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Bacterial Survival and Association with Sludge Flocs During Aerobic and Anaerobic Digestion of Wastewater Sludge Under Laboratory Conditions

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The fate of indicator bacteria, a bacterial pathogen, and total aerobic bacteria during aerobic and anaerobic digestion of wastewater sludge under laboratory conditions was determined. Correlation coefficients were calculated between physical and chemical parameters (temperature, dissolved oxygen, pH, total solids, and volative solids) and either the daily change in bacterial numbers or the percentage of bacteria in the supernatant. The major factor influencing survival of *Salmonella typhimurium* and indicator bacteria during aerobic digestion was the temperature of sludge digestion. At 28°C with greater than 4 mg of dissolved oxygen per liter, the daily change in numbers of these bacteria was approximately -1.0 $\log_{10}$/ml. At 6°C, the daily change was less than -0.3 $\log_{10}$/ml. Most of the bacteria were associated with the sludge flocs during aerobic digestion of sludge at 28°C with greater than 2.4 mg of dissolved oxygen per liter. Lowering the temperature or the amount of dissolved oxygen decreased the fraction of bacteria associated with the flocs and increased the fraction found in the supernatant.

A large number of enteric bacteria and viral pathogens 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 solids (4–6, 15, 16, 18, 20, 21), many are not completely removed during sewage treatment processes (16) and are merely transferred to wastewater sludges. The latter are further digested to stabilize sludge solids, assist in the dewatering process, sometimes generate energy (methane from anaerobic sludges), and incidentally further inactivate microbial pathogens (7). In the United States, most of the sludge is processed by anaerobic digestion. For this reason, most research has been concentrated on the fate of pathogens after anaerobic digestion of sludge. It is well established that anaerobic digestion of sludge does not completely remove bacterial or viral pathogens (8, 10, 12, 14, 17, 19). In a recent study, Dudley et al. (8) demonstrated the quantitative or semiquantitative recovery of *Pseudomonas, Staphylococcus, Mycobacterium, Clostridium, Klebsiella, Salmonella*, and *Shigella* species from anaerobically digested sludge. Laboratory experiments (3, 9, 18, 23) as well as field studies (2) have shown that anaerobic (both mesophilic and thermophilic) digestion does not completely destroy viruses. Thorough inactivation of poliovirus 1, coxsackie B3, and *Salmonella paratyphi* B was obtained after pasteurization of digested sludge (10).

Few data are available on inactivation of pathogenic and indicator microorganisms in mesophilic aerobically digested sludge. It appears that most of the work was done on thermophilic aerobic digestion of sludge. Smith et al. (21) reported that *Salmonella* and *Pseudomonas* species, fecal streptococci, and total aerobic bacteria were significantly reduced by aerobic treatment of sludge at 56°C. Drnevich and Smith (48th Annual Water Pollut. Control Fed. Conf., Miami Beach, Fla., 1975) seeded sludge with *Salmonella* spp. After 24 h of aerobic digestion at 45°C, the level of the pathogen was below detectable limits. Kabrick et al. (13) compared the survival of *Salmonella* spp., *P. aeruginosa*, bacterial indicators, viruses, and parasites during autoheated aerobic sludge digestion (54 to 65°C) and mesophilic anaerobic digestion (35°C). They found that in all cases, the microorganisms and parasites were reduced to lower levels in autoheated aerobic digesters than in anaerobic digesters.

Since little information on survival of pathogens and indicator microorganisms during mesophilic aerobic digestion of sludge was available, the present study was conducted to determine the effect of controlled variables such as detention time, sludge source, temperature, and dis-

solved oxygen, and uncontrolled variables such as pH, total solids, and volative solids on the survival of such bacteria during aerobic sludge digestion. For comparison, bacterial survival during anaerobic digestion of sludge was also studied.

MATERIALS AND METHODS

Experimental procedure: general description. Survival of bacteria and the association of bacteria with sludge flocs was studied in 11 individual trials that lasted for an average of 9 days. 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 before the experiments were started to achieve the desired operating conditions. An anaerobic sludge digester was used along with the aerobic digesters in most studies. At the beginning of each trial, bacteria were added to each of the digesters to provide 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 added to the digesters. The wasted sludge that was used for daily additions to the digesters was obtained at the beginning of each trial and was kept at 4°C. The volume of digested sludge removed and replaced with wasted sludge was determined by the detention time desired, 1/15 or 1/40 the volume for 15 or 40 days detention time, respectively.

Digesters. Aerobic digestion of sludge was carried out in 10-gallon (37.85-liter) rectangular aquaria (approximately 40 by 20 by 20 cm) containing 15 liters of sludge. Continuous mechanical agitation was provided by stirrers powered by external motors. Humidified air was added through spargers located at the corners of each tank. This arrangement permitted agitation of sludge throughout the tank and prevented the accumulation of sludge at the corners. Aquarium heaters were used to raise the temperature of digested sludge above ambient temperatures. Digesters were placed in a refrigerated incubator for digestion studies at below-ambient temperatures.

Anaerobic digestion of sludge was carried out in glass carboys containing 8 liters of sludge. The digesters were contained in a heated external water bath maintained at 28 or 34°C. Gas produced by the digester was collected in a water-filled carboy.

Sources of sludge. Aerobically digested sludge and wasted sludge were obtained from wastewater treatment plants serving the city of Gainesville (Main Street and Kanapaha plants) and one serving the University of Florida. Anaerobically digested sludge from the city of Tallahassee wastewater treatment plant was used as the initial inoculum for the anaerobic digester.

Bacteria and preparation of bacterial inocula. *Salmonella typhimurium* (ATCC 13311), *Streptococcus faecalis* (ATCC 6569), and *P. aeruginosa* (ATCC 10145) were obtained from the American Type Culture Collection. An *Escherichia coli* strain bearing the RP-4 plasmid, which confers resistance to ampicillin and kanamycin, was obtained from the Department of Microbiology and Cell Science Stock collection. Inocula for sludge digesters were prepared by growing the bacteria in 3% tryptic soy broth (Difco Laboratories, Detroit, Mich.) overnight at 37°C under static conditions. The bacteria were collected by centrifugation at 14,000 × *g* for 10 min. The bacteria were washed with and suspended in phosphate-buffered saline. The absorbance of diluted suspensions of the bacteria was determined at 550 nm to standardize inocula. Approximately 5 ml of a stock suspension with an absorbance of 10 (calculated from diluted samples) at 550 nm was added, together with fresh wasted sludge, to the digesters each day. These inocula provided approximately equal amounts (10^5/ml) of the different bacteria used.

Bacterial analyses. Samples (50 ml) of the sludge that were removed from the digesters were centrifuged at 500 × *g* for 5 min. This was sufficient to sediment the sludge flocs but did not remove appreciable numbers of individual bacteria. A 5-ml volume of the supernatant fraction was removed and replaced with 5 ml of a solution containing (per 100 ml of water): 9 g of NaCl, 0.1 g of $Na_4P_2O_7$, and 0.1 g of LubrolWX (11), all of which were obtained from Sigma Chemical Co., St. Louis, Mo. The samples were then mixed with a Tekmar homogenizer (Tekmar Co., Cincinnati, Ohio) for 1 min. The supernatant samples and the homogenized sludge samples were diluted in phosphate-buffered saline. Next, 0.1 ml of the diluted samples was spread on each of two plates of media. The media (Difco) used to enumerate the individual bacteria were as follows: KF agar (*S. faecalis*); XLD agar (*S. typhimurium*); *Pseudomonas* isolation agar (*P. aeruginosa*); MacConkey agar with 10 μg each of ampicillin and kanamycin per ml (*E. coli*); plant count agar (total aerobic bacteria). Colonies on KF agar were counted after 48 h of incubation at 37°C; colonies on all other plates were counted after 24 h of incubation at 37°C.

Physical and chemical analyses. Dissolved oxygen was measured by using a Yellow Springs Model 54A dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). Total and volatile solids were determined according to standard procedures (1). The temperature and pH of the digested sludge were also recorded.

Statistical analyses. Data were analyzed by using Duncan's multiple range test (22). Differences reported are significant at the 95% confidence level. Correlation coefficients between physical and chemical parameters and either daily change in bacterial numbers or percentage of bacteria in the supernatant were calculated by analyzing approximately 200 individual paired values (22).

RESULTS

The results obtained in one trial of 10 days are shown in Table 1. Wasted sludge from the Main Street plant was added daily to the digesters to provide a detention time of 15 days. The temperature, dissolved oxygen, pH, total solids, total bacteria of each type, and percentage of bacteria in the supernatant fraction were determined daily. Under condition I, the change in *S. typhimurium*, *S. faecalis*, and *E. coli* was approximately -1.0 $\log_{10}$/day per ml (Table 1). No

TABLE 1. Bacterial survival and association with sludge flocs during aerobic and anaerobic digestion of sludge

Condition	Temp (°C)	Dissolved oxygen (mg/liter)	pH	Total solids (g/liter)	Bacteria	Daily change in total bacteria per ml[a] (log_{10})	% of bacteria in supernatant[a]
I	28.3	2.7	6.0	20.6	*Salmonella typhimurium*	-1.24^{A}	8^{A}
					Streptococcus faecalis	-0.92^{A}	12^{A}
					Escherichia coli	-1.13^{A}	4^{A}
					Pseudomonas aeruginosa	-0.28^{B}	3^{A}
					Total aerobic bacteria	-0.34^{B}	7^{A}
II	6.2	3.7	7.4	19.6	*Salmonella typhimurium*	-0.23^{A}	23^{B}
					Streptococcus faecalis	-0.10^{A}	50^{A}
					Escherichia coli	-0.23^{A}	25^{B}
					Pseudomonas aeruginosa	-0.29^{A}	34^{B}
					Total aerobic bacteria	-0.07^{A}	21^{B}
III	28.0	0	6.3	18.4	*Salmonella typhimurium*	-0.90^{A}	51^{A}
					Streptococcus faecalis	-0.83^{A}	47^{A}
					Escherichia coli	$-0.61^{A,B}$	30^{B}
					Pseudomonas aeruginosa	-0.34^{B}	47^{A}
					Total aerobic bacteria	-0.09^{C}	33^{B}

[a] Numbers in the same column and with the same letter superscript are not significantly different.

significant difference at the 95% confidence level was observed between these values. The daily changes in *P. aeruginosa* and total aerobic bacteria were −0.28 and −0.34 log_{10}/day per ml, respectively. These values were not significantly different from each other but were different from the values for the other bacteria. The percentage of the different bacteria in the supernatant was relatively low (3 to 12%). No significant differences were observed between the values for the different bacteria.

During digestion of sludge at a lower temperature (6.2°C), the daily change of all bacteria was less than −0.3 log_{10}/day per ml. No significant differences were observed between the values for the different bacteria. At this temperature, 21 to 50% of the bacteria were found in the supernatant fraction.

Under anaerobic conditions at 28°C, the values for daily change in *S. typhimurium*, *S. faecalis*, and *E. coli* were similar (−0.6 to −0.9 log_{10} per ml) and were different from the values for *P. aeruginosa* and total aerobic bacteria. More than 30% of all bacteria were found in the supernatant fraction.

In general, the values for daily change in total numbers of *S. typhimurium* and *S. faecalis* were similar under aerobic and anaerobic conditions at 28°C and were higher (more negative) than values obtained during aerobic digestion at 6°C. The percentage of these bacteria in the supernatant fraction was lower during aerobic digestion at 28°C than during digestion under anaerobic conditions at 28°C or under aerobic conditions at 6°C. *P. aeruginosa* and total aerobic bacteria were generally more stable at 28°C in sludge subjected to aerobic or anaerobic digestion.

Additional trials were conducted, using sludge from the three different wastewater treatment plants previously described and using different sludge digestion conditions. Preliminary analysis of results obtained with sludge from the Kanapaha, Main Street, and University of Florida treatment plants indicated that the source of sludge did not significantly influence the results. Therefore, the results of trials involving sludge from these three sources were combined (Tables 2 and 3). For these trials, the temperature of anaerobic sludge digestion was raised to a value that was closer to that of certain operating plants, 34°C, and *P. aeruginosa* was not added to the digesters. Early studies indicated that the change in *P. aeruginosa* was similar to that of the total aerobic bacteria (Table 1). Also, growth of *P. aeruginosa* on media used for enumerating *S. typhimurium* and *E. coli* made counting low numbers of these two bacteria difficult.

Inactivation rates of bacteria as well as the percentage of bacteria in the supernatant that were observed during digestion of sludge under similar conditions are compared in Tables 2 and 3. Although several values are shown in these tables, certain general trends can be observed. No differences in the daily change of the bacteria studied were observed during sludge digestion at 6°C (condition D). The daily changes in *S. typhimurium* and *E. coli* were usually similar during digestion under different conditions and also greater (more negative) than the values obtained for *S. faecalis* and total aerobic bacte-

TABLE 2. Factors influencing survival of bacteria and their association with sludge flocs during aerobic and anaerobic digestion of sludge: effect of sludge digestion conditions on different bacteria

Condition	Temp (°C)	Dissolved oxygen (mg/liter)	Detention time (days)	pH	Total solids (g/liter)	Volatile solids (g/liter)	Bacteria	Daily change in total bacteria (mg/liter [$\log_{10}$])[a]	% of bacteria in supernatant[a]
A	28	4.7	15	5.7	7.5	5.4	*Salmonella typhimurium*	-1.29^{A}	7^{A}
							Streptococus faecalis	-0.82^{B}	7^{A}
							Escherichia coli	-1.26^{A}	9^{A}
							Total aerobic bacteria	-0.36^{C}	5^{A}
B	34	0	15	6.7	11.4	8.0	*Salmonella typhimurium*	-1.08^{A}	73^{A}
							Streptococcus faecalis	-0.52^{B}	$50^{B,C}$
							Escherichia coli	-1.29^{A}	59^{B}
							Total aerobic bacteria	-0.32^{B}	39^{C}
C	20	5.3	15	6.3	8.0	5.8	*Salmonella typhimurium*	-1.05^{B}	11^{A}
							Streptococcus faecalis	-0.44^{C}	9^{A}
							Escherichia coli	-1.25^{A}	13^{A}
							Total aerobic bacteria	-0.20^{D}	4^{A}
D	6	4.2	15	6.3	6.8	5.3	*Salmonella typhimurium*	-0.21^{A}	$29^{A,B}$
							Streptococcus faecalis	-0.05^{A}	30^{A}
							Escherichia coli	-0.26^{A}	34^{A}
							Total aerobic bacteria	-0.04^{A}	18^{B}
E	27	1.0	15	6.4	6.3	4.8	*Salmonella typhimurium*	-1.01^{A}	$23^{A,B}$
							Streptococcus faecalis	-0.31^{B}	12^{B}
							Escherichia coli	-1.27^{A}	33^{A}
							Total aerobic bacteria	-0.27^{B}	16^{B}
F	27	6.5	40	7.4	9.2	5.6	*Salmonella typhimurium*	$-1.26^{A,B}$	7^{A}
							Streptococcus faecalis	-0.99^{B}	7^{A}
							Escherichia coli	-1.38^{A}	11^{A}
							Total aerobic bacteria	-0.33^{C}	14^{A}

[a] Numbers in the same column and with the same letter superscript are not significantly different.

ria. Inactivation rates of *S. faecalis* were significantly higher than those of total aerobic bacteria during sludge digestion under three conditions (conditions A, C, and F) but were similar under the other conditions (conditions B, D, and E). These latter conditions differed from the first set of conditions in that the temperature (condition D) or the level of dissolved oxygen (conditions B and E) was lower.

The values for percentage of bacteria in the supernatant showed more consistent trends than the values for the change in total bacteria. Under three conditions (A, C, and F), no significant differences in the values for the different bacteria were observed. These three conditions were similar in that aerobic conditions with greater than 4 mg of dissolved oxygen per liter and temperatures of 20 to 28°C were maintained and less than 15% of the total bacteria were found in the supernatant. More bacteria were found in the supernatant fraction, and more variability was observed between the values for different bacteria, during sludge digestion at low temperature (6°C, condition D) or during digestion with reduced levels of dissolved oxygen (conditions B and E). The highest percentage of bacteria in the supernatant was found during sludge digestion under anaerobic conditions (condition B). The next highest values were found during aerobic sludge digestion at 6°C or at 27°C with the level of dissolved oxygen reduced to 1 mg/liter (conditions D and E). The lowest levels of bacteria in the supernatant were found during aerobic sludge digestion at greater than 20°C with greater than 4 mg of dissolved oxygen per ml (conditions A, C, and F).

Correlation coefficients between physical and chemical parameters and either the daily change in bacterial numbers or the percentage of bacteria in the supernatant were calculated for the sludge undergoing aerobic digestion (Table 4). Temperature was the variable most highly correlated with the daily change in bacterial numbers (Table 4). These values (average r value of -0.48) were highly significant, with probabilities of $P \leq 0.0001$ for three of the bacteria studied. Significant ($P < 0.01$) positive correlations were found between the daily change in bacterial numbers and total solids or pH, but the r values were lower. No significant correlations were

TABLE 3. Factors influencing survival of bacteria and their association with sludge flocs during aerobic and anaerobic digestion of sludge: influence of sludge digestion conditions on individual bacteria[a]

Condition	Temp (°C)	Dissolved oxygen (mg/liter)	Detention time (days)	pH	Total solids (g/liter)	Volatile solids (g/liter)	Bacteria	Daily change in total bacteria (mg/liter [$\log_{10}$])	% of bacteria in supernatant
A	28^{B}	4.07^{B}	15^{B}	5.7^{D}	$7.5^{C,D}$	5.4^{B}	*Salmonella typhimurium*	-1.29^{A}	7^{C}
B	34^{A}	0.0^{D}	15^{B}	6.7^{B}	11.4^{A}	8.0^{A}		$-1.08^{A,B}$	73^{A}
C	20^{C}	5.3^{B}	15^{B}	6.3^{C}	$8.0^{B,C}$	5.8^{B}		-1.05^{B}	11^{C}
D	6^{D}	4.2^{B}	15^{B}	6.3^{C}	$6.8^{C,D}$	5.3^{B}		-0.21^{C}	29^{B}
E	27^{B}	1.0^{C}	15^{B}	6.4^{C}	6.3^{D}	4.8^{B}		-1.01^{B}	23^{B}
F	27^{B}	6.5^{A}	40^{A}	7.4^{A}	9.2^{B}	5.6^{B}		$-1.26^{A,B}$	7^{C}
A	28^{B}	4.7^{B}	15^{B}	5.7^{D}	$7.5^{C,D}$	5.4^{B}	*Streptococcus faecalis*	-0.82^{A}	7^{C}
B	34^{A}	0.0^{D}	15^{B}	6.7^{B}	11.4^{A}	8.0^{A}		-0.52^{B}	50^{A}
C	20^{C}	5.3^{B}	15^{B}	6.3^{C}	$8.0^{B,C}$	5.8^{B}		-0.44^{B}	9^{C}
D	6^{D}	4.2^{B}	15^{B}	6.3^{C}	$6.8^{C,D}$	5.3^{B}		-0.05^{C}	30^{B}
E	27^{B}	1.0^{C}	15^{B}	6.4^{C}	6.3^{D}	4.8^{B}		-0.31^{B}	12^{C}
F	27^{B}	6.5^{A}	40^{A}	7.4^{A}	9.2^{B}	5.6^{B}		-0.99^{A}	7^{C}
A	28^{B}	4.7^{B}	15^{B}	5.7^{D}	$7.5^{C,D}$	5.4^{B}	*Escherichia coli*	-1.26^{A}	10^{C}
B	34^{A}	0.0^{D}	15^{B}	6.7^{B}	11.4^{A}	8.0^{A}		-1.29^{A}	59^{A}
C	20^{C}	5.3^{B}	15^{B}	6.3^{C}	$8.0^{B,C}$	5.8^{B}		-1.25^{A}	13^{C}
D	6^{D}	4.2^{B}	15^{B}	6.3^{C}	$6.8^{C,D}$	5.3^{B}		-0.26^{B}	34^{B}
E	27^{B}	1.0^{C}	15^{B}	6.4^{C}	6.3^{D}	4.8^{B}		-1.27^{A}	33^{B}
F	27^{B}	6.5^{A}	40^{A}	7.4^{A}	9.2^{B}	5.6^{B}		-1.38^{A}	11^{C}
A	28^{B}	4.7^{B}	15^{B}	5.7^{D}	$7.5^{C,D}$	5.4^{B}	Total aerobic bacteria	-0.36^{A}	5^{C}
B	34^{A}	0.0^{D}	15^{B}	6.7^{B}	11.4^{A}	8.0^{A}		-0.32^{A}	39^{A}
C	20^{C}	5.3^{B}	15^{B}	6.3^{C}	$8.0^{B,C}$	5.8^{B}		$-0.20^{A,B}$	4^{C}
D	6^{D}	4.2^{B}	15^{B}	6.3^{C}	$8.0^{B,C}$	5.3^{B}		-0.04^{B}	18^{B}
E	27^{B}	1.0^{C}	15^{B}	6.3^{C}	6.3^{D}	4.8^{B}		-0.27^{A}	16^{B}
F	27^{B}	6.5^{A}	40^{A}	7.4^{A}	9.2^{B}	5.6^{B}		-0.33^{A}	$14^{B,C}$

[a] Numbers in the same column and with the same letter superscript are not significantly different.

observed between the daily change in bacterial numbers and dissolved oxygen, volatile solids, or detention time. Temperature was also negatively correlated with the percentage of bacteria in the supernatant. The dissolved oxygen was highly correlated with the percentage of bacteria in the supernatant ($P \leq 0.0001$), except for the total aerobic bacteria ($P < 0.01$).

When correlations were made by using data obtained under all digestion conditions, temperature was correlated with the change in bacterial numbers (mean $r = -0.39$) but not with the

TABLE 4. Correlations between physical and chemical parameters and daily change in bacterial numbers or percentage of bacteria in the supernatant during digestion of sludge under aerobic conditions[a]

Bacteria	Correlation (*r*) between physical and chemical parameters and:				
	Daily change in no. of bacteria[b]			% of bacteria in supernatant[c]	
	Temp	Total solids	pH	Dissolved oxygen	Temp
Salmonella typhimurium	−0.55*[d]	0.38	0.24	−0.32*	−0.24
Streptococcus faecalis	−0.51*	0.41	0.26	−0.56*	−0.28
Escherichia coli	−0.57*	—[e]	0.30	−0.36*	−0.33*
Total aerobic bacteria	−0.27	0.28	—	−0.26	−0.34*
Mean	−0.48	0.36	0.27	−0.38	−0.30

[a] Conditions A, C, D, E, and F as described in Table 2.

[b] Correlations between the daily change in bacterial numbers and dissolved oxygen, detention time, and volatile solids were not significant ($P > 0.01$).

[c] Correlations between the percentage of bacteria in the supernatant and pH, detention time, total solids, and volatile solids were not significant ($P > 0.01$).

[d] Asterisks indicate highly significant values ($P \leq 0.0001$); other values were significant at $P < 0.01$.

[e] Values with $P > 0.01$ are not given.

TABLE 5. Correlations between physical and chemical parameters and either daily change in bacterial numbers or percentage of bacteria in the supernatant during digestion of sludge under aerobic and anaerobic conditions[a]

Bacteria	Correlation (r) between physical and chemical parameters			
	Daily change in bacterial numbers[b]		% of bacteria in supernatant[c]	
	Temp	pH	Dissolved oxygen	Volatile solids
Salmonella typhimurium	−0.46*[d]	0.22	−0.58*	−0.57*
Streptococcus faecalis	−0.38*	0.24	−0.39*	−0.51*
Escherichia coli	−0.51*	0.24	−0.35*	−0.23
Total aerobic bacteria	−0.22	0.11	−0.55*	−0.34
Mean	−0.39	0.16	−0.47	−0.41

[a] Values were calculated by using data for all digestion conditions described in Table 2.
[b] Correlations between daily change in bacterial numbers and dissolved oxygen, detention time, total solids, and volatile solids were not significant ($P > 0.01$).
[c] Correlations between percentage of bacteria in the supernatant and temperature, detention time, pH, and total solids were not significant ($P > 0.01$).
[d] Asterisks indicate values that are highly significant ($P \leq 0.0001$); other values were significant at $P < 0.01$.

percentage of bacteria in the supernatant (Table 5). The pH but not the total solids, volatile solids, or dissolved oxygen was correlated with the change in bacterial numbers. Both the dissolved oxygen and the volatile solids were correlated with the percentage of bacteria in the supernatant. The correlation between dissolved oxygen and percentage of bacteria in the supernatant was highly significant for all bacteria studied ($P \leq 0.0001$).

The temperature, pH, and total solids were not correlated with the percentage of bacteria in the supernatant.

In all of the previously described experiments, bacteria were added with fresh sludge to the digesters daily. The values for change in total bacteria therefore reflect changes that occurred over a 24-h period. It was therefore of interest to determine whether the rate of daily change in bacteria would vary with time. This could indicate that a fraction of the added bacteria was more resistant to inactivation during sludge digestion and might persist for a longer period of time than would be expected from considering only short-term inactivation rates. To determine whether a persistent fraction of bacteria was present, digesters were operated under aerobic conditions without the addition of bacteria or sludge. Two digesters were operated at ambient temperature (27°C), and one was operated at 5°C. All digesters were operated with greater than 4 mg of dissolved oxygen per liter. The rate of change in total numbers of *S. typhimurium* did not change appreciably over 9 days of incubation (Fig. 1), indicating that a persistent fraction of bacteria was not present in the original inoculum. Similar figures were obtained for *S. faecalis* and total aerobic bacteria (data not shown). *E. coli* was not added because it could grow on the XLD agar that was used for enumeration of *S. typhimurium* and could interfere with counting low levels of these bacteria.

DISCUSSION

Unlike the anaerobic digestion process, in which relatively constant levels of temperature with little or no dissolved oxygen are maintained, the aerobic digestion process is subject to fluctuations in temperature and dissolved oxygen according to the weather and operating conditions prevailing at a particular treatment plant. Therefore, temperature and dissolved oxygen are important factors to consider in studying the efficiency of aerobic sludge digestion. Our initial studies (Table 1) have shown that the

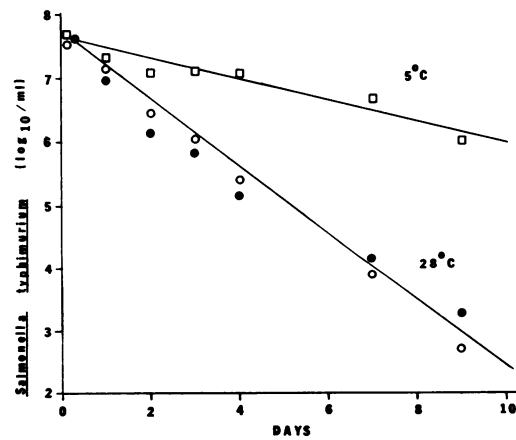

FIG. 1. Survival of *S. typhimurium* during aerobic digestion of sludge without addition of fresh sludge. Symbols: ○ and ●, bacteria in digesters incubated at 28°C; □, digester maintained at 5°C.

inactivation rates of *S. typhimurium*, *S. faecalis*, *E. coli*, *P. aeruginosa*, and total aerobic bacteria were higher at 28°C than at 6°C in aerobically digested sludge. We have also shown that the inactivation rates of three bacteria (*S. typhimurium*, *S. faecalis*, and *E. coli*) were similar under both anaerobic and aerobic digestions at 28°C. There were differences in inactivation rates of the different bacteria studied under aerobic or anaerobic conditions at 28°C, but not in those studied at low temperatures. *P. aeruginosa* and total aerobic bacteria were more stable at 28°C than were the other bacteria. There were generally more bacteria in the supernatant at 6°C under aerobic conditions or at 28°C under anaerobic conditions than at 28°C under aerobic conditions.

Additional studies were conducted under different conditions. Correlation coefficients between physical and chemical parameters and either the daily change in bacteria numbers or the percentage of bacteria in the supernatant were determined separately for sludge digested under aerobic conditions and for sludge digested under both aerobic and anaerobic conditions.

The temperature of sludge digestion was the variable most highly correlated negatively with both the change in bacterial numbers and the percentage of bacteria in the supernatant during aerobic sludge digestion. The temperature likely affects both the rate of bacterial metabolism and the activity of protozoan predators. As the temperature is lowered, both of these activities would be reduced. This could explain the observed negative correlation between temperature and the change in bacterial numbers. Both the total solids and the pH had positive, significant, but low values of correlation with the change in bacterial numbers. This suggests that values closer to neutrality are more favorable to bacterial survival than are low pH values. Also, increasing the level of solids may be protective for bacteria.

When sludge digestion under aerobic and anaerobic conditions was considered, the temperature was again found to have a significant negative correlation with the change in bacterial numbers but not with the percentage of bacteria in the supernatant. The high percentage of bacteria found in the supernatant during anaerobic sludge digestion at high temperature likely eliminates the correlation between the temperature and the percentage of bacteria in the supernatant that was observed during aerobic sludge digestion. Since both the highest levels of volatile solids and percentage of bacteria in the supernatant were found during anaerobic digestion of sludge, the significant correlation found between these variables is not surprising. The other parameters measured (total solids, temperature, and pH) were not correlated with the percentage of bacteria in the supernatant.

Bacterial type influenced the rate of inactivation. Total aerobic bacteria and *S. faecalis* were generally more stable than *S. typhimurium* or *E. coli*. In a previous study, Berg and Berman (2) found *S. faecalis* to be more stable than fecal or total coliforms during mesophilic or thermophilic digestion of sludge. These authors also found *S. faecalis* to be a better bacterial indicator for the presence of viruses in digested sludge than total or fecal coliforms.

In summary, the survival of bacteria during aerobic sludge digestion under laboratory conditions was influenced by temperature, total solids, pH, and the type of bacteria. The association of bacteria with sludge flocs during aerobic sludge digestion was influenced primarily by dissolved oxygen, and to a lesser degree by temperature. When digestion under aerobic and anaerobic conditions was considered, temperature again was the variable most highly correlated with the change in bacterial numbers. The pH and type of bacteria, but not dissolved oxygen, volatile solids, or total solids, were correlated with the change in bacterial numbers. Both volatile solids and dissolved oxygen, but not pH, temperature, or total solids, were correlated with the percentage of bacteria in the supernatant when both anaerobic and aerobic conditions were considered.

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