QUANTITATIVE ASPECTS OF THE MICROFLORA OF
AN OVERLAND FLOW SPRAY IRRIGATION
SEWAGE DISPOSAL SYSTEM

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AN OVERLAND FLOW SPRAY IRRIGATION
SEWAGE DISPOSAL SYSTEM

THESIS

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By
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CHAPTER I

INTRODUCTION

The problem of sewage disposal is as old as mankind itself. Solutions to this problem have ranged from disposing of the sewage by the easiest obvious means, to diverting raw sewage down a system of sewers into a river, and finally to today's systems which include (1) screening, (2) chemical precipitation, (3) lagooning, (4) sand filters, (5) biological filtration, (6) trickling filters, (7) activated sludge processes, and (8) several combinations of these systems (3).

In 1947 Hanover Canning Company, Hanover, Pennsylvania, introduced an innovation in the disposal of industrial wastes by spraying the overflow from their purifying lagoons onto a field (6). This process was subsequently employed by C. W. Thronthwaite in Seabrook Farms, Seabrook, New Jersey (5). This application was based on the concept that water from industrial use was not radically different from water accidentally contaminated by contact with dead animal bodies. He established a 200-acre tract of highly permeable wooded...
waste land to receive 10 million gallons per day (MGD) of plant effluent. Subsequent investigations showed that forest land was not requisite. They also showed that semipermeable soils function as well as permeable soils in waste purification. In 1960, when the Campbell Soup Company started plant operations in Paris, Texas, the existence of a clay C horizon or subsurface soil type did not prevent the use of this type of system.

Originally, Campbell Soup Company employed 300 acres of grassland which were graded into a series of terraces with an average slope of 3 per cent. Sprinkler heads were installed at the top of each slope, the acreage planted in grasses, and in 1962, surface spraying with waste effluent began. In 1965 an additional 200 acres were added so that the present area includes 500 acres. These 500 acres originally maintained a varied flora consisting, primarily, of several strains of Bermuda grasses, clovers, Johnson grasses, and other local range plants. This native vegetation was supplemented with northern high-moisture-resistant-grasses. The fields were planted with reed canary, fescue, and red top. At the present time reed canary is the predominant species, having overgrown the imported as well as the local plants. This observation
is in direct opposition to reports (2) from other fields, where it has been observed that alta fescue becomes predominant after a given period of growth. At the present time approximately 3.2 MGD of process waste water are treated in this field. This water is sprayed in eight-hour cycles. The plant waste waters are collected in a sump in the pump house, degreased, and the larger solid vegetable particles removed. Processing plant equipment and machines are washed with caustic soda each third work shift. These caustic soda washings are included in the water which reaches the field. Yet, even though the pH of the plant effluent going onto the field varies widely, the pH of the runoff water from the field varies only slightly from neutrality. Furthermore, the BOD reduction is consistently in the range of 98-99 per cent.

Extensive studies have been carried out at other plants in order to determine the best method of waste disposal (3), the best type of cover grass (2), the effects of bactericidal chemicals (1), the destruction of slime layers (7), and various other aspects of this problem. Since a major portion of the purification is probably carried out by the microbial population in the soil, it was of interest to determine how these organisms are affected by this type
of waste water. It was the purpose of this investigation to attempt to quantitate the microflora of the fields which receive the effluent from the Campbell Soup Plant, Paris, Texas, and to determine whether or not there is a correlation between the numbers and types of organisms present and the efficiency of purification of the system.


CHAPTER II

MATERIALS AND METHODS

Media

In order to quantitate different segments of the microbial population, certain selective media were used. The sections of the total populations which were chosen were as follows: aerobic heterotrophic bacteria (AHB), *Streptomyces*, non-symbiotic nitrogen fixers, algae, and fungi.

A modification of Pramer's medium (2, pp. 35-37) was used for the enumeration of AHB. The medium consisted of the following: 0.5 per cent yeast extract and 0.5 per cent glucose added to Pramer's basal medium, which contained 0.05 per cent $\text{K}_2\text{HPO}_4$, 0.01 per cent $\text{KNO}_3$, 50 milliliters of soil extract (made by autoclaving African violet soil and water in a ratio of 1 to 3 for one hour and then filtering), and 15 grams of agar. The pH was maintained at 6.5 to 7.0. This medium was found to give more luxuriant growth than the basal medium and colony counts were more accurate.

Algae, both green and blue-green, were grown in a mineral salts medium and the number present estimated by the Most
Probably Number method of Burtok (2, pp. 25-27).

Fungal counts were made on Rose-Bengal medium as described by Cooke.

The medium of Augier as modified by Vela and Wyss for growing Azotobacter in soil isolations (3) was used for the non-symbiotic nitrogen fixers.

**Streptomyces** were determined by the method described by Pramer (2, pp. 35-37).

**Methods**

Soil samples were taken within two hours after spraying of waste water on the field had terminated. Four samples were taken from the top of each slope, near the sprinkler heads, and four samples from about 2/3 of the way down the slope. Four soil samples were also taken from an unused lot which served as control. Each set of four samples was mixed to give a homogenous mixture. From this mixture about 20 grams of wet soil were added to an equal amount of water and the pH determined. A separate sample of about 60-70 grams was removed from the soil mixture and weighed for a percent moisture determination. A third sample, of 10 grams, was added to 90 ml of water and serial dilutions were made. Quadruplicate spread plates were made using 0.4 ml of each of
three dilutions for each type of agar used. This plating took place within four to six hours after sampling.

The plates for fungi counts and the tubes used for algal counts were incubated 72 hours and four weeks, respectively, at room temperature. All other plates were incubated at 30°C. The AHB plates were incubated for 24 to 36 hours; non-symbiotic nitrogen fixers were counted in about three days, and the Streptomyces colonies were counted in five days.


CHAPTER III

RESULTS AND DISCUSSION

Efficiency of the system is indicated by the data compiled by R. S. Kerr Water Research Center, Ada, Oklahoma, in conjunction with the present study (3). Some average measurements of water purification are presented in Table 1. These data clearly indicate that this method of waste treatment is very efficient.

TABLE I

AVERAGE DEGREE OF PURIFICATION OF PLANT EFFLUENT*

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Per Cent Reduction</th>
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<tr>
<td>BOD</td>
<td>98-99</td>
</tr>
<tr>
<td>TN₂</td>
<td>80-90</td>
</tr>
<tr>
<td>VSS</td>
<td>95</td>
</tr>
<tr>
<td>TOC</td>
<td>95</td>
</tr>
</tbody>
</table>

BOD - Biological Oxygen Demand  
TN₂ - Total Nitrogen  
VSS - Volatile Suspended Solids  
TOC - Total Organic Carbon

* Analyses from R. S. Kerr Water Research Center, Ada, Oklahoma.
pH values of the test lot soils vary from 6.10 to 7.20 while those on the control lot vary from 4.50 to 6.20. The plant effluent pH is as high as 10–11 when the machinery is washed with caustic soda after each third shift, but the field effluent and soil pH vary only slightly from neutrality. This illustrates another advantage of this system, namely, the ability to withstand shock loadings of this type. Table 2 shows the pH of the five sample sites as a function of time.

### TABLE II

**pH CHART**

<table>
<thead>
<tr>
<th></th>
<th>G11T</th>
<th>G11B</th>
<th>Y1T</th>
<th>Y1B</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td>Apr</td>
<td>6.91</td>
<td>7.04</td>
<td>6.7</td>
<td>6.48</td>
<td>5.7</td>
</tr>
<tr>
<td>May</td>
<td>7.05</td>
<td>7.05</td>
<td>5.6</td>
<td>6.51</td>
<td>6.20</td>
</tr>
<tr>
<td>Jun</td>
<td>7.20</td>
<td>7.00</td>
<td>6.91</td>
<td>7.20</td>
<td>5.31</td>
</tr>
<tr>
<td>Jul</td>
<td>7.00</td>
<td>7.15</td>
<td>7.10</td>
<td>6.80</td>
<td>5.10</td>
</tr>
<tr>
<td>Aug</td>
<td>6.60</td>
<td>6.65</td>
<td>6.95</td>
<td>6.80</td>
<td>5.70</td>
</tr>
<tr>
<td>(3) Oct</td>
<td>6.10</td>
<td>7.00</td>
<td>6.15</td>
<td>6.40</td>
<td>4.50</td>
</tr>
<tr>
<td>(30) Oct</td>
<td>6.7</td>
<td>6.96</td>
<td>6.70</td>
<td>7.00</td>
<td>5.7</td>
</tr>
</tbody>
</table>

By the sampling method used, the soil moisture content determined varied from 13.5 to 55.5 per cent. The lowest figure is associated with a slow-down in plant operations.
during which the volume of effluent fell to approximately 900,000 gallons per day while the higher figure represents a more typical value while the plant is in full production and the effluent value is some 3.2 MGD. Water content in the control fields during the same period of time varied from 1.0 to 18.4 per cent. The variations in per cent moisture would indicate that a water holding capacity was determined instead of a real per cent moisture. These data are shown in Table 3.

**TABLE III**

**PER CENT MOISTURE**

<table>
<thead>
<tr>
<th></th>
<th>G11T</th>
<th>G11B</th>
<th>Y1T</th>
<th>Y1B</th>
<th>C</th>
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<tbody>
<tr>
<td>Apr</td>
<td>37.4</td>
<td>31.5</td>
<td>38.3</td>
<td>24.9</td>
<td>18.4</td>
</tr>
<tr>
<td>May</td>
<td>36.5</td>
<td>29.8</td>
<td>12.4</td>
<td>14.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Jun</td>
<td>36.8</td>
<td>27.8</td>
<td>55.5</td>
<td>33.2</td>
<td>15.4</td>
</tr>
<tr>
<td>Jul</td>
<td>34.7</td>
<td>25.2</td>
<td>39.8</td>
<td>43.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Aug</td>
<td>32.5</td>
<td>13.5</td>
<td>48.7</td>
<td>34.8</td>
<td>1.0</td>
</tr>
<tr>
<td>(3) Oct</td>
<td>27.4</td>
<td>21.7</td>
<td>32.2</td>
<td>27.7</td>
<td>11.1</td>
</tr>
<tr>
<td>(30) Oct</td>
<td>51.5</td>
<td>40.4</td>
<td>54.6</td>
<td>39.4</td>
<td>9.4</td>
</tr>
</tbody>
</table>
One of the objects of the study was to compare the total populations on the control with those of the test lots, as well as variations within the test lots. This was accomplished by determining the average number of organisms in each lot. Figure 1 is a graph of these data. The data indicate that the average total population of the test lots is, without exception, higher than the average population found in the control lot. This difference in total population between the test lots and the control lot is as one would anticipate, considering the added nutrient content of the spray and the maintenance of a moist environment in the soil.

These data also seem to indicate that there is a greater degree of fluctuation in the populations of the test lots and may reflect variations in organic loading in the spray.

The data which were collected also permitted a comparison of $Y_1$, which has received spray for six years, and $G_{11}$, a lot which has been in use only two years. Figure 2 shows this comparison by time of sampling. Even though both lots are equally efficient in purifying the plant effluent, the new lot always has a lower microbial population. Averaging the populations from these two lots (Figure 1) was thought to be valid because even though $G_{11}$ has a lower count than $Y_1$, it is usually higher than the control. (See Figure 3).
Figure 1--Total Microbial Population on Control and Test Lots
Figure 2—Total Microbial Population on Test Lots G-11 and Y-1*

*Y₁ was not sampled until June
Figure 3—Total Population, $G_{11}$ vs Control
Total microbial populations at two locations on each slope were compared by the methods described above. These data are presented in Figure 4. Except for the July sample the total population on the top of the slope was higher than the population near the bottom. Again, the most plausible explanation for this is that the concentration of nutrients is greater under the sprinkler heads. These nutrients are present both as soluble carbon compounds and as particulate material resulting from vegetable processing. After spraying, the particulate material settles out, providing a continuing source of carbon, and the soluble material is partially oxidized by the microflora at the top of the slope. When the water reaches the bottom of the slope, much of the carbon has been removed so that substrate becomes a limiting factor with regard to the number of organisms which can be supported. The decrease at the top of the slope in the July sample might be explained by the presence of an inhibitor in the effluent; for example, okra has been shown to inhibit bacterial growth on an effluent (2). However, supportive evidence for this case is lacking. As with the data in Figure 1, the highly variable nature of the population is illustrated.
A second aspect of this study was to determine the effects of the effluent on various segments of the microbial population. Using appropriate selective media, the populations of AHB, *Streptomyces*, fungi, algae, and non-symbiotic nitrogen fixers were enumerated. Several attempts were made to quantitate protozoa but with no success. Undoubtedly, they are present but were not detectable by the method used (3, p. 22), even in dilutions as low as 1:10. Quantitation of anaerobes was also done. However these studies were discontinued since it was found that 95 to 99 per cent of all anaerobes were facultatively anaerobic. Nitrogen-fixing blue-green algae are also present but were not routinely tested for.

Although the percentage fluctuates greatly, the greatest fraction of the total population on both the test and control lots was found to consist of AHB plus *Streptomyces*, with the AHB making up the bulk of the population as a rule. (See Figures 5 and 6). The only exception to this was in the October 30, 1968, sampling period at which the non-symbiotic nitrogen-fixing population was found to make up 77 and 49 per cent of the total population on the control and test lots, respectively. The algae and fungi together consistently were
Figure 5—Percent of Total Population Accounted for by Aerobic Heterotrophic Bacteria (AHB) and *Streptomyces* (S) on the Control Lot
Figure 6--Per cent of Total Population Accounted for by Aerobic Heterotrophic Bacteria (AHB) and **Streptomyces** (S) on the Test Lot
found to make up less than 2 per cent of the total population, with the fungi normally being found to be the lower of the two. This order of concentration of these particular types of organisms in soil is not atypical (1) and thus adds weight to the premise that the efficiency of the system is due to an increase in the total heterogenous microbial population rather than an increase in any one segment of the population.
CHAPTER BIBLIOGRAPHY


CHAPTER IV

CONCLUSION

This investigation was an attempt to quantitate various segments of the microbial flora and to relate the percentage of the total on an average test lot to a control lot and to relate total populations to each other in varying ways.

The average of the four test lot sampling sites has a consistently higher total population of microorganisms than the populations of the control lot. This is expected since the nutrient content of the test lot is increased daily by the sprayed effluent. The spray also allows bacterial forms which are readily subject to dessication to abound since a more moist environment is provided. The implication of these results is that the microbial population plays a significant role in the degradation of the organic matter in the plant effluent.

Support of this thesis is provided by a comparison of the populations on the top and bottom of the test lots. Almost without exception the top of the slope, close to the sprinkler heads, has a higher count. One could speculate
that the decrease in July could be due to an inhibitor which was degraded before it reached the 2/3 sampling site, or to a plating error.

With regard to variations between test lots, it was found that the lot which had been in use longer had a larger population. This could be due to an increase in accumulated organic substances that are degraded slowly by select microbial populations in the field, and thus to a greater reserve supply of nutrients. However, it was observed that the older lot has less of a slope than the newer lot. This more gentle slope would increase the retention time of plant effluent on the slope which would, in turn, permit a greater increase in the population. Which of these two explanations is more valid would require additional experimentation.

The population fractions, in descending order, are AHB, streptomycetes, non-symbiotic nitrogen fixers, algae and fungi. If, indeed, the higher the count the more active the group of organisms, then the AHB and Streptomyces would account for the greatest part of the degradative activity of the soil population. However, even those organisms which occur as minor portions of the population might be very important with regards to the purification process if their metabolic activity is high.
In summary, these data indicate that the purification of this type of waste is due, at least in part, to the soil microflora as reflected by numbers on the test versus control lots and the top versus the bottom of the test lots. Based on the types of organisms selected for, the bulk of the purification is due to the metabolic activity of the AHB plus *Streptomyces*.

Furthermore, the purification of the effluent is apparently due simply to an increase in relative numbers of the types of populations on the test lots as opposed to the control lot. That is, the frequency of occurrence on both test and control lots is AHB, *Streptomyces*, non-symbiotic nitrogen fixers, algae, and fungi in descending order.
CHAPTER V

SUMMARY

This investigation was an attempt to quantitate the microflora of a field receiving effluent from a canning company whose effluent contents vary widely. It includes total and relative counts of five major portions of the microbial population with the only major section not sampled being protozoa. The populations enumerated were fungi, algae, non-symbiotic nitrogen fixers, *Streptomyces* and aerobic heterotrophic bacteria.

The findings were as expected in the relationship between test and control lots, in that larger populations were found on the test lots where available nutrients are at a higher concentration. Counts averaged about $170 \times 10^6$ organisms per gram dry weight of soil on the test lots and about $16 \times 10^6$ organisms per gram dry weight of soil on the control lot.

The data show no major shifts in the relative abundance of the organisms tested for on the test versus the control lots, indicating that the purification of this type of
effluent is primarily due to an increase in the total soil microbial population in response to the added nutrients from the plant effluent.
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