## AEROBIC SEWAGE STABILIZATION AT ELEVATED TEMPERATURES.

by

Lewis R. Brown, David W. Cook, John C. Mickelson, and Robert G. Tischer

# Department of Microbiology Mississippi State University

### INTRODUCTION

Previous studies conducted in this laboratory have shown that the thermophilic bacterium <u>Bacillus circulans</u> could effect a reasonable reduction in the Chemical Oxygen Demand (COD) of normal domestic sewage. The results of these studies were sufficiently encouraging to warrant investigating the possibility of designing an aerobic sewage disposal system operated at thermophilic temperatures. In this connection, it is of importance to know not only the performance of this system in terms of removing the Biochemical Oxygen Demand (BOD) and suspended solids of the influent but also to evaluate the system in terms of its ability to destroy pathogenic microorganisms in the sewage. This paper will describe some of the attributes of an aerobic thermophilic sewage treatment process and the affect of this process on microorganisms contained in normal domestic sewage.

#### PROCEDURES

The reaction vessel employed in these studies was a two-liter roundbottom flask equipped with a thermometer well. The flask was fitted with a two-hole rubber stopper and mixing and aeration were accomplished by bubbling in pre-heated air. Excessive moisture loss was prevented by means of a water-cooled condenser attached to the air outlet. The entire assembly was enclosed in an incubator. The substrate was one liter of normal domestic sewage obtained from the State College, Mississippi, sewage disposal plant after passage through the bar screen.

The BOD, COD, and suspended solids were determined in accordance with standard methods (3). The multi-tube fermentation technique using phenol red lactose broth as the substrate was employed for estimating the number of coliform organisms, and the tests were conducted in accordance with standard methods (3).

Plate counts were carried out using Plate Count Agar (Difco) and the conventional pour plate technique.

Thermal death time studies were conducted in screw-capped test tubes employing 10 ml amounts of suspending medium into which a given concentration of the test organism was inoculated. After inoculation, the tubes were immersed in water baths at the appropriate temperatures and, at the testing times, were removed from the water baths and cooled quickly with running water. The tubes were then assayed for viable organisms by streaking 1/10 ml onto the surface of the appropriate medium with subsequent incubation of the plates at 35  $\pm$  0.5 C. The organisms employed in this study were <u>Salmonella paratyphi</u>, <u>Shigella sonnei</u>, an unidentified streptococcus isolated from normal domestic sewage, and a laboratory culture of <u>E</u>. coli.

The assaying media employed were MacConkey Agar for <u>Salmonella</u> <u>paratyphi</u> and <u>Shigella</u> <u>sonnei</u>, MacConkey Agar and Eosin Methylene Blue (EMB) Agar for the coliform organisms, and m-Bacto Enterococcus Agar for the enterococci.

### RESULTS

Preliminary studies indicated that it would not be necessary to add a starter culture of thermophilic organisms to normal domestic sewage; for by simply aerating at 55 C, reasonable reductions in BOD were achieved. Representative data from such experiments are shown in Table I. The BOD values and percent reduction in BOD fluctuated from batch to batch but averaged more than 60% BOD reduction.

Similar studies in which a culture of <u>B</u>, <u>circulans</u> was added to the raw sewage showed BOD reductions in the same general range. The only difference was that in some cases when a large inoculum was used the bio-precipitation phenomenon reported earlier (1) occurred.

Since the thermophilic population in the normal domestic sewage increases considerably during treatment at elevated temperatures, the question then arises as to what is the fate of the coliforms and other mesophiles in the sewage during this treatment. To help answer this question, fresh domestic sewage was aerated at 55 C without the addition of thermophiles. It seemed (Table II) that coliform organisms had survived the treatment for 24 hours. It was found, however, that the positive tubes from the presumptive test failed to confirm using EMB Agar.

Experiments similar to the above were conducted at temperatures of 65 C. Representative data from these studies are presented in Table III. In regard to the coliform population, it was found that 90% of the positive presumptive tubes from the original sample contained coliform organisms as shown by positive confirmed and completed tests. Conversely, all of the positive presumptive tubes from the one and 24 hour samples failed to confirm the presence of coliforms when Brilliant Green Lactose Bile Broth (BGLB) was employed as the confirmatory medium. Further tests revealed that the positive tubes from the one hour and 24 hour presumptive tests contained spore-forming rods.

Estimates of the coliform density of sewage were conducted using both Phenol Red Lactose Broth and Brilliant Green Lactose Bile Broth, and it was found that both results agreed within the limits of the test. Consequently, all subsequent estimates of coliforms were made using Brilliant Green Lactose Bile Broth. In the next series of tests, 250 ml of medium from a previous run were used as the inoculum. The sewage was placed in a reaction vessel and quickly brought to the desired temperature in a hot water bath. The flasks were constantly shaken during this period. Representative data from tests run at 55 C. are shown in Table IV, and it can be observed that by just heating to 55 C., 92.2% of the coliform organisms were destroyed.

A reduction of 99.99% in the coliform population of sewage was achieved by merely heating to a temperature of 65 C. (Table V). After one hour of treatment at 65 C., 100% of the coliforms were destroyed. The total count of organisms growing at 35 C. dropped slowly after a large initial drop. The thermophilic count dropped initially but then increased greatly over the 24 hour period.

In other experiments conducted at 65 C., samples were collected after 6 and 24 hours and analyzed for suspended solids, BOD, and COD in addition to collform population and thermophilic population. Representative data from this type of experiment are given in Table VI. The suspended solids determinations were run both before and after one hour of settling. The BOD and COD values are total values: <u>i. e</u>., the whole sewage without any settling.

Thermal death time studies were run employing three suspending media: (1) sterile domestic sewage (sterilized by autoclaving), (2) nutrient broth, and (3) distilled water. Within the limits of detection of the tests, it was found that <u>Escherichia coli</u>, and the enterococcus were killed within 30 minutes at 55 C. and within 10 minutes at 65 C. in all of the suspending media.

The results of similar studies using the enteric pathogens <u>Salmonella paratyphi</u> and <u>Shigella sonnei</u> are shown in Figure 1. Once again, within the limits of detection of the tests, it can be seen that the organisms were destroyed within a matter of minutes.

#### DISCUSSION

The activated sludge sewage disposal process is generally regarded as the most efficient of the sewage disposal processes now in use. Some of the advantages of this type of system are a clear, sparkling and non-putrescible effluent, freedom from offensive odors during operation, high BOD and suspended solids removal (approximately 90%), removal of better than 90% of the bacteria, control within limits of the degree of nitrification, possible commercial value of the sludge, and low installation cost.

Unfortunately, however, there are also many disadvantages, including the requirement of constant skilled attendance, the large volume of sludge produced, the difficulty in disposing of the sludge, and the uncertainty concerning the degree of treatment expected under all conditions. Probably the chief disadvantage of the system is its lack of stability. The laboratory system described in this paper would probably be considered a modified activated sludge process, but it differs from the classical activated sludge process in a number of respects.

One of the fundamental differences is that the classical activated sludge is made up of bacteria, fungi, protozoa, rotifers, and sometimes even nematodes, depending upon the waste involved and the length of time of treatment. On the other hand, the only microorganisms encountered thus far in the process herein described are thermophilic bacteria, practically all of which are sporeformers. The obvious advantage of such a situation is that the microbial population is much more stable. Additionally, nitrification of the sewage, and the subsequent problems arising therefrom, are eliminated since the nitrifying organisms are not able to exist at the temperature employed.

Another difference between the systems is in the disposal of the sludge produced. It is generally agreed that wet activated sludge can only be used as a fertilizer when handled with caution. Heat-dried activated sludge, however, does appear to be safe for reasonable agricultural use. In the thermophilic process, however, all normal pathogens would be destroyed and thus the sludge from such a system could be used as a fertilizer without heat treatment. One of the problems which could arise from its use, however, is the canning of vegetables grown in the presence of an unusually high concentration of the thermophilic sporeformers.

It should be emphasized that the sewage used in these investigations was not pre-settled and, furthermore, the BOD values reported for the effluent were made on the total effluent without final settling. Therefore, the BOD removal cannot be compared to the removals obtained in other sewage treatment processes where a number of steps are involved.

Probably the major disadvantage of a thermophilic system would be the apparent increased cost of installation and operation. As reported earlier, this problem may be circumvented by the use of a cheap energy source such as a solar furnace or atomic energy. Before a final evaluation can be made, a thorough investigation of the entire system must be taken into consideration, including the need for primary treatment, the disposal of the sludge, and the post aeration treatment (settling and chlorination).

It seems perfectly clear from the results reported here, that the need for chlorinating the effluent from a thermophilic process would be precluded since the sewage is in essence pasteurized. The thermal death time studies have shown that all of the detectable organisms employed in the test were destroyed in a matter of minutes, while the process itself will undoubtedly require hours at 55 C. or 65 C. Further, it was observed that the colliform population is in essence completely destroyed in normal domestic sewage during treatment and the total number of mesophilic organisms is drastically reduced. Undoubtedly, many of the mesophilic organisms are facultative thermophiles. Thus, this type of disinfection (heat) does not spare bacteria protected in organic matter as is the case with chlorination.

- 36 -

Contrary to some early reports on thermophilic sewage stabilization, aerobic treatment of normal domestic sewage at elevated temperatures merits considerable research emphasis. This type of treatment may well prove to be the method of choice in the relatively near future.

#### ACKNOWLEDGEMENTS

This investigation was supported in part by Research Grant No. WP-193(C1) from the Division of Water Supply and Pollution Control, U. S. Public Health Service, and by funds provided by the United States Department of the Interior as authorized under the Water Resources Research Act of 1964, Public Law 88-379.

#### REFERENCES

- Tischer, R. G., Brown, L. R. and Cook, D. W. Decomposition of Wastewater by Thermophilic Microorganisms. J. Water Pol. Cont. Fed. <u>34</u>, 1244-1255 (1962).
- Tischer, R. G., Cook, David W. and Brown, L. R. The Effect of Inorganic Ions on the Stabilization of Domestic Sewage by a Thermophilic Bacterium. Dev. Ind. Microbiol. 4, 268-274 (1963).
- "Standard Methods for the Examination of Water and Wastewater." 11th Edition, American Public Health Association, New York (1960).

	BOD		
Sample	Concentration	Reduction	
	mg/l	%	
Original	140		
18 hours (total)	56	60.0	
30 hours (settled 1 hour)	37		

# Table I. The effect of aerobic treatment at 55°C on the Biochemical Oxygen Demand of normal domestic sewage.

Table II. The effect of aeration at 55°C on the mesophiles in normal domestic sewage.

Sample	Co	liforms*	Plate Count at 35 C	Plate Count at 20°C		
		Reduction				
	No./ml	%	No./ml	No./ml		
0 hours	92,000		1,350,000	370,000		
1 hour	4	99.99	57,000	8,900		
2 hours	540	99.4	69,000	1,560		
24 hours	92	99.9	**	200		

\* Using Phenol Red Lactose Broth (PRLB)

\*\* not counted

# Table III. The effect of aeration at 65°C on the coliforms in normal domestic sewage.

Sample		Coliforms*
		Reduction
	No./ml	%
0 hours	345,000	
L hour	79	99.97
24 hours	24	99.99

\* using Phenol Red Lactose Broth (PRLB)

Table IV. The effect of aeration at 55°C on the coliforms in normal domestic sewage using Brilliant Green Lactose Bile Broth (BGLB).

Coliforms		
	%	
220,000		
	92.2	
	100.0	
	100.0	
	100.0	

\* After Heating to 55°C.

Sample	Co	liforms*			
All and the second second		Reduction	Plate Count at 35°C	Plate Count at 55°C	
1. S.	No./ml	%	No./ml	No./ml	
0 hours	426,000		100,000,000	300,000	
AF** - 0 hours	11	99.99	300,000	78,000	
l hour	0	100.00	26,000	160,000	
4 hours	0	100.00	78,000	290,000	
24 hours	0	100.00	24,000	29,000,000	

Table V. Effect of aeration at 65°C on the microflora of normal domestic sewage.

\* Using Brilliant Green Lactose Bile Broth

\*\* After Heating to 65°C.

Table VI. The effect of aerobic treatment of normal domestic sewage at 65°C on the coliforms, thermophiles, suspended solids and Biochemical and Chemical Oxygen Demands.

	Suspended Solids						
Sample	Plate Coliform Count organisms* at 55 <sup>°</sup> C	Count	Whole	Settled 1 hour	BOD Reduc- tion	COD Reduc- tion	
	No./ml	No./ml	mg/l	mg/l	mg/1 %	mg/1 %	
0 hours	7,9000,000	450,000	211.3	109.9	416	579	
6 hours	0	21,000,000	178.8	47.0	254 38.9	409 29.4	
24 hours	0	1,600,000	167.8	12.0	102 75.5	301 48.2	

\* Using Brilliant Green Lactose Bile Broth.

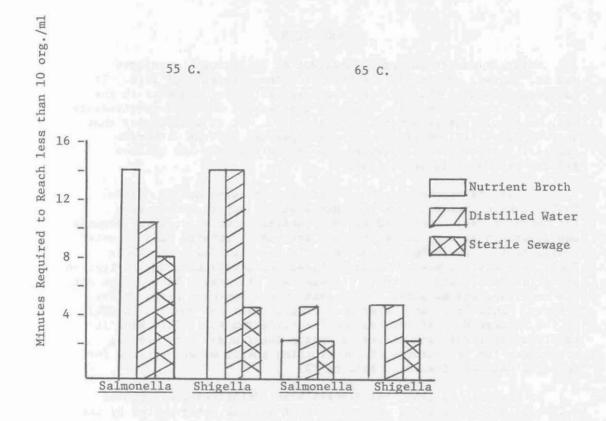


Figure 1. Thermal Death Time Studies.

- 41 -