A STUDY OF THE FIVE-DAY BIOCHEMICAL OXYGEN DEMAND OF TANNERY WASTE

Thesis for the Degree of B. S.

MICHIGAN STATE COLLEGE

Stanley J. Mogelnicki

1939

A Study of the
Five-Day Biochemical Oxygen Demand
of Tannery Waste

A Thesis Submitted to

The Faculty of

MICHIGAN STATE COLLEGE

of

AGRICULTURE AND APPLIED SCIENCE

BY

Stanley J. Mogelnicki

Candidate for the Degree of

Bachelor of Science

June 1939

THESIS

TABLE OF CONTENTS

ACKNOWLEDGEMENT

The writer wishes to express his sincere appreciation to Mr. E. F. Eldridge for his guidance and helpful suggestions.

STATEMENT OF PROBLEM

The object of this problem is to make a study of the biochemical oxygen demand of tannery waste in an effort to find a method of measuring the true B. O. D. of the tannery waste for the comparison of its strength with sewage and other industrial wastes.

INTRODUCTION

The biochemical oxygen demand, or B. O. D., test is a determination of the amount of oxygen required by aerobic bacteria to opidize the unstable organic matter in sewage, trade waste, or stream. The test is highly valuable for measuring the degree of pollution of a stream and for comparing the relative strength of wastes. To aid in the better interpretation of the results obtained by the test, a study has been made of the five-day B. O. D. of tannery waste.

The B. O. D. Determination

The biochemical oxygen demand test is a direct application of the natural biological purification processes in streams and polluted waters, in which the unstable organic compounds of the wastes are oxidized aerobically by the bacteria and plankton to simple end products. In this test, conditions are maintained artificially so that the oxidation can proceed undisturbed during the period of incubation; and the organic matter present in a sample is measured in terms of the oxygen required to oxidize it. The test is carried out by determining the dissolved oxygen content before and after the period of incubation on suitably prepared portions of the sample.

If strong waste is being tested, it is necessary to dilute the samples to such an extent that the oxygen will not be depleted before the end of the period of incubation. In preparing the desired dilutions, the essential requirement is to obtain samples that are uniform in regard to distribution of the waste.

A simple method of dilution is achieved be adding suitable

amounts of waste directly to the bottles. Dilution water is first siphoned from its container into the bottles until they are half full. The required amounts of wastes are added and the bottles completely filled with diluting water. The stoppers are inserted without entrainment of air, and a water seal is provided. The bottles are then placed in a 20 degree C. incubator for a definite period of incubation. The time of incubation is usually five days, after which each dilution and the diluting water is tested for dissolved oxygen content. The difference between the dissolved oxygen content of the diluting water and that of the dilution is the biochemical oxygen demand. If the sample has been diluted to make the test, the loss of oxygen in the diluted portions must be multiplied by the diluting factor to obtain the oxygen demand of the original sample.

Certain other precautions must be taken to insure comparable results. Important among these are: clean glassware; constant incubator temperature; tightly stoppered and properly sealed bottles; proper seeding of samples where this is necessary; the use of satisfactory diluting water where dilution is required; the careful adjustment of dilutions to obtain adequate oxygen depletions; and the inclusion of blank controls on the dilution water and the seeding material where these are used.

Diluting Water

A very important factor which must be considered in the B. O. D. determination is the type of diluting water used. Because of the various diluting waters used, many discrepancies arise. Generally, the diluting water is obtained from the stream being studied; but the results obtained with the water from one stream will not be comparable with the results

from the water of another stream. Due to the non-uniformity of results arising from the use of the various diluting waters, there has been much experimentation and discussion on the subject in an effort to obtain a standard diluting water.

A study of various diluting waters was made by Messrs. E. F. Eldridge and W. L. Mallman [1]. In this study, the college tap water was used as a standard to measure other diluting waters, because it gave the highest B. O. D. result in the five-day period of incubation. According to the above writers, the diluting water need not necessarily simulate the natural occurring waters, but should be as near a perfect menstruum for the total oxygen demand of the organic matter present as is possible. The same writers view the B. O. D. test as a quantitative measure of the organic matter present and not an attempt to simulate stream conditions. Accordingly, these writers claim that the diluting water should be a mineral containing medium which has no oxygen demand, but which does furnish the proper mineral requirements and hydrogen ion concentration for the growth of the desired bacteria.

Five diluting waters with different pH values were used in this study. "The first was tap water with a pH of 7.3; the second, distilled water, pH 6.2; the third, distilled water with 300 parts per million sodium carbonate, pH 8.5; the fourth, distilled water with 300 parts per million sodium bicarbonate, pH 7.5; and the fifth, distilled water with 500 parts per million di-sodium acid phosphate, pH 7.6." Also, a synthetic water was made in an attempt to simulate the average river water by dissolving easily soluble salts containing the principal ions of the salts in the average river waters. The synthetic water was made by dissolving 170 parts per million calcium chloride ($CaCl_2 \cdot 2H_2O$), 120 parts per million magnesium sulfate ($MgSO_4 \cdot 7H_2O$), and 230 parts per million sodium bicarbonate ($NaHCO_3$) in distilled water. The pH value of the synthetic water was 7.28; the same as that of tap water. The B. O. D. values obtained with these two waters were very nearly the same. The final pH values were 7.1 for the tap water and 7.2 for the synthetic water. This showed that the products of oxidation had very little effect on the pH; however, the sewage used was a tank effluent without industrial waste, the presence of which might have made a difference.

Consideration of the results of this experiment proved that the pH value of the diluting waters is not the only significant factor in obtaining correct results, but that certain other salts found in natural waters must be present. Apparently, the quantity of these salts makes very little difference in the results, as the tap water used in these experiments contained a greater quantity than the synthetic water, yet the results were the same.

The results of the investigation showed that a synthetic water containing the mineral salts common to natural waters was superior to distilled, bicarbonate, carbonate, and phosphate waters. The two limiting factors were found: the pH value, and the mineral salt content. This study proved that the type of diluting water does greatly influence the B. O. D. results; this influence being due somewhat to the two factors mentioned.above.

Another study of diluting waters was made by Messrs. H. Heukelekian and N. S. Chamberlin. The following waters were used in their study:

1. "Stream water from Great Egg Harbor Creek, near Weymouth, in the southern section of New Jersey.

2. "Stream water from North Branch, a tributary to the Raritan, in the central section of the state.
3. "Stream water from Pequest River, tributary to the Delaware, in the northern section of the state.
4. "Sodium bicarbonate water - 100 parts per million.
5. "Sodium bicarbonate water - 500 parts per million.
6. "Synthetic water of the following composition, as suggested by Mohlman:

    $NaHCO_3$ - 65 parts per million.

    $CaCl_2$ - 10 parts per million.

    $CaSO_4$ - 15 parts per million.

    $MgSO_4$ - 10 parts per million.
7. "Phosphate water, as suggested by Theriault - 42.5 parts per million of KH_2PO_4 neutralized with N/1 NaOH.
8. "Distilled water."

Foreign matter was removed from the stream waters by filtering, then the waters were allowed to stand two weeks so as to satisfy their original B. O. D. The waters were analyzed for B. coli, dissolved oxygen, pH value, alkalinity, acidity, total solids, ash, chlorides, sulfates, and iron.

The biochemical oxygen demand determination was made in accordance with "Standard Methods". The period of incubation was five days at 20 degrees C. for each type of water.

The following conclusions were derived from the results of this experiment:

1. The B. O. D. values of the three stream waters used as diluting water were different in each case.

2. The lowest B. O. D. value was obtained from the stream water with the lowest salt concentration.

3. The B. O. D. values were similar when the artificial waters were used.

4. The B. O. D. value obtained with distilled water was low.

The results of this study are very similar to those made by Messrs. Eldridge and Mallmann.

Advantages and Limitations of Present Method

The biochemical oxygen demand determination is an invaluable test for measuring the degree of pollution of streams and for comparing the relative strength of wastes. This test has advantages over strictly chemical tests in that it simulates very closely the reaction taking place under natural conditions and includes oxygen used in both carbonaceous and nitrogenous oxidation. Such an advantage is satisfactory in the study of the polution of a single stream; but when it comes to stream pollution studies and the comparison of the relative strength of wastes, which necessitate great dilutions, the problem of getting a standard diluting water is apparently very complex.

The biochemical oxygen demand test can be applied to domestic sewage with dependable results, because the conditions in the samples when diluted for incubation are well suited to the development and activity of the organisms responsible for oxygen utilization. The dilutions are well seeded, the pH is favorable, and the organic and mineral food supply is natural for the type of organisms present.

This is also true of certain industrial wastes, such as milk waste; however, there are numerous other wastes that are not favorable, such as white water from a paper mill, the Steffens waste from a beet sugar factory, and the tanning liquors from a tannery. These wastes have certain characteristics to which the organisms must become adjusted before they become active. In some cases, it may be impossible for the organisms to properly adjust themselves even when apparently all conditions such as pH, etc. have been made favorable.

Some of these wastes are alkaline, some acid, some sterile, and some contain only special organic and inorganic compounds. Alkalinity and acidity can be corrected, seeding can be added, but the proper development of organisms requires a more or less balanced organic and mineral diet. Also time is required for organisms to become accustomed to changed conditions. The length of time required will depend upon the conditions to which they must accustom themselves and cannot be determined within the scope of a B. O. D. test. It may be several days or more, and if such is the case, the apparent five-day B. O. D. may be only a small part of the total B. O. D. This five-day B. O. D. cannot be compared with the five-day B. O. D. of sewage or some more favorable waste material.

With industrial wastes such as tannery waste, the apparent B. O. D. has been found to increase as the dilution of the waste was increased. Also the B. O. D. has been found to increase with an increase in seeding.

The answer to the cause of this variation in results seems to be in the ratio of number of organisms represented by the quantity of seeding, to the amount of material composing the food supply. Since it is common practice to add the same amount of seeding to each dilution regardless of the amount of waste added, it seems that the number of organisms present will affect the utilization of oxygen. If this is the case, then the greater the dilution of the waste, the greater will be the ratio of organisms to food and the higher will be the apparent B. O. D. If this is correct, then the standardization of the B. O. D. test so that it can be used with sterile wastes, such as tannery waste, will be difficult.

EXPERIMENTAL

To aid in the better interpretation of the B. O. D. of tannery waste, the following study was made with six diluting waters.

Procedure

For this study the following diluting waters were used:

1. Standard diluting water (distilled water with 300 parts per million of $NaHCO_3$).
2. Standard diluting water with 10 parts per million of KCl.
3. Standard diluting water with 15 parts per million of NH_4Cl.
4. Standard diluting water with 42.5 parts per million of Na_3PO_4.
5. Standard diluting water with 10 parts per million of KCl and 15 parts per million of NH_4Cl.
6. Standard diluting water with 10 parts per million of KCl, 15 parts per million of NH_4Cl, and 42.5 parts per million of Na_3PO_4.

The standard diluting water was made by aerating five gallons of distilled water containing 300 parts per million of $NaHCO_3$ until the dissolved oxygen content was above 6 parts per million. The water was allowed to stand overnight. From this container, three liters of the standard diluting water were siphoned into each of five one-gallon bottles, and the required amounts of salts were added as specified above. These waters were allowed to stand until equilibrium was established.

The procedure planned for each diluting water was to run a series of tests composed of 4/10 per cent and 1/10 per cent dilutions of tannery waste incubated for five days at 20 degrees C. Each series consisted of 54 bottles—9 bottles for each diluting water as follows: 3 bottles of diluting water blanks, 3 bottles of 4/10 per cent dilution, and 3 bottles of 1/10 per cent dilution. One bottle from each of the three groups was not seeded; one bottle from each of the three groups was seeded with one c.c. of sewage; and one bottle from each of the three groups was seeded with two c.c. of sewage. The sewage was obtained just previous to seeding and was first filtered before being used.

A duplicate of this series had been made before it was found that the 4/10 per cent dilutions were giving desired results. In an effort to have comparable results, dilutions of 2/10 per cent and 1/10 per cent were used in the next series, but even the 2/10 per cent dilution did not give desired results, so that only the results of the 1/10 per cent dilutions were able to be used. In this second series, the same procedure detailed above was followed except for the seeding. Three c.c. and four c.c. seedings were used instead of one c.c. and two c.c.

The B. O. D. was calculated by taking the difference between the dissolved oxygen content of the diluting water blank and that of the dilutions. This method eliminated from the computation the B. O. D. of the sewage, which was automatically taken care of.

Results

The B. O. D. values obtained from the various waters without seeding were variable. Also, for any single diluting water, the B. O. D. value of the larger dilution was different from that of the smaller dilution. The cause of these differences is not known as there are many times during the test where an error may arise due to technique. However, the same results were obtained from a duplicate series and this tends to eliminate errors due to technique.

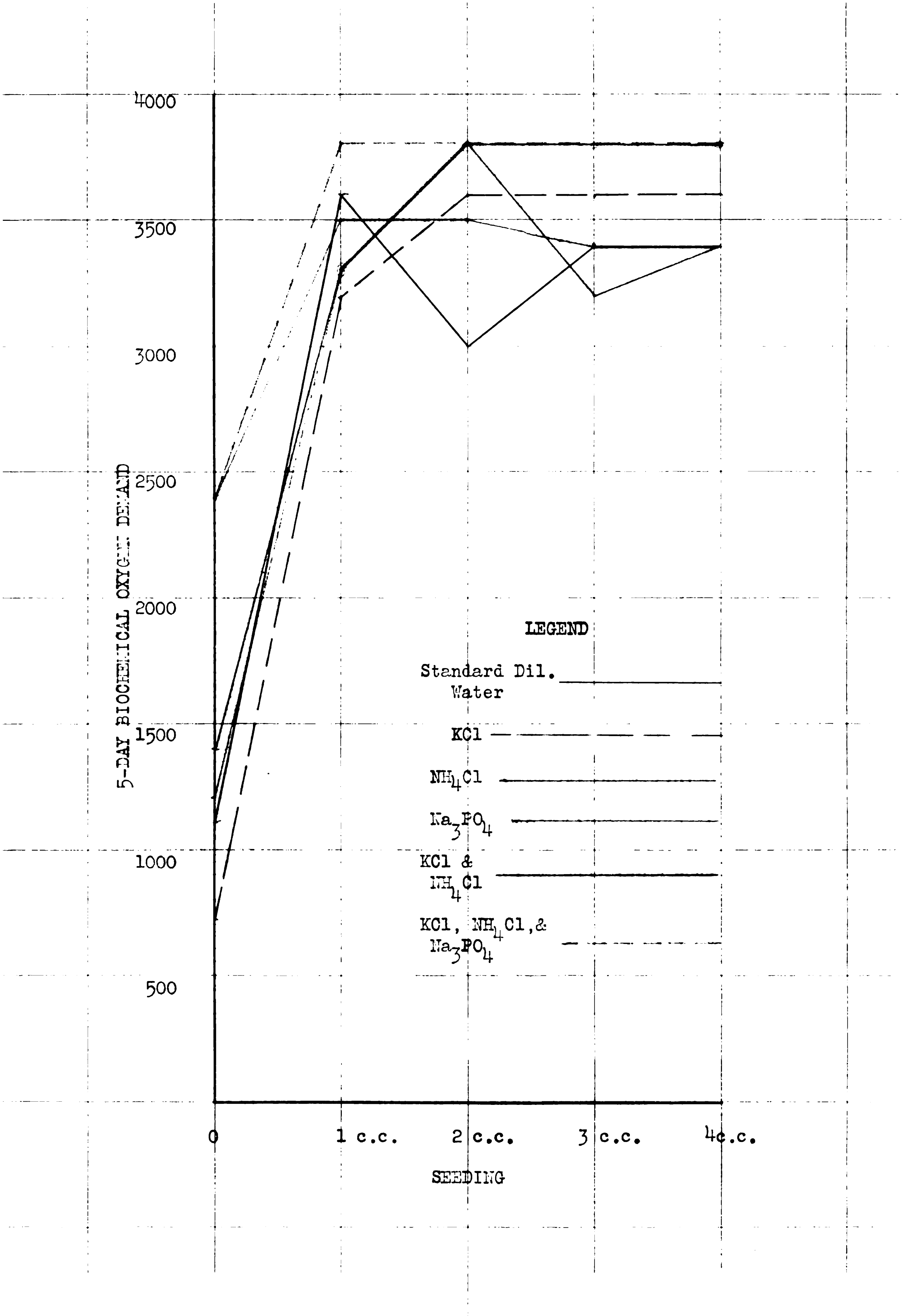
The phosphate water gave the highest B. O. D. without seeding, while the standard diluting water gave the lowest, and the other waters, not including any water which contained phosphate, were likewise low. The reason for the high result with the phosphate water may be due to the increased bacterial activity in the presence of phosphate, which, according to Mallmann[3], is necessary for the proper metabolic action of micro-organisms.

There were no marked differences in the B. O. D. values obtained with all the waters with all seedings; however, there are some discrepancies in the results, but these may be due to variation in the number of organisms supplied in the seeding.

The results can best be seen from Table I and Chart I.

Table I

	Series I				Series II			
Seedings	None		1 cc.	2 cc.	None		3 cc.	4 cc.
Dilutions	.4%	.1%	.1%	.1%	.2%	.1%	.1%	.1%
Standard dil. water	650 800	660 800	3400 3000	3800 3200	500	400	3600	3600
KCl	750 700	800 1400	3600 3600	3200 2800	1000	400	3400	3400
NH_4Cl	700 1100	1000 1800	3400 3200	3800 3800	1000	600	3200	3400
Na_3PO_4		2400 2400	3200 3800	3800 3200	2800	2400	3400	3400
KCl & NH_4Cl	700 850	1200 1200	3400 3200	4000 3600	2200	1200	3800	3800
KCl, NH_4Cl, & Na_3PO_4		2400 2400	3800 3800	3800 3800	3000	2800	3800	3800


4000
3500
3000
2500
2000
1500
1000
500
5-DAY BIOCHEMICAL OXYGEN DEMAND
0
1 c.c.
2 c.c.
3 c.c.
4 c.c.
SEEDING
LEGEND
Standard Dil. Water
KCl
NH4Cl
Na3PO4
KCl & NH4Cl
KCl, NH4Cl, & Na3PO4

The B. O. D. values obtained from the various dilution waters were plotted against the seeding, as shown by Chart I. Not much increase in the B. O. D. in proportion to the seeding is shown, but this is probably due to the fact that the food supply available in such great dilutions was so small that the optimum quantity of organisms was present in one c.c. of sewage. Therefore, there was no appreciable increase of B. O. D. with an increase in seeding.

From the table it is evident that without seeding the B. O. D. values are higher for the greater dilutions. This bears out the results of previous experiments which were discussed in the introduction. These variations were duplicated on a duplicate series, which fact eliminates technique as the possible source of error.

These differences apparently are due to the ratio of the number of organisms to the amount of food supply. Also the toxicity may be increased when larger quantities of waste are used in a dilution, and thus reduces the activity of the organisms and thereby giving a low B. O. D.

Just how the phosphate diluting water may be applied to the bio-chemical oxygen demand determination, and thus determine the true B. O. D. of tannery waste, is a very difficult prediction. There are so many other factors that must be considered to standardize the B. O. D. determination, that the problem of getting a standard diluting water for all wastes is apparently too complex. For theroetical studies, however, more research along this line might bring forth a standard diluting water.

Conclusions

1. The presence of phosphate in dilution water gives the highest B. O. D.
2. Seeding with sewage definitely increases the B. O. D.
3. More research is necessary before any recommendation can be given for the use of a special diluting water.

BIBLIOGRAPHY

(1) "The Influence of Diluting Water on the Biochemical Oxygen Demand", by E. F. Eldridge and W. L. Mallmann, Michigan State College, Engineering Experiment Station Bulletin, Vol. 7, no. 1, 1931.

(2) "The Effect of the Dilution Water on the Biochemical Oxygen Demand Determination", by H. Heukelekian and N. S. Chamberlin, Sewage Works Journal, Vol. 3, no. 2.

(3) "The Influence of Phosphates on the Metabolism of Bacteria", Michigan Academy of Science, Vol. 14, 617, 1931.

(4) "Studies of Biochemical Oxygen Demand of Trade Wastes", by E. F. Eldridge, Engineering News Record, July 3, 1930.

(5) "Biochemical Oxygen Demand of Industrial Wastes", by E. F. Eldridge , Michigan State College Engineering Experiment Station, Bulletin no. 78 , 16 - 19, 1938.