SEWAGE STABILIZATION ACTIVITIES OF SEWAGE THERMOPHILES

BY

C. M. Ladner, Dr. L. R. Brown, and Dr. R. G. Tischer

Department of Microbiology
Mississippi State University

INTRODUCTION

For the last several years, investigations have been conducted in our laboratory into the possibilities of developing a high temperature sewage stabilization process. Tischer, Cook, and Brown (1962) reported that a high temperature stabilization process might be of value in treating normal domestic sewage. Attributes of such a system include the destruction of pathogenic bacteria during processing and the ability to control the microbial population, since there are fewer organisms capable of growing at temperatures of 55°C and higher. Brown, Cook, Mickelson, and Tischer (1966) demonstrated that the enteric pathogens in the genera Salmonella and Shigella were destroyed within minutes at the treatment temperatures employed and, that the Biochemical Oxygen Demand and Chemical Oxygen Demand reductions were comparable to those obtained with a mesophilic treatment process. These studies also indicated that during the aeration of normal domestic sewage at elevated temperatures, populations in the neighborhood of 10^7 to 10^8 thermophiles per ml rapidly developed.

This paper will report on the isolation of organisms responsible for the stabilization process and report on some of their characteristics.

PROCEDURES

The organisms employed in this study were obtained as follows: Normal domestic sewage was aerated on a New Brunswick Model FS-614 fermentor for 48 hours. Plate counts were then made from the material using Bacto-plate count agar and the spread plate technique. The plates spread with one-tenth ml of the 10^{-5} dilution contained approximately 150 colonies per plate. Representative colonies were picked and purified by the conventional streak plate technique. A total of 9 isolates were obtained and were selected initially on the basis of differences in colonial morphology. Identification procedures were carried out in accord with the "Manual of Microbiological Methods" (1957).

Manometric studies were conducted in the following fashion: Cells were grown on enriched sewage agar prepared by adding 0.5% peptone, 1.0% glucose, and 1.7% Bacto-agar to filtered normal

domestic sewage. Sterilization was affected at a 121°C for 20 minutes. Bottle slants were prepared, inoculated with a broth culture of the desired organism, and incubated at 55°C for 17 hours. The cells were then harvested into physiological saline, washed 4 times, and re-suspended in the same medium. Standard manometric techniques as described in Umbreit, Burris, and Stauffer (1964) were employed. Dow-Corning high vacuum grease was employed in place of vaseline because of the high temperatures employed. The substrates tested were concentrated sewage (prepared by concentrating normal domestic sewage fourfold with heat and vacuum), 1% peptone, and 1% glucose.

RESULTS

According to "Bergey's Manual of Determinative Bacteriology" (1957), seven of the isolates conformed most closely to Pseudomonas aeruginosa (achromogenic strain) and two of the isolates were classified in the genus Bacillus.

Figures 1-9 demonstrate the ability of the various isolates to oxidize the concentrated sewage, peptone, and glucose. Pseudomonas isolate No. 1 and Pseudomonas isolate No. 3 oxidized peptone at a much faster rate than either the concentrated sewage or the glucose. The differences between oxidative rates observed for the three substrates were not nearly so marked in Pseudomonas isolate No. 4, Pseudomonas isolate No. 6, Pseudomonas isolate No. 7, Pseudomonas isolate No. 9, and Pseudomonas isolate No. 11. It is interesting to note that Pseudomonas isolate No. 6 oxidized the concentrated sewage at a faster rate than it did either the glucose or the peptone. The oxidative rate observed for the concentrated sewage with all seven Pseudomonas isolates seemed to level off quite rapidly, suggesting that the readily oxidizable materials in the sewage were consumed rather quickly. Bacillus isolate No. 12 oxidized the concentrated sewage and peptone at nearly the same rate and both slightly better than glucose; whereas, Bacillus isolate No. 16 utilized peptone to a greater extent than it did either the glucose or the concentrated sewage.

Table 1 shows the oxygen consumption rates for the 9 sewage thermophiles on all three substrates. It may be observed that all of the isolates, with the exception of Pseudomonas isolate No. 6 gave their highest Q_{02} for peptone. Bacillus isolate No. 12 gave a higher Q_{02} for concentrated sewage than did any other isolate including Pseudomonas isolate No. 6, however, the Bacillus isolate No. 12 was higher on the peptone than it was on the concentrated sewage. Pseudomonas isolate No. 3 was the most effective in utilizing peptone and especially glucose, since the Q_{02} for glucose was 74.1, which was considerably greater than any of the other isolates.

Table 2 shows the respiratory quotients (RQ) for all 9 isolates

on the three substrates. It may be observed that the RQ values for the concentrated sewage ranged from 0.43 to 0.76. The RQ values for peptone ranged from 0.41 to 0.66 and the RQ values for glucose ranged from 0.41 to 0.73. Pseudomonas isolate No. 3 had the highest RQ on all three substrates indicating that a greater percentage of the carbonaceous material utilized was present terminally as carbon dioxide.

SUMMARY

A total of 9 thermophiles were isolated from normal domestic sewage after aeration at 55°C. Initially, all isolates demonstrated differences in colonial morphology although identification procedures indicated that 7 of the isolates were Pseudomonas aeruginosa (achromogenic strain) and 2 of the isolates belonged in the genus Bacillus. It would appear that all of the isolates were compatible since they were obtained from plates derived from the same dilution of the treated sewage.

All seven Pseudomonas isolates demonstrated different oxidative abilities on the substrates employed. By the same token the two isolates characterized as belonging to the genus Bacillus demonstrated different oxidative abilities both from each other and from the Pseudomonas isolates.

ACKNOWLEDGMENT

This investigation was supported in part by funds provided by the United States Department of Interior as authorized under the Water Resources Research Act of 1964, public law 88-379.

REFERENCES

Breed, R. S., Murray, E. G. D., and Smith, N. R.
Bergey's Manual of Determinative Bacteriology
Seventh Edition, Williams and Wilkins Company, Baltimore, Maryland (1957).

Brown, L. R., Cook, D. W., Mickelson, J. C., and Tischer, R. G.
Aerobic Sewage Stabilization at Elevated Temperatures
Proceedings of the Water Resources Research Institute Conference, 33-41 (1966).

Society of American Bacteriologists
Manual of Microbiological Methods
McGraw-Hill Book Company, Inc., New York, New York (1957).

Tischer, R. G., Brown, L. R., and Cook, D. W.
Decomposition of Wastewater by Thermophilic Microorganisms
J. Water Pol. Cont. Fed. 34, 1244-1255 (1962).

Umbreit, W. W., Burris, R. H., and Stauffer, J. F.
Manometric Techniques
Fourth Edition, Burgess Publishing Company, Minneapolis, Minnesota (1964).

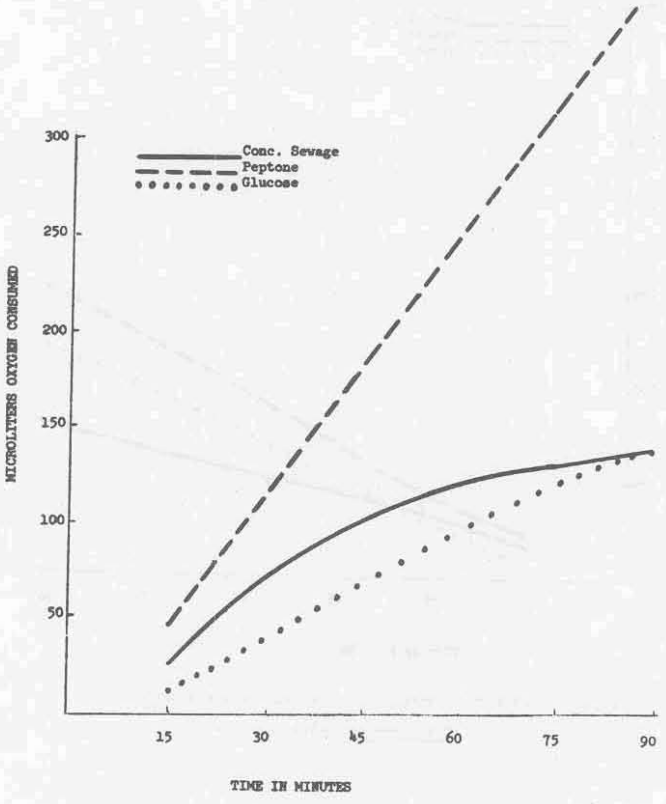

Fig. 1 Oxygen Consumption by a Cell Suspension of Pseudomonas isolate No. 1.

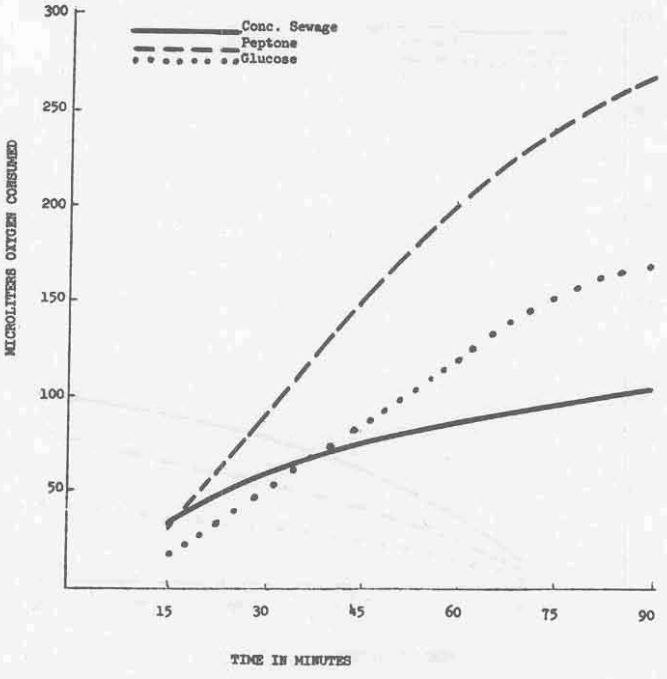

Fig. 2 Oxygen Consumption by a Cell Suspension of Pseudomonas isolate No. 3.

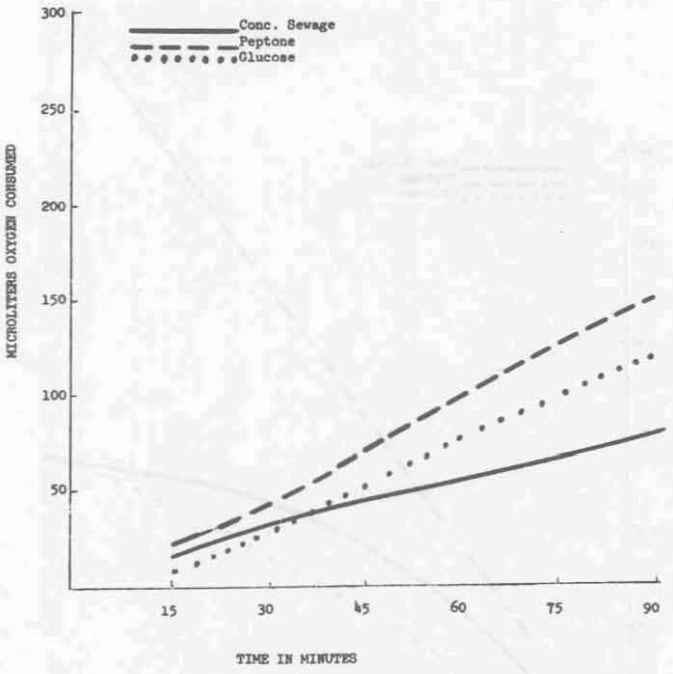

Fig. 3 Oxygen Consumption by a Cell Suspension of *Pseudomonas* isolate No. 4.

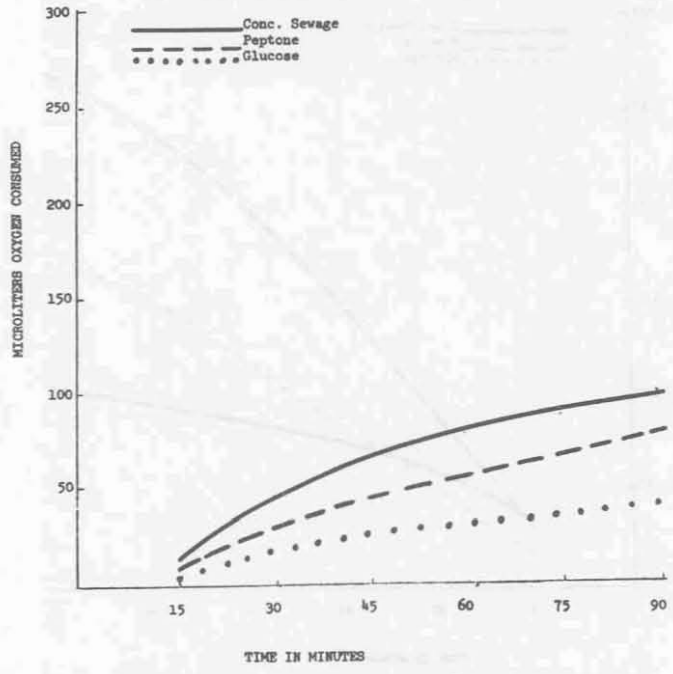

Fig. 4 Oxygen Consumption by a Cell Suspension of *Pseudomonas* isolate No. 6.

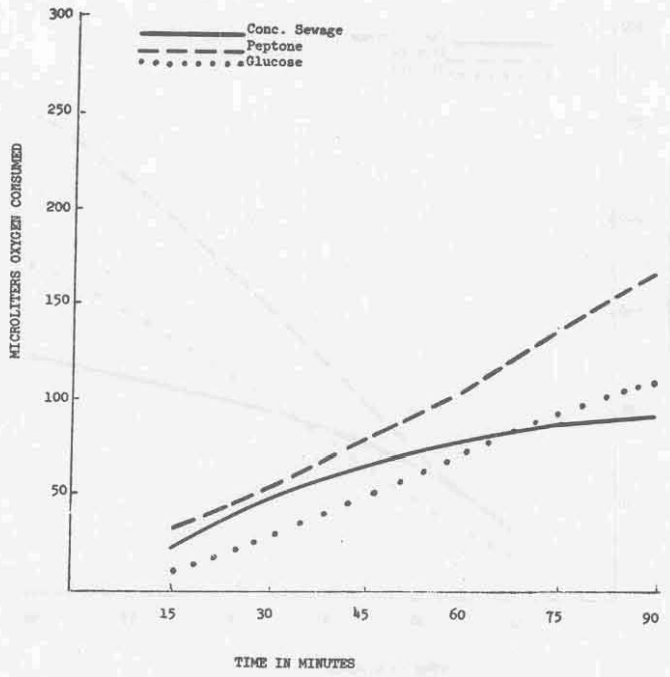

Fig. 5 Oxygen Consumption by a Cell Suspension of Pseudomonas isolate No. 7.

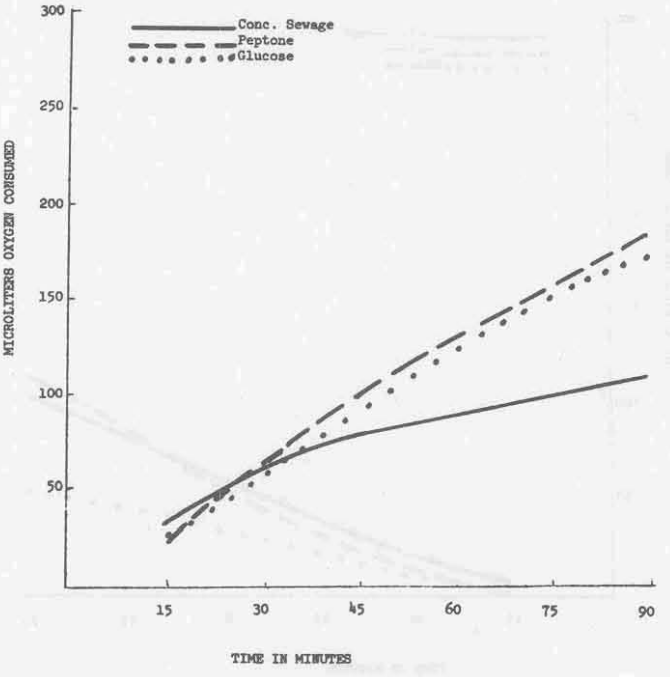

Fig. 6 Oxygen Consumption by a Cell Suspension of Pseudomonas isolate No. 9.

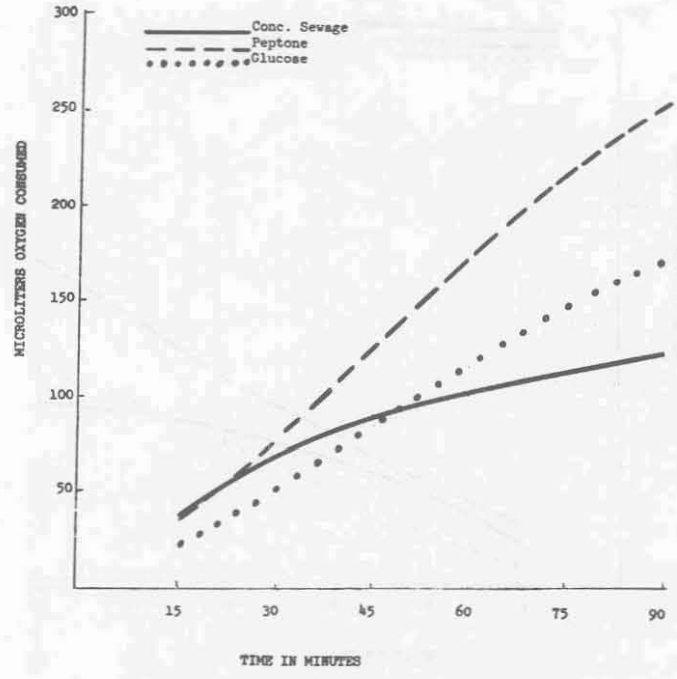

Fig. 7 Oxygen Consumption by a Cell Suspension of Pseudomonas isolate No. 11.

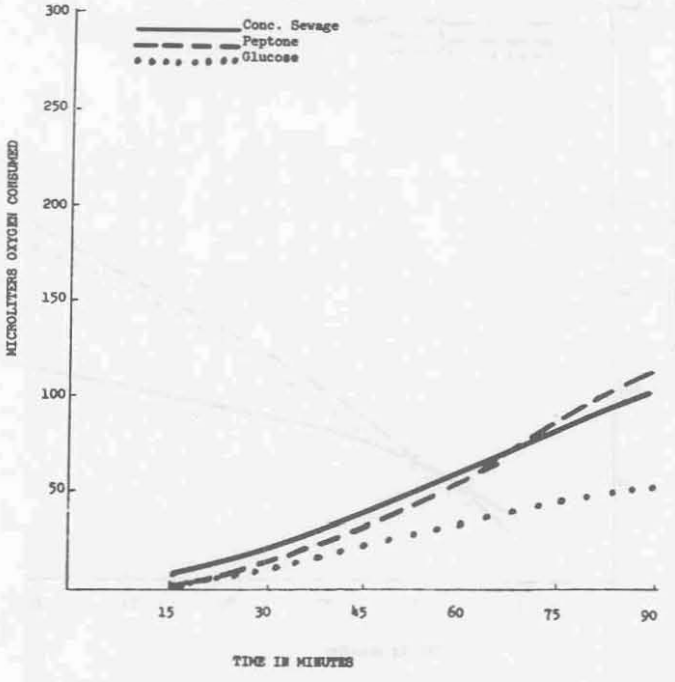

Fig. 8 Oxygen Consumption by a Cell Suspension of Bacillus isolate No. 12.

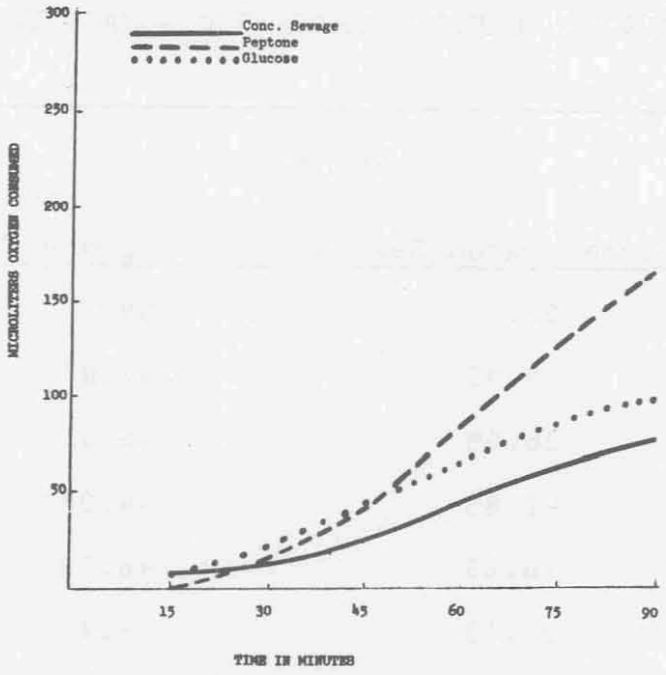

Fig. 9 Oxygen Consumption by a Cell Suspension of *Bacillus* isolate No. 16.

Table 1. OXYGEN CONSUMPTION RATES FOR SEWAGE THERMOPHILES

Isolate Number	Q_{O_2} for Concentrated Sewage	Peptone	Glucose
1	23.05	99.8	35.55
3	24.40	102.07	74.18
4	16.69	45.51	38.39
6	48.85	44.04	22.02
7	16.83	46.59	31.94
9	23.33	58.73	54.36
11	28.92	83.87	57.21
12	60.37	79.12	32.72
16	28.33	64.30	33.16

Table 2. RESPIRATORY QUOTIENTS FOR SEWAGE THERMOPHILES

Isolate Number	Respiratory Quotient for Concentrated Sewage	Peptone	Glucose
1	0.43	0.59	0.62
3	0.76	0.66	0.73
4	0.59	0.63	0.62
6	0.68	0.45	0.41
7	0.75	0.57	0.66
9	0.63	0.51	0.68
11	0.75	0.62	0.66
12	0.64	0.41	0.48
16	0.65	0.59	0.62