APPLIED MICROBIOLOGY, Dec. 1969, p. 994-1001
Vol. 18, No. 6

Survival of Human Pathogens in Composted Sewage

B. BEAUFORD WILEY AND STEPHEN C. WESTERBERG
Department of Microbiology, College of Medicine, University of Utah, Salt Lake City, Utah 84112

Received for publication 14 July 1969

Studies were conducted to assess the effectiveness of an aerobic composter in destroying pathogens that may possibly be present in raw sewage sludge. Experiments conducted in this study were designed to determine whether or not selected indicator organisms (i.e., *Salmonella newport*, poliovirus type 1, *Ascaris lumbricoides* ova, and *Candida albicans*) could survive the composting process. The results of the assay showed that after 43 hr of composting, no viable indicator organisms could be detected. The poliovirus type I was the most sensitive, being inactivated within the first hour, whereas *C. albicans* was the most resistant, requiring more than 28 hr of composting for its inactivation. The data from this study indicated that aerobic composting of sewage sludge would destroy the indicator pathogens when a temperature of 60 to 70 C is maintained for a period of 3 days.

Nearly all American towns and cities with populations in excess of 2,500 have sewage disposal plants. It has been estimated that 36.5 lb of wet sewage solids (sludge) are produced per capita per year (8). Therefore, in one year a town with a population of 2,500 would produce in excess of 90,000 lb of sewage solids. Means employed for final disposal of sewage solids (sludge) present a problem to every city or community. Three means generally used for disposing of digested sewage sludge are (i) discharging it into deep water of a lake, sea or ocean, (ii) utilizing it as a fertilizer by spreading it upon farm land, and (iii) using it for making land fills. All three methods produce hazards to public health (8).

If sewage sludge could be processed in such a way as to yield a product that could be sold as a safe odor-free fertilizer, then part of the cost of sewage treatment and disposal could be deferred. Composting of human organic waste offers such a possibility.

To establish whether composting would destroy any pathogens that may be present in the sludge used as a raw material, a composting process utilizing aerobic conditions without added heat was used. An apparatus designed to carry out this process was obtained from the Eimco Corp., Salt Lake City, Utah. For this work, pathogens representing each of four kinds of organisms commonly found in the human intestinal tract (i.e., a bacterium, a virus, a fungus, and ova of a metazoan parasite) were chosen as an indicator of the behavior of each kind of organism during the composting process.

MATERIALS AND METHODS

Salmonella newport (University of Utah strain U-225) was chosen as the bacterial indicator since *Salmonellae* appear to be commonly present in sewage and are among the most important pathogens found there. Poliovirus type 1 (Sabin vaccine strain from Eli Lilly & Co., Indianapolis, Ind.) was used as the indicator virus since poliovirus is also often present in sewage (9) and can be propagated with relative ease on a continuous cell line such as human amnion cells (FL). Ova of the helminth *Ascaris lumbricoides* were used as an indicator of ova destruction. The ova of this organism are commonly found in sewage and were found to be the most resistant of any ova or cysts studied by Cram (2). For the fungal organism, *Candida albicans* (University of Utah strain U-250) was chosen. This organism is often part of the flora of the intestinal tract of man and has been frequently isolated from sewage. Although this organism is common on the mucous membranes and in feces in a high percentage of healthy individuals, it can be the cause of pathogenic processes in these habitats (7).

The research was conducted in three phases. The first phase was a study of the thermal death times and thermal death points for each of the indicator organisms. This was conducted to predict the possible effect the composting process would have on each of the organisms. The second phase was concerned with testing the techniques that would be used to assay the final compost for the presence of the indicator organisms and also to determine the size of inoculum needed to assure detection of them. The last phase concerned the inoculation of the composter and assay of the final product for the presence of the indicator organisms that may have survived the process.

Thermal death time for S. newport. A temperature of 60 C was chosen for this study since this was the lowest temperature measured in the composter during

its operation. Six tubes (13 by 100 mm) containing 1.9×10^9 *S. newport* organisms suspended in 3 ml of Nutrient Broth (Difco) were placed in a water bath at 60 C. At 10-min intervals, a tube was removed and 10-fold dilutions in nutrient broth were made. All the dilution tubes were kept chilled in an ice bath until pour plates could be prepared for counting. All salmonella plate counts in this research were made after a 3-day incubation period at 37 C. A Quebec Colony Counter (Spencer Lens Co., Buffalo, N.Y.) was utilized for counting the plates.

Thermal death point for S. newport. Nutrient broth cultures containing 2.6×10^9 organisms each were held for 30 min in water baths at temperatures varying in 5 C increments from 35 to 65 C. Dilutions and plating were carried out as in the thermal death time procedure.

Thermal death time for poliovirus type 1. Human amnion (FL) cells were chosen for this investigation. The FL cell line was obtained from the Utah State Department of Health, Salt Lake City, Utah. Monolayer cell cultures for propagation of virus were prepared by using 32-oz (960 ml) prescription bottles inoculated with 10.8×10^6 cells in 50 ml of Melnick's growth medium (MH). This medium was composed of Hanks balanced salt solution plus 5% calf serum, 0.5% lactalbumin hydrolysate, penicillin G (500 units/ml), and streptomycin (50 micrograms/ml). Stationary tube tissue cultures were also prepared by using screw-cap tubes (16 by 150 mm) inoculated with 2×10^5 cells in 2 ml of growth medium. The cultures were used after they reached confluence in 4 to 5 days. Seven screw-cap tubes (13 by 100 mm) containing 3.2×10^5 tissue culture infective doses ($TCID_{50}$) of poliovirus in 1 ml of growth medium were incubated in a water bath at 60 C. At 5-min intervals from 0 to 30 min, a tube was removed and 0.3 ml of the virus suspension was transferred to each of three stationary tube tissue cultures containing growth medium and incubated at 35 C. The cells were examined daily for cytopathic effect (CPE) for a period of 5 days. All the virus experiments included controls which consisted of uninoculated and known infected cells.

Thermal death point for poliovirus. Volumes (1-ml) of poliovirus (3.2×10^5 $TCID_{50}$/ml) in growth medium were held for 30 min in water baths at temperatures from 45 to 60 C at 5 C intervals. At the end of the 30-min incubation period, the virus suspensions were transferred to confluent cultures of FL cells incubated and examined for CPE.

Thermal death time for A. lumbricoides ova. *A. lumbricoides* worms were obtained from local abattoirs. The female worms were cut longitudinally down the midline and the uteri were removed. The uteri were homogenized in saline for 2 min by using an homogenizer (VirTis Corp., Gardiner, N.Y.). This procedure yielded a suspension of ova with a concentration of 1.7×10^5 ova/ml. A temperature of 60 C was chosen to study the thermal death time for these ova. Eight screw-cap tubes (13 by 100 mm) each containing a 1-ml suspension of *A. lumbricoides* ova in saline (1.7×10^5 ova/ml) were placed in a water bath at 60 C. One tube was held at room temperature to serve as a control. Ova from the control tube were observed after 21 days to establish the percentage of viable ova in the total population. At 10-min intervals a tube was removed from the water bath and incubated at room temperature (23 C) for a period of 21 days. At the end of the incubation period, one drop of the suspension of ova from each sample was placed on a microscope slide, mounted with a cover slip, and examined microscopically under the low-power objective (×100) with reduced light. Ova were considered viable if the protoplasm had become segmented or embryonation had occurred.

Thermal death point for A. lumbricoides ova. Suspensions (1 ml) of ova in saline were held for a period of 30 min in water baths at temperatures varying in 5 C increments from 50 to 70 C. At the end of the 30-min time period, the tubes were removed and allowed to incubate for 21 days at room temperature. The samples were then examined for viability as described above in the thermal death time procedure.

Thermal death time for Candida albicans. A temperature of 70 C was chosen for this study since earlier studies indicated that *C. albicans* could withstand 60 C for more than 90 min. Six tubes (13 × 100 mm) of *C. albicans* (3.9×10^7 cell/ml) suspended in 3 ml of Sabouraud Broth (Difco) were placed in a water bath at 70 C. At 10-min intervals, a tube was removed and 10-fold dilutions were carried out. All the dilution tubes were kept chilled in an ice bath until plates could be made for counting. Pagano Levin Agar (Difco) was employed as a plating medium. The plates were incubated at room temperature for 5 days after which colony counts were made.

Thermal death point for C. albicans. Sabouraud Broth cultures of *C. albicans* (5.2×10^7 cells/ml) were incubated for 30 min in water baths at temperatures varying in 5 C increments from 35 to 80 C. Dilutions and platings were carried out as in the thermal death time study for *C. albicans*.

Isolation of S. newport from sewage sludge. Volumes (1 ml) containing known numbers of *S. newport* ranging from 5 to 50,000 organisms/ml were added to each of 5 tubes containing 9 ml of raw sewage sludge and mixed for 1 min with a Vari-Whirl mixer (Van Waters & Rogers, Inc., San Francisco, Calif.). A 1-ml amount from each sludge sample was then inoculated into 9 ml of gram-negative (GN) broth (Difco). The GN broth cultures were incubated at 41.5 C for 6 hr. Spino (15) reported that the isolation rate of *Salmonella* was greatly increased when incubated at this elevated temperature. After the 6-hr incubation period, one loopful from each broth culture was streaked onto Selenite Brilliant Green (SBG) agar and incubated at 37 C for 36 hr. The formula and use of this highly selective SBG broth was described by Kenner et al. (10). A 1.5% concentration of agar was added to the SBG broth for preparing plates. Colonies resembling *Salmonella* on the SBG plates after the 36-hr incubation period were confirmed by the following procedure. Suspected *Salmonella* colonies were transferred to Triple Sugar Iron (TSI) agar (Difco) slants and incubated for 24 hours at 37 C. TSI slants

demonstrating typical *Salmonella* reactions (i.e., alkaline slant, acid butt, with H_2S production) were typed with group C_2 antiserum (Difco) by using a slide agglutination test. Identification based upon positive group C_2 agglutinations were confirmed by using urea agar slants, dulcitol broth, lysine iron agar slants, and lactose broth.

Techniques for isolation of poliovirus from sewage sludge. Volumes (1 ml) of growth medium containing known numbers of poliovirus type 1 ranging from 5 to 5,100 $TCID_{50}$ were added to 100 ml of sterile sewage sludge followed by mixing in an homogenizer. After mixing for 10 min, 10-ml samples were removed, suspended in 100 ml of sterile saline, and centrifuged at 3,400 × *g* for 1 hr in a centrifuge (model GLC-1; Ivan Sorvall, Inc., Norwalk, Conn.). After centrifugation, the two-phase aqueous polymer system adapted for concentration of enterovirus in sewage by Lund (11) was employed for concentration of poliovirus particles. The chemicals used were sodium dextran sulfate 2000 (DS; 20% solution, w/v; Pharmacia, Uppsula, Sweden) and polyethylene glycol (PEG; 30% solution, w/v; Carbowax 6000, J. T. Baker Co.). The 100-ml sample containing poliovirus was adjusted to *p*H 7.2 with 0.01 M phosphate buffer after the addition of 10 ml of 5 M NaCl solution, 29 ml of PEG solution, and 1.3 ml of DS solution. This mixture was homogenized for 1 hr with an homogenizer. The sample was then transferred to a 250-ml separatory funnel and placed at 4 C for 24 hr. After 24 hr, the DS bottom phase of 1 to 1.3 ml was collected. To precipitate the DS, 0.67 ml of a 3 M KCL solution was added for each milliliter of this phase leaving the virus in the supernatant fluid. This was ether treated and assayed for virus. A 1-ml amount from each dilution was inoculated in triplicate into stationary tube FL cell tissue cultures and incubated at 35 C. The stationary tube cultures were inspected daily for CPE as an indication of the presence of infective virus particles.

Neutralization tests. FL cell tissue cultures, prepared in stationary tubes, were incubated at 35 C until confluent monolayers were formed. After the removal of the growth medium, the cultures were washed three times with phosphate-buffered saline, and maintenance medium was added. The immune serum, prepared in rabbits, (poliovirus type 1; Microbiological Associates, Bethesda, Md.) was diluted 1:5 in maintenance medium, mixed with equal volumes of the virus suspension, and incubated at 35 C for 30 min. One tube contained equal volumes of a 1:5 antiserum dilution and maintenance medium to establish that the serum was free from cytotoxic activity. One tube contained equal volumes of virus suspension and maintenance medium to serve as a virus control. After incubation, 1-ml volumes of the serum-virus mixtures were inoculated into the FL tissue culture tubes by using three cultures for each serum-virus mixture. The inoculated cultures were incubated at 35 C and then examined for neutralization of viral CPE after 3 days, since virus controls exhibited CPE at that time.

Techniques for isolation of A. lumbricoides ova from sewage sludge. Volumes (1 ml) of saline containing known numbers of *A. lumbricoides* ova ranging from 1 to 1,200 ova/ml were added to each of four tubes containing 9 ml of raw sewage sludge. The ova and sludge were mixed for 1 min with a Vari-Whirl mixer, filtered through two layers of gauze, and centrifuged at 736 × *g* for 45 to 60 sec with a Sorvall GLC-1 centrifuge. A zinc sulfate centrifugal-flotation procedure (3) as modified by Pihl (*personal communication*) was used for concentration of the ova in the sample as follows.

After the first centrifugation, the supernatant fluid was discarded and the sediment was washed three times by the addition of 5 ml of saline followed by centrifugation at 736 × *g*. After the third wash cycle, the supernatant fluid was discarded and 5 ml of 34% (w/v) zinc sulfate solution (1.20 specific growth) was added to the sediment. The sediment was resuspended, centrifuged at 736 × *g* for 45 to 60 sec, and brought to a stop over a 2-min period. Additional zinc sulfate was added to each sample to bring the meniscus up to a point just above the top of the tube. After a 10-min waiting period, a cover slip was touched to the meniscus to remove the floating ova and mounted on a clean slide for microscopic examination.

Technique for the isolation of C. albicans from sewage sludge. Volumes (1 ml) containing known numbers of *C. albicans* ranging from 1 to 1,400 organisms/ml in Pagano Levin broth were added to each of four tubes containing 9 g of sewage sludge and mixed for one min by using a Vari-Whirl mixer. Ten-fold dilutions were prepared from each sludge sample with Sabouraud Dextrose Broth (Difco) as a diluent. Each dilution (1 ml) was plated, and then incubated for 5 days at room temperature before counting. The highly selective and differential medium used for the primary isolation of *C. albicans* by the pour-plate technique was a modification of Pagano Levin Base (Difco) described by Stedham et al. (16).

Colonies of *C. albicans* on this medium appear pink to red from the reduction of a tetrazolium salt to a colored formazan. Organisms producing this color were subsequently confirmed as being *C. albicans* by their production of chlamydospores when transferred to Rice Extract Agar (Difco) and incubated at room temperature for 5 days.

The composting process. The process was carried out through three stages. The first stage consisted of mixing the incoming sewage sludge from the sewage treatment plant's primary settling tank with a flocculating agent to aid in water removal by vacuum filtration. During the second stage, the sludge-flocculant residue from the filter was fed into the composting bin for the final composting.

The composting bin consisted of a chamber of 40 ft^3 capacity with four augers, one in each corner, for mixing of the compost material. Air was forced up through the bottom of the bin to create the necessary aerobic conditions. The composter was designed in such a way as to allow the compost material to travel in only one direction by passing up and down from auger to auger. The fresh sludge entered one corner (i.e., inflow point) of the bin and, after about 4 to 5 hr, would reach the far corner (outflow point), after traveling the length of each of the four augers. The composted material was then recycled back to the

inflow point and the process was repeated. This continuous recycling took place for a period of 5 days before the composting process was considered finished. The temperature within the composter during processing varied between 60 and 76 C.

Inoculation of the composter. The inoculum of indicator organisms was added to the composter by suspending a 1,000-ml, drop-formed separatory funnel containing the organisms over the inflow area of the composter. A 500-ml volume containing *S. newport* (2.7×10^{13} organisms), *C. albicans* (2.04×10^{10} organisms), and *A. lumbricoides* ova (1.4×10^{8}) was added continuously in this manner over a period of 1 hr. At a later date, a 200-ml inoculum of poliovirus type 1 containing 6.4×10^{7} $TCID_{50}$ was added to the composter in the same way. On both occasions, samples of the compost were collected from the surface in sterile containers 1 hr after the inoculation at the inflow point and then three times daily at the outflow point. All of the compost samples were kept refrigerated until assays could be run.

Final assay of the finished compost. Figure 1 is a flowsheet describing the isolation procedures employed in the final assay of the composted product. Ten 1-g portions from each compost sample were added directly to 9-ml volumes of GN broth and treated in the manner described previously for isolation of *S. newport* from sewage sludge. One 10-g portion from each compost sample was homogenized in 10 ml of sterile physiological saline for 2 min with a VirTis homogenizer. This 20-ml volume was then added to 100 ml of additional saline, and the assay for poliovirus was carried to completion in the same manner described previously in the technique for isolation of poliovirus from sewage sludge. Ten 1-g portions from each compost sample were each emulsified in 5 ml of physiological saline and incubated at room temperature for a period of 21 days. After the incubation period, the samples were mixed for 2 min with a Vari-Whirl mixer, and the zinc sulfate flotation concentration procedure was carried out as described earlier in the technique for isolation of *A. lumbricoides* from sewage sludge. Ova counts and viability determinations were carried out also in the manner described previously. Ten 1-g portions of each compost sample were added directly to 15-ml volumes of molten, modified Pagano Levin agar cooled to 48 C for pour plates. The plates were incubated at room temperature for a period of at least 48 hr. Suspected colonies of *C. albicans* were confirmed by their production of chlamydospores when transferred to Rice Extract Agar and incubated at room temperature.

RESULTS

Thermal death time and thermal death point determinations. The thermal death time for *S. newport* at 60 C was found to be 40 min. Starting with an initial population of 1.9×10^{9} organisms per ml, the counts fell to zero after subjecting the organisms to 60 C for a period of 40 min. The thermal death point for this organism, when held for 30 min, was 65 C. The thermal death time for poliovirus type 1 when 3.2×10^{5} $TCID_{50}$ were held at 60 C was 5 min. The thermal death point determined for a 30-min period was 55 C.

The thermal death time for *A. lumbricoides* ova when a suspension of 1.7×10^{5} viable ova per ml was held at 60 C was 60 min. When the ova were subjected to this temperature for this period of time and subsequently incubated at room temperature for 21 days, no maturation of the ova occurred. The thermal death point was 65 C when the ova were held at the specified temperatures for a period of 30 min. The ova control established that 73% of the population were viable at the start of the experiment. The actual number of ova used in the study was $2.3 \times$

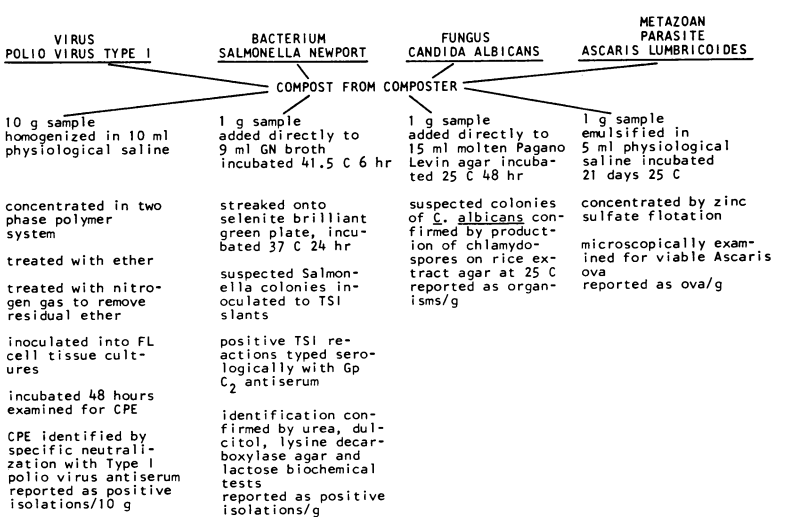

FIG. 1. *Assay for indicator organisms in composted solid human waste.*

10^5/ml, giving a viable ova count of 1.7×10^5 ova per ml. The thermal death time at 70 C for *C. albicans* was 60 min. A rapid reduction of viable yeast cells occurred within the first 20 min, and then the count varied only slightly through 50 min. At 60 min the count had fallen to zero. The thermal death proved to be 80 C when the organism was held at this temperature for 30 min. When 5.2×10^7 organisms were incubated at 80 C for 30 min, all were killed.

Isolation of indicator organisms from sewage sludge. The GN broth enrichment, incubated at elevated temperature, proved to be an effective method of isolating *S. newport* from raw sewage sludge. The only organisms other than *S. newport* that were able to grow on the SBG solid culture medium were species of *Pseudomonas*, *Proteus*, *Aerobacter*, and *Alcaligenes*. Colonies of these organisms exhibited a similar appearance after 24 hr; however, after 36 hr *S. newport* colonies could be recognized by their characteristic curled margin and deep brick-red color resulting from the reduction of the selenite. It was found that, when as few as 50 *S. newport* organisms were present in 1 g of raw sewage sludge, four out of five colonies picked from the SBG agar plates after enrichment subsequently proved to be *S. newport*. Thus, if this concentration of organisms could be established in the composter, then reisolation of the organisms could be relatively certain. The technique was capable of detecting the organism when 50 organisms were present in 1 g of compost; thus, 2.7×10^{12} organisms were inoculated into the composter, giving a theoretical concentration within the compost of 2.4×10^6 organisms per g. This concentration far exceeded the minimal concentration necessary for detection. The method employed for isolating the poliovirus proved to be adequate when the concentration of virus was at least 160 $TCID_{50}$ per 10 g of sludge. Ten grams were assayed rather than 1 g since this larger sample size would facilitate isolation of the virus if it were present in small numbers. The amount of poliovirus inoculated into the composter was 6.4×10^7 $TCID_{50}$. This gave a theoretical concentration of 560 $TCID_{50}$ per 10 g of compost. The experiments conducted to ascertain the sensitivity of the isolation procedure for ova of *A. lumbricoides* showed that, when at least 12 ova per g of sewage sludge were present, isolation could be made. When 12 ova were known to be present in the one gram sample, at least two ova could be isolated; thus, 1.4×10^8 ova were introduced into the composter, yielding a theoretical concentration of 1.2×10^2 ova per g of compost. This concentration well exceeded the minimal concentration of ova necessary for detection. The use of modified Pagano-Levin medium for the primary isolation of the *C. albicans* from the sewage sludge proved highly satisfactory. When as little as 14 *C. albicans* yeast cells were initially present per gram of sewage sludge, isolations could be made and confirmed. Ninety-five per cent of the colonies which grew on this medium proved to be *C. albicans*. An inoculum of 2.0×10^{10} *C. albicans* organisms was inoculated into the composter, yielding a theoretical concentration of 1.7×10^4 cells per g of compost. This represented 1,000 times more organisms per g than was necessary to assure detection.

Final assay of the composted product for the presence of indicator organisms. One hour after the addition of the *S. newport* organisms to the composter, all the samples taken from the inflow area were positive for the organism. The actual number of organisms was not determined in the assay since enrichment procedures were employed. Table 1 shows that by the 25th hr postinoculation, all the samples were negative for *S. newport*. Samples were taken periodically through 220 hr, with no further isolations of the indicator organism.

All the samples assayed for the poliovirus were negative. Several times the cells displayed apparent CPE, but when the fluids from these tubes were passed to fresh cell cultures, no further CPE occurred, indicating the tissue CPE was due to nonspecific cytotoxicity rather than a virus.

The only viable *A. lumbricoides* ova that were isolated came from the samples taken at the inflow area 1 hr after inoculation of the composter as indicated in Table 2. At 4 hr postinocu-

TABLE 1. *Isolation of Salmonella newport*[a] *from composted sewage sludge*

Hr after inoculation	No. of samples assayed	Positive isolations[b]
1[c]	5	5/5
4	5	1/5
19	5	5/5
25	5	0/5
28	5	0/5
48	5	0/5
48–220	80	0/5

[a] *S. newport* organisms (2.7×10^{12}) were inoculated into the composter at the inflow point.

[b] Actual concentration of organisms was not determined since enrichment procedures were employed.

[c] This sample was taken from the inflow point; all subsequent samples were taken from the outflow point.

TABLE 2. *Isolation of Ascaris lumbricoides ova*[a] *from composted sewage sludge*

Hr after inoculation	No. of samples assayed	Mean no. of ova per sample	Mean no. of viable ova per sample
1[b]	5	35	30.8
4	5	8	0
19	5	16	0
23	5	6.8	0
26	5	3.2	0
46	5	0.2	0
46–50	10	0.8	0
50–218	70	0	0

[a] *A. lumbricoides* ova (1.4×10^8) were inoculated into the composter at the inflow point.

[b] This sample was taken at the inflow point; all subsequent samples were taken at the outflow point.

TABLE 3. *Isolation of Candida albicans*[a] *from composted stewage sludge*

Hr after inoculation	No. of samples assayed	Samples positive	Mean no. of organisms per sample
1[b]	10	10/10	TNTC[c]
4	10	10/10	3.4
19	10	0/10	0
28	10	3/10	0.3
43	10	0/10	0
52	10	0/10	0
67	10	0/10	0
76	10	0/10	0

[a] *C. albicans* organisms (2.0×10^{10}) were inoculated into the composter at the inflow point.

[b] Samples taken at the inflow point; all subsequent samples were taken at the outflow point.

[c] Colonies too numerous to count accurately.

lation, an average of eight ova per sample were isolated, but none of the ova exhibited segmentation or embryonation after incubation for 21 days. At 19 hr after inoculation, the ova that were isolated showed evidence of disintegration by rupturing of the outer shell. From 23 through 50 hr, only fragments of the ova were present. No trace of any ova could be found from 50 through 218 hr postinoculation. A total of 110 samples were assayed.

Samples taken for *C. albicans* at the inflow area 1 hr after inoculation yielded pour plates that were so heavily overgrown that colony counts could not be made. Table 3 indicates that 4 hr postinoculation, the samples taken at the outflow area yielded positive isolations from each of the ten 1-g samples. All samples taken after 28 hr were negative. A total of 80 samples taken over a 76-hr period were assayed.

DISCUSSION

When sewage sludge is composted, it is vitally important that the composting process be operated in such a way as to insure thermophilic bacterial activity. This bacterial activity is necessary to produce sufficiently high temperatures over an adequate period to render the composted product free from pathogenic organisms. Good mixing with proper aeration of the sewage sludge in the composter will achieve this thermophilic condition when other conditions such as moisture content are met (4). However, temperature alone is not a guarantee that all pathogens will be destroyed. Experiments conducted in this study were designed to determine whether pathogens could possibly survive thermophilic temperatures present during composting. This was achieved by introducing indicator organisms into the composter and periodically testing for their survival. The composter developed by the Eimco Corp. fulfilled the requirements for thermophilic activity (4) and maintained a temperature range, within the compost, from 60 to 70 C.

The results obtained from the experiments conducted in this study indicated that the composter was capable of destroying the selected pathogens and thus could destroy any pathogens with similar thermal sensitivities that might be present in the raw sewage sludge. The thermal death time and thermal death point determinations for the bacterial indicator *S. newport* showed that it was destroyed when subjected to 60 C for 40 min or 65 C for 30 min. These conditions were met in the composter, suggesting that this organism could be destroyed. Poliovirus type 1, the viral indicator, was shown to be inactivated by 5 min at 60 C. Since this is the lowest temperature in the thermophilic range achieved in the composter, this virus should likewise be easily destroyed. The metazoan parasite indicator, *A. lumbricoides* ova, was shown to be killed by a 60-min exposure at 60 C or 30 min at 65 C. From a consideration of the temperatures attained in the composter, it is reasonable to assume that this indicator would not survive the composting process. The fungal indicator, *C. albicans*, was found to be more resistant to heat than the other organisms. This organism was found to be killed when exposed to 70 C for 60 min or 80 C for 30 min. The thermal death points and thermal death times for the indicator organisms were conducted in media other than that found in the composter, since these tests were designed to provide only an approximation of the thermal sensitivities and were not meant to duplicate the conditions found in the composter. Since the composting process continued for 5 days at tem-

peratures from 60 to 75 C, it is suggested that the *C. albicans* organisms would also be destroyed. Each of the indicator organisms thus exhibited thermal death time and thermal death point characteristics, suggesting they would be inactivated when subjected to the composting process.

Samples taken at the inflow area of the composter revealed that the indicator organisms, with the exception of the poliovirus, survived well during the first hour after inoculation. Some samples taken at the outflow area were positive for *C. albicans* for up to 28 hr postinoculation.

Survival for this period of time could be explained on the basis that *C. albicans* was protected from continuous exposure to temperatures of 60 to 70 C by the organic matter in the sludge. Our preliminary experiments indicated to us that this organism was more heat-resistant than any of the other organisms. A further explanation could be that the organism was found in some instances after 28 hr because the sensitivity of our assay for *C. albicans* was greater than that for *S. newport* or poliovirus. The sensitivity of our assay for *Ascaris* ova enabled us to detect ova in samples 26 hr after inoculation of the composter although the ova were not viable.

The poliovirus was undetectable 1 hr after inoculation. When the virus suspension was inoculated, the surface temperature of the compost in the composter was between 55 and 60 C. Considering this, it was reasonable to conclude that the virus was rapidly inactivated due to heat sensitivity. One must also consider another possible factor. Clarke (1) observed that simple mixing of poliovirus with sewage at room temperature resulted in a loss greater than 75% of the original virus within 5 min after its addition to the raw sewage. In the same article he found that a 99% reduction of the virus occurred within 45 min. Since no CPE was detected during the virus assay, it can be assumed that other enteroviruses with similar thermal sensitivities present in the compost were likewise inactivated.

There are reports in the literature of attempts to determine if pathogenic organisms can survive composting (16), but most of the reports were based on attempts to isolate at the end of the composting process, any surviving pathogens that may have been already present in the original material (13). Other investigators (5, 6, 14) simply concluded that since certain high temperatures were attained during the process, then pathogens should have been destroyed. There are even those who believe that because no one had become ill from eating crops fertilized with sewage compost then the composting process must have destroyed any pathogens that may have been present (12). There were no reports found in the literature where organisms representing four groups of pathogens were employed as indicators of a composting process's effectiveness in destroying pathogens.

Pathogen destruction during composting may occur as a result of thermal killing by sufficiently high temperature and by antibiotic action or by the decomposing organisms or their products (4). Evidence from this study indicated that the time-temperature conditions of this composting process were sufficient to destroy all of the indicator organisms. Whether other factors contributed to the destruction of the indicator pathogens was not studied, but must be considered. From the results of this study, it seems clear that when sewage sludge is composted in the manner described here a final product can be obtained which would be safe and could be investigated for use as a soil conditioner or fertilizer. It is reasonable to assume that problems of hygiene involving sewage sludge disposal could be solved by composting of sludge, thus producing a product which could be returned to the soil as a soil conditioner or possibly as a fertilizer.

ACKNOWLEDGMENTS

This work was carried out under subcontract number 137-363-8-21 between the Eimco Corp., Salt Lake City, Utah 84110, and the University of Utah. The original source of the funds was a Public Health Service Grant, 86-67-103, from the Solid Wastes Program, National Center of Urban and Industrial Health, Cincinnati, Ohio.

The data presented herein was submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Utah by Stephen C. Westerberg.

LITERATURE CITED

1. Clarke, N. A., R. E. Stevenson, S. L. Chang, and P. W. Kabler. 1961. Removal of enteric viruses from sewage by activated sludge treatment. Amer. J. Public Health 51: 1118–1129.
2. Cram, E. B. 1943. The effect of various treatment processes on the survival of helminth ova and protozoan cysts in sewage. Sewage Works J. 15:1119–1131.
3. Faust, E. C., and P. F. Russell. 1957. Clinical parasitology, 6th ed., p. 951–952. Lea & Febiger Publishers, Philadelphia.
4. Golueke, C. G., B. J. Card, and P. H. McGauhey. 1954. A critical evaluation of inoculums in composting. Appl. Microbiol. 2:45–53.
5. Golueke, C. G., and H. B. Gotaas. 1954. Public Health aspects of waste disposal by composting. Amer. J. Public Health 44:339–348.
6. Grindrod, J. 1961. Six years of refuse composting in Britain. Public Works 92:110–111.
7. Harrison, T., R. Adams, I. Bennett, Jr., W. Resnik, G. Thorn, and M. Wintrobe. 1962. Principles of internal medicine, 4th ed., p. 1062. McGraw-Hill Book Co., Inc., New York.
8. Hoover, C. B. 1928. Public health engineering, sewage sludge. Amer. J. Public Health 18:363–364.
9. Kelly, S. and W. W. Sanderson. 1960. Density of enteroviruses in sewage. J. Water Pollut. Contr. Fed. 32:1269–1273.

10. Kenner, B. A., S. W. Rockwood, and P. W. Kabler. 1957. Isolation of members of the genus Salmonella by membrane filter procedures. Appl. Microbiol. 53:305–307.
11. Lund, E., C. E. Hedstrom, and O. Strannegard. 1966. A comparison between virus isolations from sewage and from fecal specimens from patients. Amer. J. Epidemiol. 84:282–286.
12. Perold, I. S. 1951. Compost's value overrated. Public Health (Johannesburg, South Africa) 15:70.
13. Reeves, J. B. 1959. Sanitary aspects of composted sewage sludge and sawdust. Sewage Ind. Wastes 31:557–564.
14. Schulze, K. L. 1962. Continuous thermophilic composting. Appl. Microbiol. 10:108–122.
15. Spino, D. F. 1966. Elevated-temperature technique for the isolation of *Salmonella* from streams. Appl. Microbiol. 14:591–596.
16. Stedham, M. A., D. C. Kelley, and E. H. Coles. 1966. Modified Pagano Levin medium to isolate *Candida* species. Appl. Microbiol. 14:525–528.
17. Wiley, J. S. 1962. Pathogen survival im composting municipal wastes. J. Water Pollut. Contr. Fed. 34:80–90.