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Septage Treatments to Reduce the Numbers of Bacteria and Polioviruses

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Disposal of the pumped contents of septic tanks (septage) represents a possible means of dissemination of enteric pathogens including viruses, since persistence of enteroviruses in septic tank sludge for greater than 100 days has been demonstrated. The risk of exposure to potentially infectious agents can be reduced by disinfecting septages before their disposal. Of the septage disinfectants examined (technical and analytical grade glutaraldehyde, hydrogen peroxide, heat treatments, and a combination of heat and hydrogen peroxide), the treatment including hydrogen peroxide (5 mg, plus 0.33 mg of trichloroacetic acid, per ml of septage) and 55°C killed virtually all the bacteria in septage within 1 h, whereas 55°C alone inactivated inoculated polioviruses within 30 min. Virus was the most sensitive to heat, whereas fecal coliforms appeared to be the most sensitive to all chemical treatments. The responses of fecal streptococci and virus to both grades of glutaraldehyde (each at 1 mg/ml) were similar. Virus was more resistant than either fecal streptococci or total bacteria to low concentrations of hydrogen peroxide (1 to 5 mg/ml); however, virus and fecal streptococci were more labile than total bacteria to the highest peroxide concentration (10 mg/ml) examined. It is possible that the treatment combining heat and hydrogen peroxide was the most effective in reducing the concentrations of all bacteria, because catalase and peroxidases as well as other enzymes were heat inactivated, although catalase seems the most likely cause of damage. However, this most effective treatment does not appear to be practical for on-site use as performed, so further work on septage disinfection is recommended.

Septic tank-soil absorption systems are the most common method for on-site waste disposal, serving approximately one-third of the U.S. population (14). The septic tank is designed so that the solids in the incoming wastewater settle in the tank while the clarified effluent passes into a soil absorption field for final disposal and treatment. The solids that accumulate within the tank must be removed periodically so that they do not flow into the wastewater absorption field. The average septic tank requires pumping only every 2 to 5 years, or when the accumulated solids occupy roughly one-third of the tank's volume (17). Pumping removes all the solid and liquid contents, collectively known as septage.

Septage is a highly concentrated waste that possibly contains various concentrations of bacterial pathogens, enteric viruses, protozoan cysts, or parasitic ova (14–16). Enteroviruses inoculated into operating septic tanks persisted in sludge for over 100 days (S. L. Stramer, Ph.D. dissertation, University of Wisconsin, Madison, 1984). However, septage from a given septic tank will not contain pathogenic agents most of the time. In surveys in Wisconsin, *Salmonella* sp. was found in only 2 of 55 septic tanks sampled (21), and human enteric viruses were found in only 1 of 78 septic tanks (5). The virus-containing system served a family in which a child had recently received three doses of the trivalent oral poliovirus vaccine. Therefore, disposal of septage from the majority of private, on-site systems onto agricultural lands should pose virtually no public health hazard from a microbiological standpoint. On the other hand, there is the possibility that pathogens may be present at any given time. Consequently, septage must be disposed of in a manner that reduces the risk of exposure to pathogens for humans (i.e., for the septic tank pumper and those who may contact the material directly or indirectly as the result of recreational water use or the consumption of contaminated food or water). For this reason, methods of septage disinfection have been considered.

This study initially was intended to evaluate the technical and analytical grades of glutaraldehyde as septage disinfectants, as suggested by J. F. Deininger (M.S. thesis, University of Wisconsin, Madison, 1977). However, one disadvantage of glutaraldehyde is that it contains several impurities that may be considered environmental contaminants (e.g., glutaric acid, acrolein, and glutaraldoxime; 2, 7, 11, 12). Therefore, this study was broadened to include 30% hydrogen peroxide, heat, and a combination of the two. The use of 30% hydrogen peroxide (which has been used to restore clogged and failed wastewater absorption systems; 6) as a septage disinfectant was examined because hydrogen peroxide oxidizes organic materials rapidly, and the end products of its decomposition are generally regarded as safe for environmental disposal. Reductions in the numbers of total indigenous bacteria in septage samples and of inoculated fecal coliforms, fecal streptococci, and vaccine-derived poliovirus were examined for each disinfectant treatment.

MATERIALS AND METHODS

Disinfection of septage samples. Septage (3% total solids content; 1) was obtained from a continuously used septic tank that had not been pumped for over 2 years. Once obtained, the septage had been held at 4°C for several months (as it was used initially in another study). Fecal coliforms, fecal streptococci, and polioviruses were added to the material before disinfection. Viral and bacterial disinfection experiments were done separately. Just before each disinfection trial, duplicate beakers containing 250 ml of

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septage were inoculated with 18-h cultures of a septage-derived fecal coliform isolate and a fecal streptococcus isolate (both grown in tryptose soy broth; Difco Laboratories). The initial most probable numbers (MPN; 1) of fecal coliforms and fecal streptococci in the septage samples were approximately 10^6 to 10^7/ml. Poliovirus type 1 strain CHAT was obtained from the Viral and Rickettsial Registry of the American Type Culture Collection. A 24-h crude virus preparation containing all cell debris was added to septage 24 h before disinfection, mixed for 30 min after inoculation, and stored overnight at 15°C (the annual mean septic tank temperature; 16); this allowed association of the virus with solids. Earlier work in this laboratory showed that approximately half of the poliovirus added to septic tank sludge was taken up overnight by solids (K. M. Green, Ph.D. dissertation, University of Wisconsin, Madison, 1976). The following day, virus-inoculated septage was mixed for 30 min, and 25-ml samples were removed for virus titration; initial concentrations were approximately 10^6 PFU/ml.

After the time zero samples were removed for both bacterial and viral analyses, disinfectant was added to one beaker of septage; the second beaker served as the untreated control. During all disinfection experiments, both the experimental and control beakers were held at ambient temperature (unless otherwise indicated) and mixed continuously. Samples (25-ml volumes for viruses and approximately 10-ml volumes for indicator bacteria) were removed after 1, 3, and 6 h (as well as 15, 30, and 90 min for several experiments).

Bacterial assays. Pour plates were used initially to determine the total numbers of bacteria (plate count agar; Difco) and of fecal streptococci (KF Streptococcus agar; Difco). Pour plates were discontinued for the fecal streptococci because septage solids interfered with colony detection at low dilutions; more importantly, the agar-based medium appeared to be more toxic than the primary enrichment medium used in the MPN procedure to sublethally injured fecal streptococci. The inability to recover damaged cells from disinfected wastewaters on selective media has been described frequently (1, 8, 9). Total bacteria in the septage samples could be assayed by pour plates because plate count agar is nonselective, so that injured organisms can be resuscitated. Samples (1 ml) were added to empty 15- by 100-mm petri dishes (usually two or more), followed by plate count agar. After 48 h at 35°C, all surface and subsurface bacterial colonies were counted and used to calculate CFU/100 ml. Septage samples for fecal coliform analyses were inoculated into lactose broth (Difco), followed by inoculation of EC medium (Difco), from all lactose broths demonstrating gas formation after 24 and 48 h at 35°C (1); lactose broth was used in lieu of lauryl tryptose broth because the former should increase the recoveries of injured coliforms. EC broths were incubated at 44.5°C in a covered water bath for 24 h, after which the numbers of tubes with gas formation were used to determine the MPN/100 ml. Fecal streptococci were enumerated similarly; however, azide dextrose broth (Difco) was the primary enrichment medium, followed after 48 h by inoculation of ethyl violet azide broth (Difco) from those azide dextrose broths in which growth had occurred. All media were incubated at 35°C. MPN results were determined from the numbers of ethyl violet azide tubes demonstrating growth after 48 h (1).

Viral assays. To 25 ml of septage (collected at each time point from both the control and disinfected suspensions) was added 25 ml of a solution of 50% 0.1 M phosphate-buffered saline and 50% fetal calf serum. The slurry of septage, phosphate-buffered saline, and serum was homogenized in an Omni-Mixer (Sorvall, Inc.) for 5 min. The homogenized slurry was adjusted to pH 9.0 with 3 N NaOH and disrupted further, to facilitate virus elution from sludge solids, on ice water in a sonic bath (Branson Sonic Power Co.; model B-220 sonifier) at 55 kHz and 125 W for 20 min. After sonication, samples (in Oak Ridge-style, polypropylene centrifuge tubes) were clarified at 16,500 × *g* for 30 min at 4°C (Sorvall; model RC-2B). The virus-containing supernatants were collected, readjusted to pH 7.0 with 3 N HCl, and filtered through a decreasing porosity series of 25-mm diameter, cellulose triacetate membrane filters (Gelman Sciences, Inc.). Filter-sterilized samples were stored frozen until titered. This procedure was found to recover 58 to 80% of the added poliovirus (Stramer, Ph.D. dissertation).

Viruses were titered by the plaque method on confluent monolayers of Buffalo green monkey (BGM) cells (Microbiological Associates). BGM cells were grown in Eagle minimum essential medium containing Earle balanced salts solution (GIBCO Laboratories) and supplemented with 10% fetal bovine serum (GIBCO) and the following antibiotics: 5.0 μg of fungizone per ml, 100 U of penicillin G sodium per ml, and 100 μg of dihydrostreptomycin sulfate per ml. The overlay medium also contained neutral red (0.002%) and Noble agar (1%; Difco). After decanting the growth medium from the flasks, groups of four flasks (per sample to be titered) were inoculated, each with 0.5 ml (four flasks were used to compensate for the twofold dilution that resulted during virus elution). After a 2-h adsorption period, 5 ml of overlay medium was added to each flask. Once the agar solidified, flasks were inverted and incubated at 37°C for a total of 5 days. Plaques were counted after 3, 4, and 5 days; at the end of 5 days, cells were stained (after removal of the agar overlay) with an aqueous solution of 1.85% formaldehyde, 25% ethanol, 0.85% sodium chloride, and 0.5% crystal violet to guarantee that all plaques had been scored. Titers were recorded as PFU per milliliter.

Investigations with glutaraldehyde. Technical grade (Eastman Chemicals) and analytical grade (Electron Microscopy Sciences) glutaraldehyde were obtained as 25% (wt/vol) stock solutions. The former was supplied by the manufacturer in 500-ml quantities and stored at 4°C, whereas the latter was supplied frozen in 10-ml ampules and stored at −10°C, as recommended by the manufacturer. The glutaraldehyde concentration of each was determined before use. The refractive index of glutaraldehyde rises above that of water by 0.0168 for each 10% increase in the glutaraldehyde content (Deininger, M.S. thesis). However, a more reliable method of determining the glutaraldehyde concentration is by sodium sulfite titration (18). A linear relationship was established between the glutaraldehyde concentration determined by titration and that determined by refractive index. Therefore, by using a standard curve (Fig. 1), measurements of the refractive index of a glutaraldehyde solution were substituted for the sodium sulfite titration. Concentrations of technical grade glutaraldehyde ranged from 21.4 to 21.7% by this method, whereas analytical grade glutaraldehyde ranged from 23.7 to 24.0%, when the solutions were assayed immediately upon receipt. After aldehyde determinations, stock solutions (50 mg/ml) of both grades were prepared, adjusting for the loss in aldehyde activity from the stock (250 mg/ml) supplied by the manufacturers; of the stock at 50 mg/ml, 5 ml was added to 250 ml of septage. Therefore, the final concentration was 1 mg/ml.

Investigations with hydrogen peroxide and heat. Three different concentrations of hydrogen peroxide (supplied as a 30% stock; LaPine Chemicals) were tested in septage at

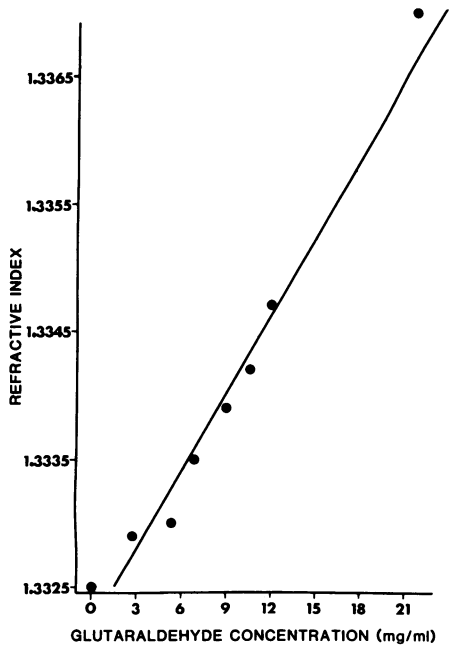

FIG. 1. Standard curve obtained when glutaraldehyde concentrations were determined by both refractive index and aldehyde titration, $y = 1.33 + (2.09 \times 10^{-4})x$; $r^2 = 0.98$, $P < 0.01$.

concentrations of 1, 5, and 10 mg/ml. Trichloroacetic acid (TCA; 0.067, 0.33, and 0.67 mg/ml, respectively) was added along with peroxide to retard catalase-mediated peroxide degradation (4). Septage was held at 50°C (for viruses only) and 55°C (for bacteria and viruses) in water baths while the septage was mixed with an Omni-Mixer. In the last series of experiments, 5 mg of hydrogen peroxide per ml (plus 0.33 mg of TCA per ml) was added to the septage held at 55°C.

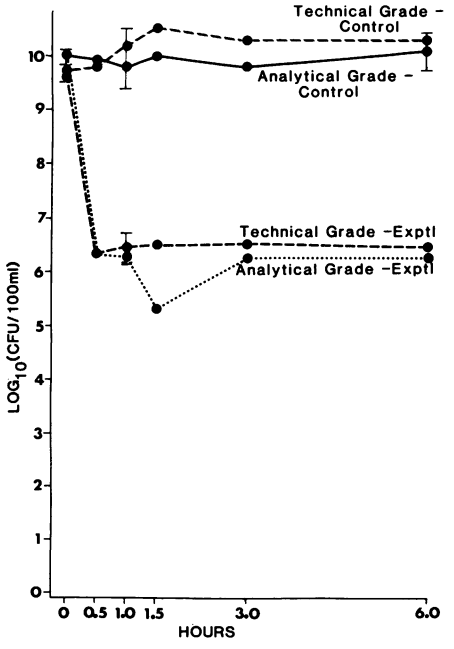

FIG. 2. Effects of analytical and technical grade glutaraldehyde on the total bacteria [$\log_{10}$(CFU ± standard error of the mean) per 100 ml] in septage. Experiments involving analytical and technical grades were done on separate occasions; therefore a control was present for each. The standard error of the mean is represented when the interval is larger than the size of the symbol.

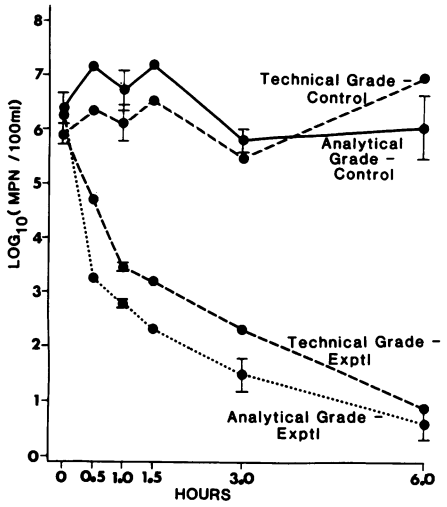

FIG. 3. Effects of analytical and technical grade glutaraldehyde on fecal coliforms [$\log_{10}$(MPN ± standard error of the mean) per 100 ml] in septage. Experiments involving analytical and technical grades were done on separate occasions; therefore a control was present for each. The standard error of the mean is represented when the interval is larger than the size of the symbol.

RESULTS

Bacterial susceptibility to glutaraldehyde. Both technical and analytical grades of glutaraldehyde (1 mg/ml) reduced the total bacterial population in septage by slightly greater than 3 $\log_{10}$ units within 30 min (Fig. 2). The total bacterial

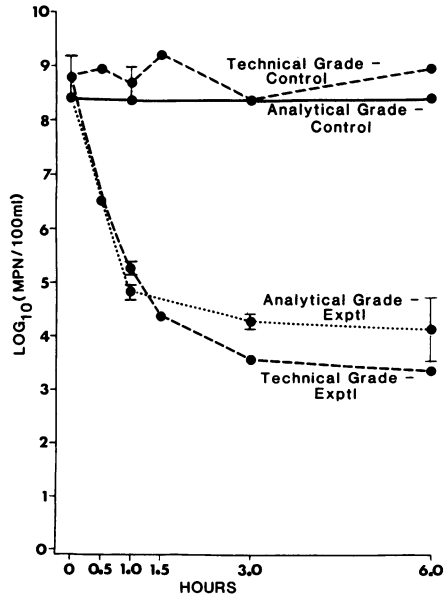

FIG. 4. Effects of analytical and technical grade glutaraldehyde on fecal streptococci in septage. Experiments involving analytical and technical grades were done on separate occasions; therefore a control was present for each. The standard error of the mean is represented when the interval is larger than the size of the symbol.

population was affected only during the first 30 min; no further reduction occurred from 30 min to 6 h (the value recorded with analytical grade at 1.5 h was based on an unpaired observation and may have been anomalous). Analytical grade glutaraldehyde was slightly more effective against fecal coliforms inoculated into septage, from which an approximate 5 to 6 $\log_{10}$ reduction occurred after 6 h (Fig. 3). In contrast to the rapid rate of decline observed only during the first 30 min for total bacteria, the rate of decline for fecal coliforms was more nearly linear, although it was also more rapid during the first 30 min. Fecal streptococci in septage showed a slightly greater susceptibility to technical than to analytical grade glutaraldehyde (after 6 h, a 5 $\log_{10}$ reduction with technical grade versus a 4 $\log_{10}$ reduction with analytical grade; Fig. 4). As observed with the total bacterial population and fecal coliforms, the most rapid decline occurred early (within 30 min to 1 h). No reductions were observed in the controls of any bacterial group.

Bacterial susceptibility to hydrogen peroxide and heat. At 1 mg of hydrogen peroxide per ml, no reductions of total bacteria or fecal streptococci occurred even after 6 h (Fig. 5 and 6). However, fecal coliforms were more susceptible to hydrogen peroxide, as was observed for glutaraldehyde; a 3 $\log_{10}$ reduction of fecal coliforms in septage occurred after 6 h even with only 1 mg of hydrogen peroxide per ml (Fig. 7). At 5 mg of hydrogen peroxide per ml, total bacteria and fecal streptococci were reduced 1 to 2 $\log_{10}$ units within 3 to 6 h, whereas fecal coliforms were reduced by approximately 6 $\log_{10}$ units. At the highest hydrogen peroxide concentration (10 mg/ml), total bacteria were reduced 4 $\log_{10}$ units, fecal streptococci were reduced greater than 5 $\log_{10}$ units, and fecal coliforms were reduced 7 $\log_{10}$ units after 6 h. The total bacterial population and fecal coliforms responded similarly when treated with 10 mg of hydrogen peroxide per ml or when held at 55°C; only fecal streptococci had a lesser reduction at 55°C than with 10 mg of hydrogen peroxide per ml (after 6 h, greater than 5 $\log_{10}$ reduction in fecal streptococci occurred with 10 mg of hydrogen peroxide per ml, versus a 4 $\log_{10}$ reduction at 55°C). When 5 mg of hydrogen peroxide per ml and a 55°C heat treatment were combined (in the presence of 0.33 mg of TCA acid), the decline of total

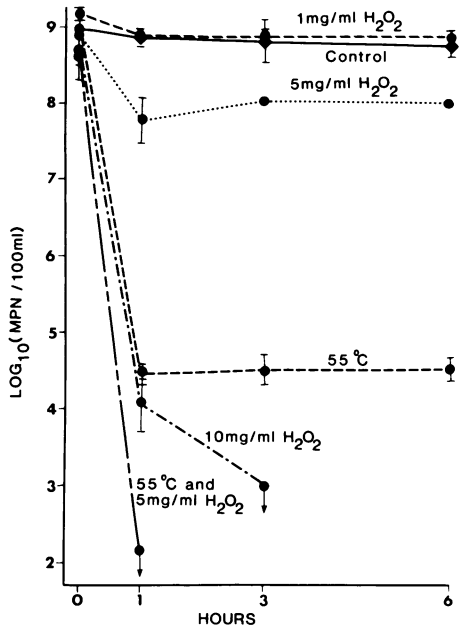

FIG. 6. Effects of hydrogen peroxide and heat on fecal streptococci [$\log_{10}$(MPN ± standard error of the mean) per 100 ml] in septage. The standard error of the mean is represented when the interval is larger than the size of the symbol. The arrows indicate that the levels were below the limit of detection.

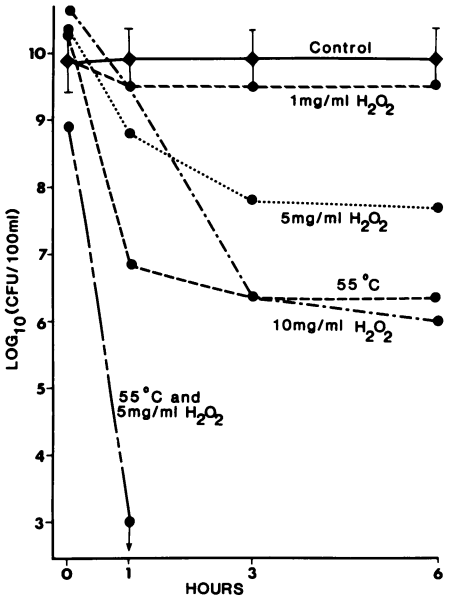

FIG. 5. Effects of hydrogen peroxide and heat on total bacteria [$\log_{10}$(CFU ± standard error of the mean) per 100 ml] in septage. The standard error of the mean is represented when the interval is larger than the size of the symbol. The arrow indicates that the level was below the limit of detection.

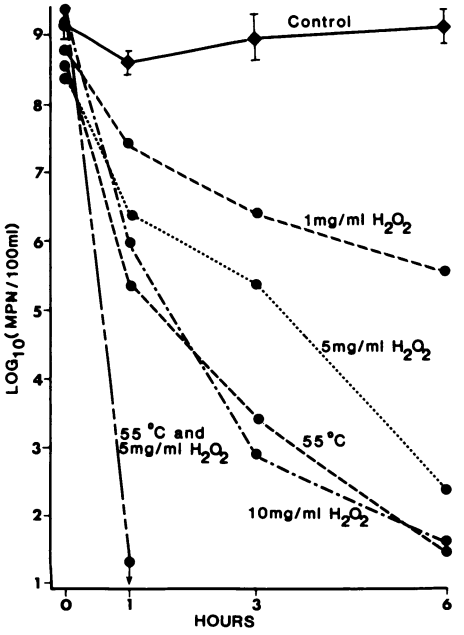

FIG. 7. Effects of hydrogen peroxide and heat on fecal coliforms [$\log_{10}$(MPN ± standard error of the mean) per 100 ml] in septage. The standard error of the mean is represented when the interval is larger than the size of the symbol. The arrow indicates that the level was below the limit of detection.

bacteria and all fecal indicators was dramatic. Greater than 6 to 7 $\log_{10}$ reductions of all groups occurred within 1 h, versus the 6 h that was necessary for equivalent or lesser reductions to occur when the two treatments were used separately.

Poliovirus suceptibility to glutaraldehyde, hydrogen peroxide, and heat. Polioviruses were more resistant than the total bacterial population, fecal coliforms, or fecal streptococci to 5 mg of hydrogen peroxide per ml, as no poliovirus reduction occurred during 6 h (Fig. 8); however, a reduction of almost 4 to 5 $\log_{10}$ of polioviruses in septage occurred after 3 h of treatment with 1 mg of either analytical or technical grade glutaraldehyde per ml or with 10 mg of hydrogen peroxide per ml. The poliovirus concentrations continued to decline after 3 h. Polioviruses were somewhat sensitive to 50°C (greater than a 2 $\log_{10}$ inactivation in 90 min; Fig. 9), but were the most sensitive to 55°C, at which greater than a 5 to 6 $\log_{10}$ reduction in polioviruses occurred in 15 to 30 min. Since heat alone was very effective, it was unnecessary to test the concurrent effects of 55°C and 5 mg of hydrogen peroxide per ml.

DISCUSSION

Studies of septic tank dosing have demonstrated that the majority of polioviruses introduced with stools will be retained within the septic tank for at least 100 days (Stramer, Ph.D. dissertation). Bacterial pathogens, if present, will probably occur in the sludge in even greater concentrations than viruses, as was indicated by greater proportions of fecal coliforms and fecal streptococci than of stool-derived polioviruses that sedimented. The majority of septic tanks will not contain any pathogens; however, if the septic tank is pumped while pathogenic agents introduced into the septic tank sludge are still infectious, a potential health threat exists. Surface application of septage onto agricultural lands is one method of disposal; illegal disposal (e.g., roadside dumping and discharge of the untreated material into waterways) also occurs frequently. Regardless of the method of septage disposal, it would be desirable to disinfect the material before its final discharge. Disinfectants used for drinking water are not applicable to sludge; chlorine and ozone are both bound by solids, and the former gives rise to chlorinated compounds. Several disinfectants for septage have been considered (Deininger, M.S. thesis); the most effective in reducing the numbers of bacteria and viruses were alkaline formaldehyde (at pH 9.0) and glutaraldehyde (at pH 7.1). Saitanu and Lund (13) have also found glutaraldehyde to be an effective viricide even for enteroviruses suspended in 10% calf serum. However, both alkaline formaldehyde and glutaraldehyde are depleted rapidly by their reaction with wastewater solids. Glutaraldehyde contains many impurities as a result of its manufacture and chemical instability, and the products of its reaction with septage are unknown (2, 11, 12). These impurities and unknown products make glutaraldehyde unsuited to this application, since the disinfectant used should be safe to handle and leave no toxic residue.

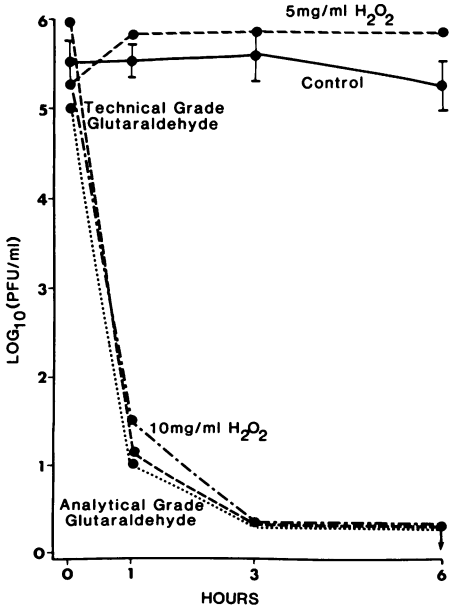

FIG. 8. Response of poliovirus type 1 [$\log_{10}$(PFU ± standard error of the mean) per ml] in septage to technical and analytical grades of glutaraldehyde and to 5 and 10 mg of hydrogen peroxide per ml. The standard error of the mean is represented when the interval is larger than the size of the symbol. The arrow indicates that the level was below the limit of detection.

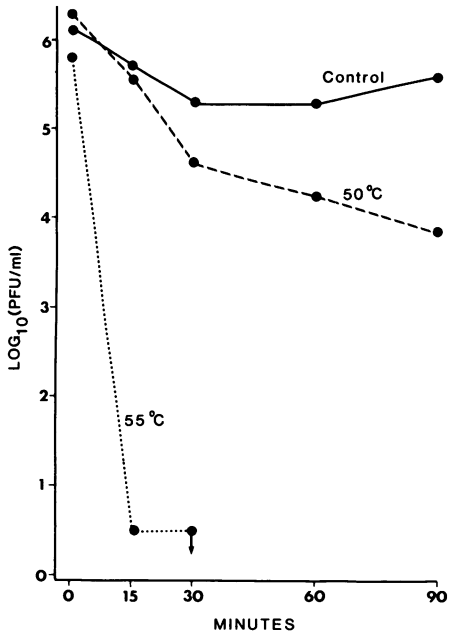

FIG. 9. Response of poliovirus type 1 [$\log_{10}$(PFU ± standard error of the mean) per ml] in septage to 50 and 55°C. The arrow indicates that the level was below the limit of detection.

For comparative purposes, glutaraldehyde (both analytical and technical grades, since only analytical grade, which would be too costly for septage disinfection, had been examined previously) was the first disinfectant examined in this study. Analytical and technical grades of glutaraldehyde appeared to be comparable in reducing fecal coliforms by 5 to 6 $\log_{10}$ units, fecal streptococci and polioviruses by 4 to 5 $\log_{10}$ units and the total numbers of aerobic bacteria by just greater than 3 $\log_{10}$ units. Glutaraldehyde is a rapid cross-linking agent for protein (2, 7, 11, 12); its rapid reactivity was observed in septage, as the vast majority of the decline of all tested agents was observed within the first 30 min to 1 h after application. Nonlinear rates of inactivation of coxsackieviruses by glutaraldehyde have also been described (13). The cost of septage treatment with technical grade glutaraldehyde at present prices would be approximately $10 for a

typical 4,000-liter (ca. 1,000-gal) septic tank, whereas that for analytical grade would be over $2,000.

Bacteria in septage appeared to be as susceptible to 10 mg of hydrogen peroxide per ml and to 55°C as to technical and analytical grades of glutaraldehyde; the responses of bacteria and viruses to 10 mg of hydrogen peroxide per ml were comparable to those observed with glutaraldehyde. Hydrogen peroxide disinfection has advantages over that of glutaraldehyde: hydrogen peroxide decomposes into innocuous end products so that no toxic residue would remain after disinfection. Because of its ability to oxidize organic materials and the predicted absence of toxic end products, hydrogen peroxide is currently used for the restoration of clogged septic tank wastewater absorption systems (6). Septage from a 4,000-liter (1,000-gal) septic tank would require 80 liters (20 gal) of 50% hydrogen peroxide at a present cost of $20 if a final concentration of 5 mg/ml is desired or $40 if 10 mg/ml is desired (this excludes the cost of the TCA). If septage is heated to 55°C while in the septic tank pumper's truck, the hydrogen peroxide concentration can be kept at 5 mg/ml; this combined treatment virtually sterilized septage in 1 h.

A combined treatment with heat and a chemical disinfectant should be more effective than one with a chemical alone because bacterial enzymes and viruses are relatively sensitive to heat; bacterial cells become more susceptible to chemical damage after heating. Both TCA and heating to 55°C probably inhibit bacterial catalase, increasing the cells' susceptibility to hydrogen peroxide. Increased susceptibility to hydrogen peroxide during heating may well have resulted from thermal destruction of catalase, because catalase is a thermolabile enzyme; after 20 min at 37°C no catalase activity is apparent in *Escherichia coli*, even at concentrations of hydrogen peroxide lower than those used in this study (20).

TCA was added principally to control foaming caused by hydrogen peroxide's decomposition and was clearly less effective than heating to 55°C in enhancing the antibacterial action of the disinfectant. Addition of 1.2 to 4.8 mg of TCA per ml (two- to sevenfold higher concentrations than were used in this study) reduces the violent decay of hydrogen peroxide; however, no significant increase ($P > 0.05$) in hydrogen peroxide efficiency (in restoring the infiltrative capacity of clogged solids) is noted (4). The addition of TCA adds cost and another compound that needs to be measured into, and subsequently discharged with, the septage. TCA may be unnecessary if the hydrogen peroxide is applied outside the pumper's truck as the septage is being discharged.

Despite the evident need, a safe and effective septage disinfectant has not yet been identified; investigations of other disinfectants (e.g., lime) continue (3, 10, 19). Disinfection of septage by some appropriate method is desirable because the material is handled during septic tank pumping and discharge, and it is likely that the practice of illegal disposal of septage along roadsides and in waterways will continue.

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