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Response of Bacteria in Wastewater Sludge to Moisture Loss by Evaporation and Effect of Moisture Content on Bacterial Inactivation by Ionizing Radiation

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Two studies were carried out to determine the influence of moisture content on the survival of bacteria in raw wastewater sludge. The first study involved the effect of water loss by evaporation on the bacterial population. The second used these dewatered samples to measure the effects of moisture content on the inactivation of bacteria in sludge by ionizing radiation. Both studies involved survival measurements of six representative fecally associated bacteria grown separately in sterilized sludge as well as survival data on bacteria indigenous to sludge. Growth of bacteria was stimulated in sludge during the initial phase of moisture removal by evaporation, but the reduction of moisture content below about 50% by weight caused a proportional decrease in bacterial numbers. In comparison with the original sludge, this decrease reached about one-half to one order of magnitude in all dried samples except those containing *Proteus mirabilis*, which decreased about four orders of magnitude. The rates of inactivation of bacteria by ionizing radiation in sludge were usually modified to some degree by variations in moisture content. Most bacteria were found to be somewhat protected from ionizing radiation at reduced moisture levels. The largest effect was found with *Salmonella typhimurium*, whose radiation resistance approximately doubled in dried sludge. However, no excessively large D_{10} values were found for any bacterial species tested.

Reduction of moisture content is a common method of sludge treatment at most wastewater treatment plants. At least one method of sludge dewatering, sand-bed drying to low moisture levels, is also used as a means of sludge stabilization and pathogen reduction. Loss of water by evaporation that occurs during this process has been shown to cause a large decrease in the numbers of enteric viruses in sludge (12). Reimers et al. (8) have reported that very few viable parasite ova are recoverable from drying beds containing sludge with less than 20% moisture. If dewatering sludge by evaporation were to greatly reduce its population of enteric bacteria, sand-bed drying would be an excellent method to eliminate all major groups of infective pathogens in sludge.

Treatment with ionizing radiation is another method of disinfection that has been investigated with many different organisms in a variety of media. Inactivation of enteric bacteria in sewage (6, 7, 9, 11, 14, 15) and liquid wastewater sludge (2, 4, 6, 7, 13, 14) by irradiation has also been studied by a number of investigators. These reports show that fecally associated bacteria are generally quite easily inactivated by ionizing radiation in these aqueous environments. A preliminary report from this laboratory suggested, however, that this may not be true in dewatered sludges (3). In that report, it was indicated that the apparent D_{10} value (the amount of irradiation required to inactivate 90% of a population of a particular organism or group of organisms) for total coliforms in dried raw sludge varied from 17 to 353 krad. However, in liquid sludge the D_{10} value for coliforms was consistently 20 to 30 krad.

Because of the potential use of sand-bed drying for inactivation of sludge pathogens and subsequent use of ionizing radiation to further disinfect dried sludges, it was important to know the separate and combined effects of the drying process and ionizing radiation on the survival of enteric bacteria. Therefore, these studies were performed with six representative fecally associated bacterial species grown in seeded raw sludge. To determine the general applicability of

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these results, comparative studies were made with bacteria indigenous to raw sludge.

MATERIALS AND METHODS

Bacteria. Cultures of *Klebsiella* sp., *Enterobacter* sp., and *Proteus mirabilis* used in these experiments were provided by John Uhlrich (Department of Microbiology, University of New Mexico School of Medicine, Albuquerque). Representative strains of *Escherichia coli* and *Streptococcus faecalis* were isolated from raw sludge, and the culture of *Salmonella typhimurium* was obtained from the American Type Culture Collection. Bacterial inocula were grown in Trypticase soy broth (BBL Microbiology Systems).

Growth and detection of bacteria in sludge. Raw sludge containing about 5% solids was obtained from the Albuquerque sewage treatment plant and sterilized by treatment with 6 Mrad of gamma radiation. This material was inoculated with about 4×10^6 colony-forming units (CFU) of a selected organism per g of sludge solids and incubated at 37°C for 48 h. Samples were then blended for 2 min at high speed, and serial dilutions were made in saline solution. For samples containing *Klebsiella* sp., *Enterobacter* sp., or *E. coli*, 0.1-ml fractions were spread on prepoured M-coliform agar plates and enumerated after 24 h of incubation at 37°C. Samples (0.1 ml) with *S. typhimurium* or *P. mirabilis* were spread on plates containing Hektoen enteric (HE) agar and enumerated after 24 h at 37°C. *S. faecalis* samples (1 ml) were assayed by pour plating with KF *Streptococcus* agar and counted after 48 h at 37°C, essentially as described in *Standard Methods for the Examination of Water and Wastewater* (1).

To recover indigenous CFU, samples of raw sludge were blended at high speed for 2 min and immediately plated on three separate agars: nutrient, HE, and KF *Streptococcus*. Samples (0.1 ml) of the appropriate dilutions were spread on both nutrient and HE agars, and CFU were enumerated after 24 h at 37°C. Colonies on HE agar were separated into three groups distinguishable by color: H_2S producers (black or black-centered colonies), lactose fermenters (yellow colonies), and others (colorless or green colonies). HE agar was used for this purpose because it provided the best separation of different groups of *Enterobacteriaceae* among 11 agars tested. Detection of indigenous fecal streptococci on KF *Streptococcus* agar was done as described for seeded *S. faecalis*.

It should be noted that thorough decontamination of the blenders used in these and other experiments required a treatment of more than 30 min in concentrated hypochlorite solution (bleach) or autoclaving. Lesser decontamination procedures resulted in survival of a significant number of residual bacteria in the blenders and carryover into subsequent samples.

Dewatering of sludge by evaporation. Either sterilized raw sludge containing individual bacterial species grown to saturation levels or fresh, untreated raw sludge with indigenous organisms was poured to a depth of about 1 cm in glass drying pans and dewatered by natural evaporation at 21°C. Samples taken at different moisture levels were adjusted to their original water content (about 5% solids) by blending for 2 min at high speed with calculated volumes of water and assayed immediately on duplicate plates for viable organisms to determine the effects of dewatering. Loss of water was essentially complete in all the drying pans after about 7 days, when the dried sludge contained approximately 95% solids.

Gamma irradiation of dewatered sludge samples. Samples of liquid sludge with about 5% solids and samples of dewatered sludge containing either about 50 or 95% solids were irradiated with a 95-kCi ^{60}Co source encapsulated in stainless-steel pins (dose rate, 26 krad/min). The source was stored in a water pool and raised above water level in a shielded cell to irradiate samples. Survival of both seeded and indigenous bacteria in liquid and dewatered sludges was determined by direct plating (in duplicate) in the manner described above. At least three samples were assayed at each radiation dose and moisture content. Samples containing less than 10% moisture were always analyzed in sets of six to minimize the effect of differences in bacterial density within the dried material.

RESULTS

Effect of dewatering on the survival of bacteria in sludge. To determine the effect of dewatering by evaporation on the survival of selected bacterial species in sludge, sterilized raw sludge containing about 5% solids was inoculated with one of the six model strains of fecally associated bacteria (*E. coli*, *Klebsiella* sp., *Enterobacter* sp., *S. typhimurium*, *P. mirabilis*, or *S. faecalis*) to an initial concentration of about 4×10^8 CFU per g of sludge solids. After 48 h of incubation at 37°C, each organism reached a density of about 4×10^9 CFU per g of solids. When the recolonized raw sludge samples were dewatered by natural evaporation at 21°C, bacterial CFU were generally found to increase during the initial stages of water loss (Fig. 1). However, for all bacteria studied, the initial increase was consistently followed by a reduction in CFU with further evaporation. The size of this reduction relative to the bacterial population in the original sludge was generally one-half to one order of magnitude, but *P. mirabilis* decreased about four orders of magnitude.

The effect of dewatering on the population of indigenous bacteria in fresh raw sludge was then determined (Fig. 2). The results were quite similar to those found with the seeded sludge except that more growth usually occurred during partial dewatering. Further dewatering, however, again consistently reduced these numbers below those present in the original raw sludge. The largest decrease was about two orders of magnitude, which was found for lactose fermenters on HE agar, a classification that includes bacteria such as *Escherichia*, *Klebsiella*, and *Enterobacter*. A decrease of less than one order of

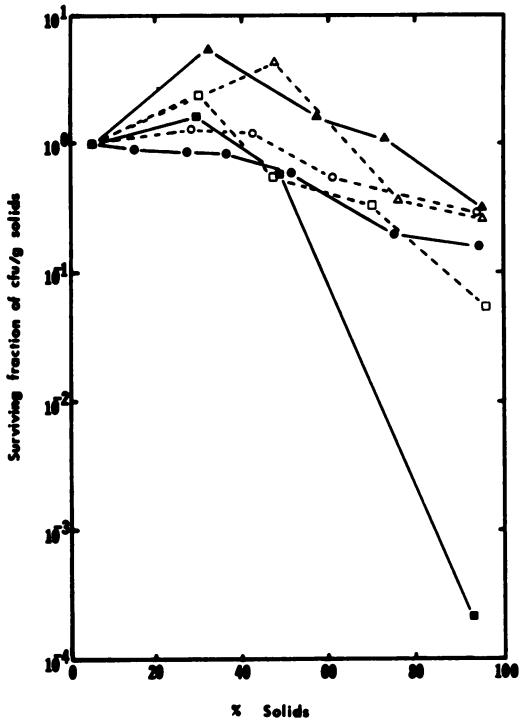

FIG. 1. *Effect of dewatering by evaporation on CFU of several selected bacteria in sludge. Raw sludge (5% solids) containing about 4 × 10⁹ CFU of a specific bacterium per g of solids was dewatered by natural evaporation at 21°C. Samples were taken at different moisture levels and assayed immediately for CFU. The surviving fraction of CFU was calculated relative to a sample held at 21°C without loss of water for the same period of time. Symbols: ●, S. faecalis; △, E. coli; ○, Klebsiella sp.; ▲, Enterobacter sp.; □, S. typhimurium; ■, P. mirabilis.*

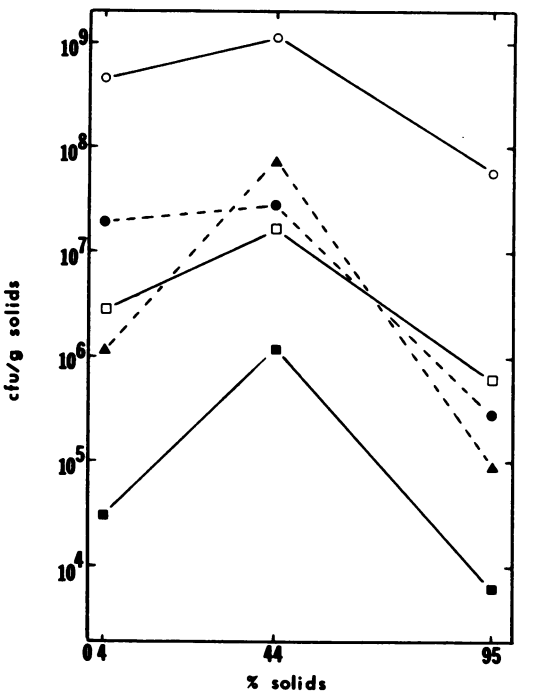

FIG. 2. *Effect of dewatering by evaporation on CFU of several groups of indigenous bacteria in raw sludge. Raw sludge (4% solids) was dewatered by natural evaporation at 21°C and assayed for recoverable CFU with nutrient agar, KF Streptococcus agar, and HE agar when samples reached 44 and 95% solids. Symbols: ○, total CFU (nutrient agar); □, fecal streptococci CFU (KF Streptococcus agar); ■, H_2S producers; ●, lactose fermenters; ▲, other CFU (HE agar).*

magnitude was observed on this same agar for H_2S producers, which included *Salmonella* and *Proteus* species, both of which are recognized pathogens.

Moisture effects on bacterial inactivation with ionizing radiation. The inactivation of bacteria with ionizing radiation in dewatered sludge was examined next. Samples chosen for this study were the original sludge (5% solids) included as a control, partially dewatered sludge (about 50% solids) included to represent sludge obtained after such processes as vacuum filtration, centrifugation, or composting, and dried sludge (about 95% solids) included to represent material obtained from a drying bed after thorough dewatering.

The effects of sludge dewatering on the inactivation rates of selected bacteria by ionizing radiation varied for the different bacterial species (Table 1). The radiation inactivation rate of

TABLE 1. *Effect of water content on the D_{10} value of selected bacteria in raw sludge*

Bacterium	Water content (% solids)	D_{10} value (krad)
E. coli	5	<22–25
	47	22–26
	95	<22–36
Klebsiella sp.	5	41–50
	42	48–92
	94	36–55
Enterobacter sp.	5	34–38
	57	36–54
	95	34–62
S. typhimurium	5	<54–71
	47	<60–77
	97	100–140
P. mirabilis	5	<24
	49	<22
	93	<50
S. faecalis	5	130–180
	51	110–160
	94	120–250

the *E. coli* strain used in this study was not detectably altered by dewatering. However, the *Klebsiella* sp. was protected in partially dewatered but not by fully dewatered sludge, and the *Enterobacter* sp. appeared to be slightly protected at both levels of dewatering.

This protective effect was much more pronounced with *S. typhimurium*, where the D_{10} value found in liquid sludge approximately doubled in dried sludge. *P. mirabilis*, the other H_2S producer studied, was very sensitive to ionizing radiation at all moisture levels. Finally, it was found that *S. faecalis* was, as expected, the most radiation-resistant bacterium at all moisture levels and was slightly, but not greatly, protected by dewatering sludge. *S. faecalis* and other fecal streptococci have been consistently found to be more resistant to ionizing radiation than most other bacteria associated with sewage (3, 4, 6, 7, 9–11).

Lastly, the radiation inactivation rates of several classes of indigenous microorganisms in dewatered raw sludge were determined (Table 2). Indigenous fecal streptococci were found to be quite resistant to ionizing radiation in sludge as expected. However, their resistance decreased somewhat with dewatering. It is unclear why this should occur because the resistance of seeded *S. faecalis* increased slightly in dried sludge (see Table 1). Lactose-fermenting bacteria, H_2S producers, and other CFU detected on HE agar all had D_{10} values which were not unusually high in either liquid or dewatered sludge.

DISCUSSION

The data presented here support two main conclusions regarding the effects of water loss on the survival of bacteria in sludge. The first conclusion is that loss of water by evaporation in itself does not appear to be an adequate method for reducing the bacterial population in sludge. This conclusion is supported by the observations that recoverable CFU from sludge actually increased during the initial phase of evaporation and, even when taken to near dryness, the observed reduction in bacterial CFU was generally only about one order of magnitude or less.

The second conclusion supported by these data is that the rates of bacterial inactivation by ionizing radiation in sludge can be altered by changes in water content but that the amount and direction of alteration varies between bacterial species. Although the observed alteration was quite significant for some organisms, e.g., *S. typhimurium*, in no case was moisture loss found to result in an excessively large D_{10} value. This is in contrast to the results reported earlier by Brandon and Neuhauser (3). It is likely that their findings came as a result to the use of contaminated blenders, a problem which was recognized and carefully avoided in this study.

Because of possible difficulties associated with detection of bacteria in sludge and other environmental samples (5), a percentage of the bacterial population may have escaped detection throughout this study. Possibly, evaporation of water from sludge caused bacteria to become more tightly associated with sludge particles and increased the percentage which escaped detection. This does not appear to be the case with the initial water loss, which generally caused an increase in CFU recoverable from sludge. However, the decreases in CFU observed with large reductions in water content may have been partially due to decreased detectability. If this were the case, it adds support to the conclusion that evaporative drying is not an effective method of inactivating bacteria in sludge.

The implication of these results is that sand-bed drying of sludge and similar treatment processes may not provide adequate bacterial inactivation. Under field conditions, other factors such as increased temperature and ultraviolet radiation could also contribute to bacterial inactivation in drying beds. However, when anaerobically digested, sand-bed-dried sludges (>90% solids) from the Albuquerque sewage treatment plant were tested, levels of lactose fermenters and H_2S producers in excess of 10^6 CFU/g were detected on HE agar (data not shown). This finding is important in view of reports that both enteric viruses and parasite ova may be destroyed during sand-bed drying (8, 12). If it can be definitively shown that the indigenous bacteria in sludge that survive drying include pathogenic species, these bacteria may represent the most significant potential pathogenic threat in dried sludge.

It should be mentioned that the D_{10} values shown for the different bacteria at different moisture levels were derived from straight line fits of the data. A range of values was presented in order to encompass variations in recoverable

TABLE 2. *D_{10} values for indigenous microorganisms in liquid and dewatered raw sludge*

Sludge (% solids)	D_{10} value (krad)[a]			
	Fecal streptococci	Lactose fermenters	H_2S producers	Others
4	190–240	28–36	<60	<40
44	110–220	26–36	<30	<20
95	70–140	<50	<85	<60

[a] Fecal streptococci were assayed on KF *Streptococcus* agar, and the other groups were measured on HE agar.

CFU between replicate samples. However, in no instance was the D_{10} value excessively large. This leads to the conclusion that treatment with ionizing radiation could be a feasible method of ridding both liquid and dewatered sludges of bacterial pathogens.

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