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# Inactivation of Indigenous Viruses in Raw Sludge by Air Drying

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Air drying of raw sludge caused inactivation of indigenous viruses. A gradual loss of infectivity occurred with the loss of water until the solids content reached about 80%. A more rapid decline of viral infectivity occurred with further dewatering.

The increased use of wastewater sludge for land applications has stimulated research toward finding simple and reliable methods of destroying pathogenic organisms in this sewage product. One method suggested as a means of inactivating virus in sludge is air drying. It has been found that enteric viruses added to liquid raw sludge are destroyed when the sludge is dewatered by natural evaporation (7). Loss of viral infectivity is apparently due to the drying process itself because polioviruses maintained at low moisture levels in raw sludge without the loss of water remain infectious. Viral inactivation that occurs during the evaporation of water from sludge has been shown to be irreversible because viral particles disintegrate and release their RNA genomes, which are in a degraded state. A similar effect has been observed when moist soil containing viruses is air dried (10).

Because these previous studies were performed with a limited number of enteric viruses seeded into raw sludge or soil, the general applicability of air drying as a means of inactivating viruses in sludge has remained unknown. Wellings et al. (8) have reported infectious viruses in air-dried sludge. The significance of this result is uncertain because neither the initial virus concentration nor the final moisture level was noted. A recent report by McCaustland et al. (4) indicates that hepatitis A virus can survive in fecal material for 30 days at 42% relative humidity. Again, however, the initial and final concentrations of infectious viruses had not been measured, so the possible effect of drying on viral infectivity was not determinable.

The original study on the effects of evaporative drying on enteric viruses in sludge had been carried out with radioactively labeled viruses seeded into raw sludge (7). The study had been performed in this way so that high virus concentrations could be used, thus permitting accurate quantitation of viral inactivation, and the cause of infectivity losses could be determined. Because of recent advances in techniques for recovery of viruses from raw sludge (2), it is now possible to extend this study to include the effects of evaporative drying on indigenous viruses in sludge.

Raw sludge was used for this study because it has the highest concentration of infectious viruses. Sludge samples were collected during August 1980 from the Sewage Treatment Plant in Harrison, Ohio, and stored at −80°C until used. The sludge was thawed and blended before being poured 1 cm deep into a plastic tray. A separate sample of the same sludge was seeded with a high concentration of poliovirus type 1 (LSc2ab strain), blended for 5 min, and processed in parallel with the unseeded sample. Both trays were set in a chemical fume hood and allowed to air dry at 21°C. Control samples were taken immediately, placed in tight-sealing containers, and stored at 5 or 21°C. Samples were collected over a period of 4 days as the samples reached progressive degrees of dryness. To ensure that temperature was not a variable, all samples were stored in airtight containers at 21°C until the last sample was taken.

Three replicate portions of each sample were measured gravimetrically at the time of collection to determine the percentages of solids. The average of the three values was used as the solids content. All samples were processed immediately at the end of the experiment. An appropriate portion of each sample was then rehydrated with sterile distilled water to the original solids content of the sludge (about 5%). Viruses were eluted from the sludge and concentrated by the Freon-centrifugation-ether method previously described (2). Samples were serially diluted and then titrated for viruses in triplicate by plaque assay on BGM cells by the agar overlay procedure described previously (2).

A comparison of recoveries of viruses from

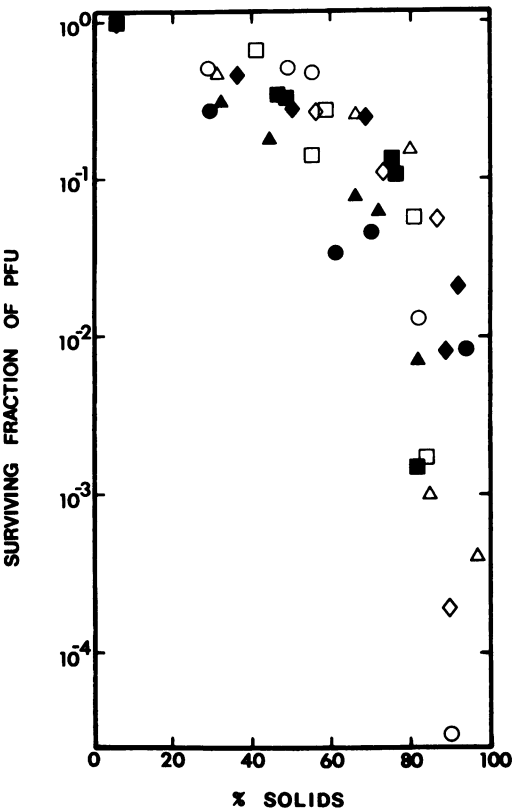


FIG. 1. Inactivation of indigenous viruses and seeded poliovirus type 1 in raw sludge during evaporative drying. Original PFU-per-milliliter values for sludge, as determined in experiments 1, 2, 3, and 4, respectively, were as follows: indigenous viruses: 285 (●), 700 (▲), 149 (■), and 276 (◆); polioviruses: $6.7 \times 10^5$ (○), $1.2 \times 10^5$ (△), $4.4 \times 10^4$ (□), and $4.2 \times 10^4$ (◇).

control sludge samples held at 5 and 21°C indicated that only a small reduction in titer occurred at the higher temperature (data not shown). Thus, temperature had a minimal effect on the survival of seeded and indigenous viruses during 4 days at 21°C in raw sludge. The effects of evaporative drying on the infectivities of viruses in sludge were determined in four separate experiments. Infectivity of seeded poliovirus and indigenous virus gradually decreased with loss of water until the solids content of the sludge reached about 80% (Fig. 1). Further evaporation caused a much more rapid decline in infectivity. This was especially evident with seeded viruses which suggests either that seeded viruses are more vulnerable during evaporative drying or that different enteric viruses are not equally sensitive during the process. The previous finding that the infectivities of polioviruses, coxsackieviruses, and reoviruses decrease more than 99.99% during evaporative drying when seeded into raw sludge (7) favors the former explanation. It is possible that the moisture content in the microenvironments in which indigenous viruses were present was actually higher than that of the environments containing seeded viruses; therefore, less drastic reductions of infectivity were seen.

A gradual decline in viral infectivity followed by a more rapid drop at low moisture levels is not unique to evaporative drying of sludge. The same effect was obtained by Yeager and O'Brien with viruses seeded into moist soil which was dried by evaporation (9). In this case, the precipitous decrease in infectivity occurred when solids increased from 97 to 99.4%. The difference between sludge and soil in solids content at which rapid decline was observed is presumably a direct reflection of the water retention capacity of the two materials. Other investigators have also reported large losses in the infectivity of viruses in soil after drying (1, 3, 5).

It is of interest that a moisture content of about 20% has been found to be required for support of bacterial growth in raw sludge (6, 11). The relationship, if any, between that observation and the finding that rapid virus inactivation during evaporation begins at about the same moisture level remains to be determined. In any event, it is apparent that evaporative drying to low moisture levels is an effective method of inactivating indigenous viruses and preventing the growth of pathogenic bacteria in sludge.

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