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Project Summary

Inactivation of Enteric Pathogens During Aerobic Digestion of Wastewater Sludge

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A study was conducted to provide data on the ability of aerobic digestion to reduce pathogens and to determine the effect of important variables. Laboratory and field studies investigated the effects of aerobic and anaerobic digestion on enteric viruses, enteric bacteria, total aerobic bacteria, and intestinal parasites. Under laboratory conditions, the temperature of the sludge digestion was the major factor influencing survival of bacteria and viruses. The survival of both bacteria and viruses was increased substantially by decreasing the temperature of sludge digestion from 28° to 7°C. Lowering the temperature or dissolved oxygen level reduced the percentage of solids-associated organisms for bacteria but not for viruses.

Bacteria were inactivated at different rates during aerobic sludge digestion. *Streptococcus faecalis* was more stable than *Salmonella typhimurium* or *Escherichia coli*. Varying detention time or source of sludge did not affect the rate of inactivation of viruses or bacteria.

Aeration of stock *Ascaris suum* ova in 0.1 N H_2SO_4 resulted in 91% embryonation. However, aeration in sludge resulted in only 19% to 50% embryonation after 30 days. Most of the ova embryonated in 0.1 N H_2SO_4 (79% to 93%) were infective for rats. In contrast, only 9% to 12% of the ova embryonated in aerobically digesting sludge were infective for rats.

Full-scale aerobic digestion of sludge reduced densities of bacteria and enteroviruses. In most cases, the reductions were close to the maxima predicted for completely mixed digesters that continually receive undigested sludge.

The number of parasitic ova varied greatly with the community served by the treatment plant. Parasitic ova were recovered from most of the samples of digested and undigested sludge from certain treatment plants but not from others. The relatively low numbers of ova recovered from the sludge samples make it difficult to evaluate the effects of sludge digestion on these agents. The inactivation rate of laboratory-grown bacteria in aerobically digested sludge was higher than that for indigenous bacteria. The indigenous bacteria were probably better protected from predation by protozoans and other animals. Predation was the major factor influencing survival of both indigenous and laboratory-grown bacteria during aerobic digestion of sludge under laboratory conditions. Predation had little effect on survival of viruses, however.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

A large number of enteric bacteria, viral pathogens, and parasitic ova may be excreted by infected individuals and may therefore be present in untreated sewage. Since a large number of these pathogens become associated with wastewater so-

lids, many are not completely inactivated during sewage treatment processes and are merely transferred to wastewater sludges. These sludges are processed further, generally by aerobic or anaerobic digestion, to improve their dewaterability, reduce their unpleasant odor, and reduce their pathogen content.

The ability of digestion processes to reduce sludge pathogens has received considerable investigation, though numerous data gaps exist. The information is particularly sparse for aerobic digestion. This investigation was undertaken to provide more definitive information on the ability of aerobic digestion to reduce pathogens and to determine the effects of important variables. The investigation studied the effects of aerobic digestion on pathogenic bacteria, bacterial indicator organisms, parasitic ova, and animal viruses.

Experimental Procedures

In laboratory experiments (which employed temperature-controlled, laboratory, aerobic and anaerobic digesters), cultured bacteria and viruses were generally used. In field studies, conducted at existing plants in the vicinity of Gainesville, Florida, indigenous microbial species were used.

Laboratory Experiments

Aerobic digestion was conducted in temperature-controlled vessels holding 15 L of sludge. Sludge was mechanically agitated, and humidified air was introduced through spargers. Anaerobic digestion was conducted in externally heated carboys holding 8 L of sludge. Bacteria and viruses charged to the digesters were grown from type cultures. *Ascaris suum* ova were obtained from the feces of naturally infected hogs. Detailed procedures for growing organisms and measuring densities are presented in the report.

Survival of bacteria and viruses and the association of these agents with sludge flocs were studied in 11 individual trials. During each trial, two to four digesters were operated under different conditions of temperature, dissolved oxygen, and detention time. Aerobically digested sludge was obtained from one of three local treatment plants and aerated for 2 to 4 days at the desired temperature before the experiments were started, thus stabilizing the sludge at the desired conditions before an experimental trial was started. An anaerobic sludge digester was started using anaerobically digested sludge obtained from Tallahassee. At the beginning of each trial, bacteria and viruses were added to each of the digesters to obtain an initial concentration of approximately 10^5/mL. On each subsequent day, a portion of sludge was removed from each digester for analysis, and a portion of wasted sludge from the plant that was the source of the aerobically digested sludge was seeded with bacteria and viruses and added to the digesters. The wasted sludge that was used for the daily additions to the digesters was obtained at the beginning of each trial and was kept at 4 °C without aeration. The volume of digested sludge removed and replaced with wasted sludge was determined by the detention time desired: 1/15 to 1/40 the volume for 15- or 40-day detention times, respectively.

Laboratory studies were also conducted on the survival of seeded *Ascaris suum* in aerobically digested sludge. Three aeration procedures were used that produced different degrees of mechanical abrasion on the ova. Aeration was provided by shaking 200 mL of sludge in 500-mL flasks on a reciprocal shaker, by mixing 200 mL of sludge in 1000-mL beakers using a magnetic stirrer, or by aerating 200-mL samples in 500-mL flasks with air diffusers connected to a small air pump. All three methods provided approximately 5 mg/L of dissolved oxygen. The aerobic digestion was run under two conditions: batch operation and daily feeding to simulate operation with 40-day residence. Samples of the sludge were examined for total recovered ova, ova embryonation, and ova infectivity in rats.

Field Studies

Sludge samples from nearby wastewater treatment plants were obtained over two periods: From January to December 1981, and from August to October 1983. The sludge samples were examined for the presence of enteric bacteria, enteric viruses, and parasitic ova. The dissolved oxygen, temperature, pH, and total solids of the sludge samples were also determined. Unlike the laboratory experiments, no organisms were seeded into the sludges; only densities of indigenous microorganisms were measured.

Results and Discussion

Bacteriological Investigations

Laboratory Studies

The influence of temperature and dissolved oxygen level on inactivation of bacteria during aerobic and anaerobic sludge digestion was studied in 11 trials, each lasting approximately 9 days. The results from one trial appear in Table 1, which presents the log daily change in bacterial densities. The log daily change is the difference between the logs of the bacterial densities of the sludge in the digester shortly after introduction of the feed sludge containing the inoculum and the sludge withdrawn from the digester 24 hr later (before addition of the next batch of feed). Table 1 shows the effects of temperature (28.3° and 6.2 °C) and dissolved oxygen concentration on the bacteria in one of the trials. These results are typical of those obtained in other trials. Wasted, aerobically digested sludge from a nearby plant was added daily to the digesters to provide a detention time of 15 days. Results show much greater reductions for *S. typhimurium, S. faecalis,* and *E. coli* than for *P. aeruginosa* and total aerobic bacteria. For all bacterial types, the log daily change was much lower at 6.2 °C than at 28.3 °C (compare Conditions I and II in Table 1). The presence or absence of air (compare Conditions I and III in Table 1) produced a small effect that was usually not significant.

The bacteria in the digesting sludge were either in the supernatant or were occluded to solids. Operation at 28.3 °C in the aerobic state showed a markedly different distribution of bacteria from the other conditions, averaging about 8% in the supernatant compared with 36% for the other conditions. Subsequent experiments confirmed that grazing by protozoa accounted for the lower proportion of bacteria in the supernatant at 28.3 °C and contributed to the greater daily reduction in bacteria obtained at these conditions.

Subsequent trials with sludges from three different wastewater treatment plants sludges showed that the source of sludge did not significantly affect the results.

In all of the previously described experiments, bacteria were added to the digesters daily along with fresh sludge. The values for change in total bacteria therefore reflect changes that occurred in the first 24 hr following bacterial addition. To determine whether the rate of daily change in bacteria would fall off with time, indicating the presence of a resistant fraction of added bacteria, digesters were operated under aerobic conditions without the addition of bacteria or sludge. Two digesters were operated at 28 °C and one at 6 °C. All digesters were operated with greater than 4 mg/L dissolved oxygen. Results with *S. typhimurium, S. faecalis,* and total aerobic count showed that curves of log density versus time were approximately linear (with negative slopes) over the

Table 1. *Bacterial Survival and Association with Sludge Flocs During Aerobic and Anaerobic Digestion of Sludge: The Influence of Sludge Digestion Conditions on Individual Bacteria*

Bacteria	Sludge Digestion Condition*	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Total Solids (g/L)	Daily Change in Total Log_{10}	Bacteria in Supernatant (%)
Salmonella typhimurium	I	28.3	2.7	6.0	20.6	$-1.24^{A\dagger}$	8^{C}
	II	6.2	3.7	7.4	19.6	-0.23^{B}	23^{B}
	III	28.0	0	6.3	18.4	-0.90^{A}	51^{A}
Streptococcus faecalis	I	28.3	2.7	6.0	20.6	-0.92^{A}	12^{B}
	II	6.2	3.7	7.4	19.6	-0.10^{B}	50^{A}
	III	28.0	0	6.3	18.4	-0.83^{A}	47^{A}
Escherichia coli	I	28.3	2.7	6.0	20.6	-1.13^{A}	4^{B}
	II	6.2	3.7	7.4	19.6	-0.23^{C}	25^{A}
	III	28.0	0	6.3	18.4	-0.61^{B}	30^{A}
Pseudomonas aeruginosa	I	28.3	2.7	6.0	20.6	-0.28^{A}	3^{B}
	II	6.2	3.7	7.4	19.6	-0.29^{A}	34^{A}
	III	28.0	0	6.3	18.4	-0.34^{A}	47^{A}
Total aerobic bacteria	I	28.3	2.7	6.0	20.6	-0.34^{A}	7^{B}
	II	6.2	3.7	7.4	19.6	-0.07^{B}	21^{A}
	III	28.0	0	6.3	18.4	-0.09^{B}	33^{A}

**Condition I = aerobic; Condition II = aerobic; and Condition III = anaerobic.*
†Figures in a single column with identical letter superscripts are not significantly different.

course of several days, indicating no persistent fraction of bacteria. The rate of inactivation was substantially lower in these experiments than when inocula were added daily (compare Tables 1 and 2). The greater reduction in supernatant bacteria at the higher temperature (Table 2) is consistent with the inoculum experiments.

Field Studies

Summarized data from field studies are presented in Table 3. Plants 1 and 2 used aerobic digesters, and Plant 3 used an anaerobic digester. For the aerobic digesters, greater bacterial reductions were achieved in Trial 2 than in Trial 1. The probable explanation for this difference was digestion temperature: Trial 1 was conducted during colder months. All of the digesters were completely mixed. With complete mixing, some sludge inevitably short-circuits to the exit with relatively short-term treatment. Bacterial reduction was calculated assuming complete mixing and destruction of bacteria except for bacteria in that portion of the feed that leaks through to the exit. Log reductions calculated on this basis (labeled "maximum possible") are presented in Table 3 where they can be compared with experimentally determined reductions. For Trial 2, experimental and calculated values are similar; this result supports the assumptions of the calculation and indicates that use of complete mix reactors may contribute to failure to achieve high bacteria or virus reductions in digesters.

Virus Investigations

Laboratory Studies

Laboratory studies determined the influence of several variables on the survival of viruses during aerobic and anaerobic digestion of sludge under laboratory conditions. The variables studied included temperature, dissolved oxygen level, detention time, virus type, and the source of the sludge used for digestion studies.

The sludge source did not significantly affect the rate of inactivation of poliovirus when sludge was aerobically digested (15-day detention time). At 28 °C and dissolved oxygen levels of 5 mg/L, the mean daily change (log_{10}) in inactivation of seeded poliovirus (Type I, L_{sc}) for sludges from three sources ranged from -0.71 to -0.97, which was not a significant difference.

The temperature of sludge digestion influenced the rate of inactivation of poliovirus (Table 4), which was highest at 28 °C, intermediate at 17.6 °C, and lowest at 5.5 °C.

Varying the dissolved oxygen between 1 and 6 mg/L at a relatively constant temperature of 28 °C did not change the inactivation rate. The mean daily change (log_{10}) ranged from -0.77 to -1.03. Anaerobic digestion of the same sludge at 32 °C produced a mean daily change (log_{10}) of -0.33, significantly lower than for aerobic digestion.

Varying the detention time of aerobic digestion between 16 and 40 days did not change the inactivation rate (log daily change) of poliovirus.

Poliovirus 1, echovirus 1, coxsackievirus B3, and the simian rotavirus SA-11 were all inactivated at similar rates during aerobic digestion of sludge at 28 °C. The range of log daily change was -0.46 to -0.77.

Aerobically digested liquid sludge when allowed to dry in a centrifuge tube from about 1 to 50 g/L over 28 days showed essentially no surviving viruses (poliovirus), whereas 5% survived in a tightly capped control.

Table 2. *Bacterial Survival and Association with Sludge Flocs During Aerobic Digestion of Sludge without Addition of Fresh Sludge*

Bacteria	Daily Change in Total Bacteria Log_{10}		Bacteria in Supernatant (%)	
	6°C	28°C	6°C	28°C
Salmonella typhimurium	-0.15	-0.48	53^{B}*	3^{D}
Streptococcus faecalis	-0.05	-0.23	80^{A}	2^{D}
Total aerobic bacteria	-0.07	-0.19	41^{C}	3^{D}

**Figures with the same letter superscript are not significantly different.*

Field Studies

Viruses were detected in samples of mixed liquor solids and aerobically digested sludge. The lowest levels were found in sludge from the second aerobic digester at the plants studied. However, the numbers of viruses were so variable that statistical analyses did not show that the differences were significant except in a few cases. Results ranged from 0 to 20

Table 3. *Summary of Field Data on Bacterial Reduction During Aerobic and Anaerobic Digestion of Sludge*

Treatment Plant*	Bacteria	Bacterial Reduction† (Log_{10}) Trial 1‡	Trial 2§
Plant 1 (aerobic digester)	Total coliforms	-1.53	-1.99
	Fecal coliforms	-1.52	—
	Fecal streptococci	-1.03	-1.63
	Aerobic bacteria	-0.95	—
	Maximum possible**	-1.86	-1.86
Plant 2 (aerobic digester)	Total coliforms	-1.02	-1.92
	Fecal coliforms	-1.09	—
	Fecal streptococci	-0.77	-1.71
	Aerobic bacteria	-0.80	—
	Maximum possible**	-1.70	-1.70
Plant 3 (anaerobic digester)	Total coliforms	—	-0.91
	Fecal streptococci	—	-1.10
	Maximum possible**	—	-1.00

* *Plant 1: Influent sludge and second of two 8.5-day residence time aerobic digesters in series*
Plant 2: Sludges in first and second of two aerobic digesters, each with 50 days of residence time
Plant 3: Influent sludge and sludge from an anaerobic digester with 10 days of residence time.

† *Log bacterial density in digested sludge - log density in undigested sludge.*

‡ *January through December 1981.*

§ *August through October 1983.*

** *Calculated assuming total destruction of bacteria except for leakage caused by complete mixing: 1:72.2 for Plant 1, 1:50 for Plant 2, and 1:10 for Plant 3.*

Table 4. *Effect of Temperature on Poliovirus (Type 1, L_{sc}) Survival in Laboratory-Scale Aerobic Digesters*

Temperature (°C)	Dissolved Oxygen (mg/L)	Total Solids (g/L)	Volatile Solids (g/L)	pH	Mean Daily Change in Virus Survival* (Log_{10})
28	5.8	10.4	4.8	5.3	-0.77^A
17.6	5.2	7.9	5.7	6	-0.5^B
5.5	5.8	7.9	5.2	5.3	-0.21^C

* *Means within the same column with the same letter are not significantly different at the p = 0.05 level.*

plaque-forming units (PFU/g) of sludge solids.

Poliovirus and coxsackievirus serotypes were isolated from the different sludge samples. Compared with wasted sludge before digestion, the aerobically digested sludge contained relatively few types of viruses.

Parasite Investigations

Laboratory Studies

Three methods of mixing (magnetic stirring, shaking, and aeration with air pumps) were used to test the effects of aerobic sludge digestion on the embryonation of *Ascaris* ova. When sludge samples were mixed mechanically by magnetic stirrers, 85% to 90% of the ova were physically destroyed. Those ova that were recovered showed gross abnormalities such as vacuolation, cracked egg coats, and granulation.

Shaking the sludge sample on reciprocating shakers during aerobic digestion resulted in 32% embryonation after 40 days of shaking and 19% embryonation after 35 days. In only one experiment were 52% of the recovered ova embryonated. No ova were embryonated in one experiment that shook the sludge sample for 49 days. Most of the nonembryonated ova in this trial were vacuolated, showed increased granulation, and were physically distorted. The percentages of unfertilized ova present in shaken sludge samples decreased within 10 to 14 days. After 10 days of shaking in one trial, 40% to 60% of the initially seeded ova were arrested in the 2-, 4-, and 8-cell stages. This result indicated that embryonation had been initiated but stopped early in the cleavage stage of development.

Aeration of aerobic sludge using an air pump produced the highest percentages of ova embryonation. After 30 to 35 days of aeration, 62% to 68% of the ova were embryonated in sludges. The percentages of nonembryonated ova consistently decreasesd over a 30- to 42-day period of aeration. Increasing the detention time of digestion up to 42 days produced little change in the percentage of the embryonation of the *Ascaris* ova. Embryonated ova recovered from aerated sludge digesters showed no distortion, vacuolation, or granulation.

The infectivity of ova recovered from sludge aerated either by shaking or with air pumps was examined in two trials. In each trial, rats were dosed with ova from sludge, embryonated ova from hog feces (positive control), and physiological saline containing no ova (negative control). In the first trial, an average of 9% (11% and 8%) of the ova recovered from shaken sludge were infective to rats, as demonstrated by the recovery of third-stage larvae from rat lungs. An average of 93% (95% and 91%) of the embryonated positive controls and no larvae from the negative controls were recovered. In the second trial, an average of 12% (10% and 14%) of the ova recovered from sludge aerated by air pumps were infective. Postivie controls showed an average infectivity of 79% (83% and 75%), and no larvae were demonstrated in the lungs of negatively controlled rats. Recovered larvae in all experiments appeared to be normal and active.

Ascaris ova are highly resistant to various acids, alkalies, and corrosive chemicals. The inner, lipoid, vitelline membrane is primarily responsible for this effect. Ova surrounded only by these membranes are resistant to these chemicals. The primary lipid component of the vitelline membrane is a waxy alcohol known as ascaryl alcohol. Ascaryl alcohol appears to be a mixture of three closely related compounds—ascosides A, B, and C. The lipid nature of this membrane makes it susceptible to organic solvents, surface active agents, and noxious gases. Possibly, the nonembryonation and physical deformities of ova may be caused by various chemicals or gases that are generated during aerobic

digestion and that destroy or alter the physiological integrity of this vitelline membrane. Once the membrane is altered, the primary protective barrier is breached, and the developing embryo is unprotected. Alterations occur at this point.

Field Studies

A number of human parasites were demonstrated in locally collected, undigested sludge and sludge obtained from aerobic and anaerobic digesters. Four human parasites were recovered from sludge: *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis,* and hookworm. These are the most common of the intestinal helminth parasites of man. The most common parasite present in sludges from the four collection sites was the common pin worm or seat worm, *E. vermicularis*. The second most common parasite was the large intestinal roundworm, *A. lumbricoides*. Hookworm and the whip worm, *T. trichiura*, were also present, but to a small extent. Sludge samples from treatment plants in Tallahassee, Florida, showed the highest incidence of indigenous parasite ova, followed (in order) by the Main Street, Kanapagha, and University of Florida treatment plants in Gainesville, Florida. Parasite ova were recovered from all sludges, regardless of their treatment.

An unexpected observation during examination of the sludge samples was the high incidence of the intestinal helminth *E. vermicularis* in Tallahassee, Florida. This parasite has a wide geographic range in the United States. Cheng reported an infection rate of 32.9% in the American population. *Enterobius* is the most common helminth infection in the United States, sometimes reaching infection rates of 60% in children and adults in various institutions in Florida. An infection rate of 26.8% was reported among students of five elementary schools in and around Tallahassee, Florida, These various studies suggest that Tallahassee, Florida, is an endemic loci of enterobiasis in the state of Florida.

Ascaris and *Trichuris* are the most common and cosmopolitan helminth infections in the world. The prevalence of these infections in the population of North America has been estimated at 4 million for *Ascaris* and 2.2 million for *Trichuris.* These two infections are still extremely common in the rural areas of the southern United States. With the prevalence of these infections in the population and with the fecundity of these parasites (*Ascaris* produces 200,000 ova/day per female, and *Trichuris* produces 1,000 to 46,000 ova/day per female), it is not surprising that these parasitic ova were found in locally collected sewage sludge.

Conclusions

Indicator and pathogenic bacteria were inactivated during aerobic digestion of sludge under laboratory conditions. Protozoans and other predators were the primary agents responsible for inactivating bacteria. Bacterial densities in undigested sludge are reduced during aerobic treatment of sludge under field conditions.

The addition of fresh, undigested sludge to aerobic digesters results in contamination of the digested sludge with undigested sludge and thus diminishes the effectiveness of the process for reducing bacterial densities.

Enteric viruses are also inactivated during the aerobic digestion of sludge under laboratory conditions. In contrast with bacteria, the presence or absence of predators had little influence on the inactivation of viruses. Aerobic treatment of sludge under field conditions reduces the density of enteric viruses. As with bacteria, contamination of digested sludge with fresh, undigested sludge leads to the presence of viruses in the digested sludge.

Aeration of *Ascaris suum* ova in the presence of 0.1 N H_2SO_4 contamination with fungi led to embryonation of more than 90% of the ova. Approximately 80% of these ova were infective for rats. In contrast, aeration in aerobically digested sludge resulted in embryonation of 50% or fewer of the ova. Only some 10% of these ova were infective for rats. Thus aerobic treatment of sludge reduces the ability of ova in the sludge to embryonate and reduces the infectivity of those ova that are embryonated. The relatively low numbers of parasitic ova in undigested and aerobically digested sludge from treatment plants made it difficult to determine the effects of aerobic sludge treatment on these ova under field conditions.

Recommendations

The presence of bacterial pathogens, enteric viruses, and parasitic ova in aerobically digested sludge suggests two major areas of future research: (1) the fate of these microbes during subsequent treatment or after disposal (including during sludge drying and application to land), and (2) modification of existing sludge treatment processes to reduce pathogens further. One modification of sludge treatment that should be considered is the aeration of sludge in batches without addition of fresh, undigested sludge. All sludge removed from digesters operated in this manner would be treated for the same length of time and would not be contaminated with the bacterial pathogens, enteric viruses, and parasitic ova that are often found in undigested sludge. The possibility of raising the temperature of sludge digestion should also be considered.

Additional studies are needed on the fate of parasitic ova during aerobic sludge digestion. Additional laboratory studies are required to confirm our findings on the effects of aerobic sludge digestion on the embryonation and infectivity of *Ascaris suum* ova. Larger volumes of sludge from treatment plants should be processed to provide the large numbers of ova needed for embryonation and infectivity studies.

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The complete report, entitled "Inactivation of Enteric Pathogens During Aerobic Digestion of Wastewater Sludge," (Order No. PB 86-183 084/AS; Cost: $11.95, subject to change) will be available only from:
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