

Leach studies. Leaching experiments on bulk samples in a Soxhlet apparatus are under way. While the tests are not completed, the relative behavior appears to be the same as for the vaporization studies.

Source efficiency calculations. Source efficiency calculations were performed for the aluminosilicate ceramic source candidates with the generalized formulation Cs₂O · Al₂O₃ · nSiO₂ described above.

Previously, source efficiency calculations were made for pure Cs₂O · Al₂O₃ · 4SiO₂ (corresponding to the natural mineral pollucite). Additional calculations have now been made for this composition adjusted for the 5 percent impurities (Mg, Fe, K, Na, Ca, and Ti) contained in material synthesized with montmorillonite as well as for the n = 6.6 material. The results are compared in Figure 12 with those for pure "pollucite" CsCl and cesium niobate (CsN). The impure pollucite curve lies so close to the pure pollucite curve that they
### Table X
Representative cesium aluminosilicate candidate materials

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>Cesium Content wt percent</th>
<th>Density g/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>263</td>
<td>Cs₂O · Al₂O₃ · 3.8SiO₂</td>
<td>43.6</td>
<td>2.89</td>
</tr>
<tr>
<td>275</td>
<td>Cs₂O · Al₂O₃ · 6.6SiO₂</td>
<td>34.0</td>
<td>2.95</td>
</tr>
<tr>
<td>273</td>
<td>Cs₂O/montmorillonite</td>
<td>39.5</td>
<td>3.17</td>
</tr>
</tbody>
</table>

### Table XI
Results of vaporization studies on candidate cesium aluminosilicates

<table>
<thead>
<tr>
<th>No.</th>
<th>Appearance of Cs⁺ T° C</th>
<th>Mass Loss wt percent</th>
<th>T° C</th>
</tr>
</thead>
<tbody>
<tr>
<td>263</td>
<td>900</td>
<td>1.0</td>
<td>1100</td>
</tr>
<tr>
<td>275</td>
<td>1100</td>
<td>0.3</td>
<td>1200</td>
</tr>
<tr>
<td>273</td>
<td>660</td>
<td>3.8</td>
<td>1100</td>
</tr>
</tbody>
</table>
are indistinguishable, and the one curve labeled "pollucite" represents both. The n = 6.6 material is shown as the curve designated as "6.6" and is lower than pollucite primarily because of decreased cesium content (40 percent for n = 4 and 34.8 percent for n = 6.6). In each of these cases, it was assumed that $^{137}$Cs comprises 30 w/o of the total cesium concentration with the remainder being inactive cesium, thereby approximating the real case.

Figure 12 also shows the calculated efficiency for a source containing calcined waste, i.e., 3 year old spent fuel that has been subjected to the Purex process to remove U and TRU, and subsequently, dried, fired, and compressed to a density of 4.8 g/cm$^3$. Prior to the drying step, it was assumed that the Gd content was doubled by Gd addition, as per the latest specification of the Barnwell re-processing plant. The average gamma-ray energy/intensity and the average mass absorption coefficient were obtained by averaging the sum of the weighted values of all contributing isotopes.* In the case of the gamma ray energy/intensity, this included decay-scheme considerations.

Figure 12 describes the gamma ray intensity at a surface point on an infinitely long cylindrical source of the various materials as a function of source radius. Using Figure 12, source materials can be compared in two ways: 1) single source equivalence, i.e., source diameter required for a material to provide the same MeV/min as a 2.54-cm diameter CsCl source, and 2) multiple source equivalence, i.e., the number of 2.54-cm diameter sources of a material required to yield the same MeV/min as a 2.54-cm diameter CsCl source. The dashed vertical and horizontal lines facilitate this analysis. The vertical line is at 1.27-cm radius. For the single source comparison, the various source radii are obtained by reading the value where the various curves are intersected by the horizontal dashed line. In the

*The composition of the waste in terms of both active and non-active isotopes was obtained from the ORIGEN Code with the following conditions: Burnup Phase 3.3 percent $^{235}$U, Power 26.47 MW, Burnup 27000 MWD, Flux $2.53 \times 10^{13}$ n/cm$^2$/sec, courtesy of S. A. Dupree, 5231, Sandia Laboratories. The primary radioisotopes at 3 years are: $^{106}$Ru, $^{106}$Rh, $^{134/137}$Cs, $^{144}$Ce, $^{144}$Pr, $^{154}$Eu.
Figure 12. Apparent surface activity of various source materials as function of source radius.
multiple source comparison, the number of sources required of a
given material is obtained by dividing the ordinate values of the
CsCl by the ordinate values of the various sources at the inter­
section of the source curves and the dashed vertical line. Results
are shown in Table XII and may differ slightly from previous data
due to density variations in the source formulations.

It is interesting to note that it requires ca. 50 calcine
sources (2.54-cm diameter) to be equivalent to one 2.54-cm diameter
CsCl source. The diameter of a single calcine source required to
replace a single 2.54-cm diameter CsCl source cannot be determined
presently, but must be >10 cm. It appears unlikely that un­
reprocessed fuel elements with high-Z uranium and transuranics would
be appreciably better than a calcine source in spite of the extra
gadolinium and dilution in the calcine. Therefore, it appears that
direct use of fuel rods or calcine may not be feasible from the
source efficiency standpoint.

Table XII
Comparison of Source Materials

<table>
<thead>
<tr>
<th>Source</th>
<th>Single Source Radius (cm)</th>
<th>Ci/C CsCl</th>
<th>Number of Sources*</th>
<th>Ci/C CsCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsCl</td>
<td>1.27</td>
<td>1.00</td>
<td>1.0</td>
<td>1.00</td>
</tr>
<tr>
<td>CsN</td>
<td>2.1</td>
<td>1.16</td>
<td>2.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Pollucite</td>
<td>2.0</td>
<td>1.09**</td>
<td>2.6</td>
<td>0.86</td>
</tr>
<tr>
<td>Cs₂O₃ · Al₂O₃ · 6.6SiO₂</td>
<td>2.2</td>
<td>0.98**</td>
<td>2.5</td>
<td>0.82</td>
</tr>
<tr>
<td>Calcine</td>
<td>---</td>
<td>---</td>
<td>47</td>
<td>---</td>
</tr>
</tbody>
</table>

*1.27 cm source radii
**density 2.9 g/cm³
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