A COMPARISON OF 20 deg. C.
BIOCHEMICAL OXYGEN DEMAND
AND 37 deg C. BIOCHEMICAL
OXYGEN DEMAND

Thesis for the Degree of B. S.

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A Comparison of 20 deg. C. Biochemical Oxygen Demand and 37 deg. C. Biochemical Oxygen Demand

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By

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STATEMENT OF PROBLEM

The object of this problem is to develop a 37 deg. C. biochemical oxygen demand test which will be comparable to the 20 deg. C. biochemical oxygen demand but at the same time decrease the time of incubation.

INTRODUCTION

The biochemical demand test (B.O.D.) is a valuable determination for measuring the degree of purification in sewage treatment and in making stream pollution surveys. It may be defined as the amount of oxygen required by aerobic bacteria to oxidize the organic matter in the sewage or stream water. Because of the discrepancies which may arise during this determination, there has been a great deal of experimentation and discussion regarding the factors influencing this test.

The B.O.D. Determination

Before discussing these factors, the standard technique and procedure will be explained.

In obtaining samples from streams, ponds or rivers a special type of sampling apparatus must be used. This equipment should be such that the liquid in the sampling bottle is collected without entrainment of air bubbles. Tests should be run as soon as possible after collection. If normal sewage 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 both to distribution of the waste and to dissolved oxygen content.

A very simple method of dilution is obtained by adding suitable amounts of sewage directly to bottles of known capacity. Dilution water is first siphoned from its container to the bottles, each bottle being about half filled. The required amounts of sewage are added and the remainder of the bottle filled with more diluting water. The bottles are then sealed by means of a water seal and placed in a 20 deg. C. incubator for a definite period of incubation. The time of incubation is usually 5 days, after which each dilution and the diluting water is tested for dissolved oxygen content. The difference between the d.o. of the diluting water and that of the dilution is the B.O.D.

Relation of Time and Temperature

This determination is based upon the fact that a polluted water containing bacteria, if exposed to oxygen will eventually become completely purified. During this time period, definite amounts of dissolved oxygen are absorbed, and since this rate of absorption of the oxygen by the polluted water is very slow, the incubation period is extended over a number of days. Also, since the reaction is biochemical, the temperature at which the test is run is carefully controlled. A variation in temperature of one degree causes a change of about 5 per cent in the specific rate of deoxygenation. Therefore, in order to obtain consistent results the temperature of incubation must be kept constant.

Experiments were run by the U. S. Public Health Service[1] primarily for the purpose of confirming the accuracy of various time and temperature correction formulas. These experiments also proved that factors other than time and temperature must be considered before a reasonable interpretation of results can be made.

Representative samples for the experiments were taken from the Ohio River. From these samples, sub-samples were made up by siphoning into 350 c.c. capacity bottles. The initial oxygen content was then determined, and the examples were incubated at 9 deg., 20 deg., or 30 deg. C. The observed oxygen demand values for the different temperatures were plotted against the period of incubation. These results, as shown in Fig. 1, showed quite a similarity between the results obtained at the different temperatures of incubation.

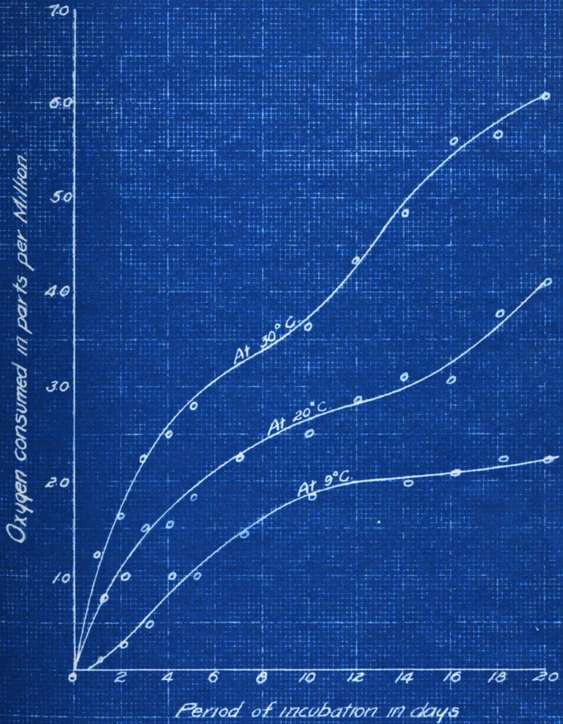
In order to illustrate the rate of deoxygenation, the 20 deg. C. (middle curve) will be used. It is evident that the rate of deoxygenation decreased uniformly during the first nine or ten days. During the next five or six days small quantities of oxygen were absorbed, but it was noted that after the sixteenth day, there was a marked increase in the rate of deoxygenation. This increase bears out a view expressed by Adeney and other British experimenters[1], that under aerobic conditions the stabilization of organic matter proceeds in two different and consecutive stages - the carbonaceous matter is first oxidized; then nitrification sets in. This second point of inflection on the curve marks the onset of the nitrification stage.

In confirming the formulas, only the average oxygen demand values corresponding to the first stage were considered. The results obtained were then compared with a formula proposed by Phelps[1]. This formula is based on the assumption that the rate of deoxygenation at any instant is directly proportional to the amount of organic matter present in the sample.

Expressed as a formula:

$$\text{Rate of deoxygenation} = \frac{d\,(La - L)}{dt} \quad \frac{-dL}{dt} = K^1 L \qquad (1)$$

where,



7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
2
4
6
8
10
12
14
16
18
20
Oxygen consumed in parts per Million
Period of incubation in days
At 30° C.
At 20° C.
At 9° C.

Fig. 1

La = oxygen absorbed during first stage

L = oxygen requirement of the sample at time, t

K^1 = a constant at given temperature

The integration of expression (1) leads to the equation

$$\log \frac{La}{L} = \log \frac{La}{La - X} = Kt \qquad (2)$$

where,

X = oxygen absorbed in t days

$K = 0.4343\ K^1$ = deoxygenation constant

Solving for X in equation (2), the following equation is obtained,

$$X = La\ (1 - 10^{-Kt}) \qquad (3)$$

The results of the observed and computed values are shown graphically in Fig. 2. In order to place all the values on a comparable basis, the data has been plotted as a percentage of oxygen absorbed during the first stage of deoxygenation instead of in parts per million. For periods of incubation of less than 8 days at 30 deg. C., 10 days at 20 deg. C., or 15 days at 9 deg. C., the agreement between the observed and the computed percentage values is very good.

In plotting the curves representing the computed values, the value of K was computed by the equation:

$$K_T = K_{20}\ (1{-}047^{T-20}) \qquad (4)$$

where,

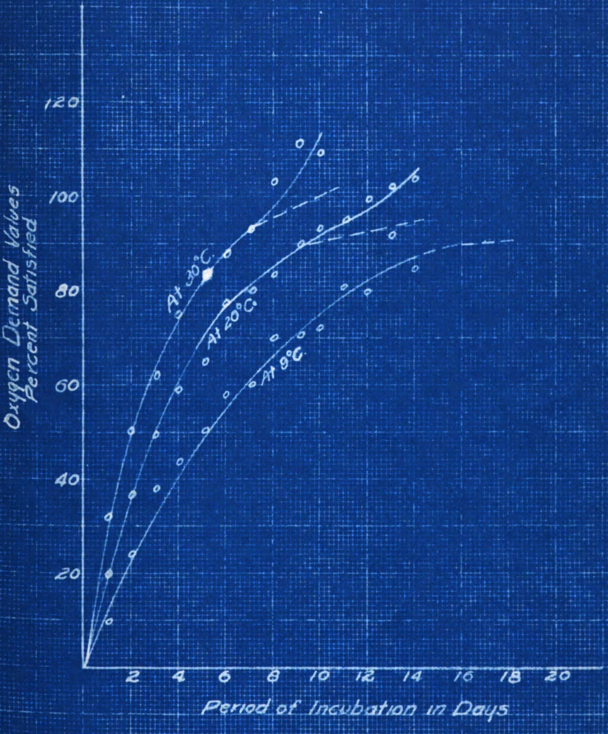

120
100
80
60
40
20
Oxygen Demand Values
Percent Satisfied
At 30°C.
At 20°C.
At 9°C.
2
4
6
8
10
12
14
16
18
20
Period of Incubation in Days

Fig. 2

K_T = the deoxygenation constant at T deg. C.

K_{20} = the deoxygenation constant at 20 deg. C. = 0.100.

If these formulas are applied to heavily polluted waters, it is possible, within certain limits, to convert the oxygen value obtained at any temperature over any period of incubation into terms of the oxygen demand value which would have been obtained under any given set of conditions.

For samples which have reached a higher degree of purification, Fig. 1, will again be used with the assumption that the five-day oxygen demand of the sample at 20 deg. C., had been determined after a preliminary conditioning period of 7 days. Under this assumption the observed depletion would have been about (2.8 - 2.3) = 0.5 parts per million. If the test had been delayed for 15 days, so that nitrification was about ready to set in, the observed loss of oxygen would have been about (4.1 - 3.1) = 1.0 parts per million. If a sewage effluent has been diluted 50 times before conducting the test, the oxygen demands would be 25 or 50 parts per million, depending on how much preliminary purification the sample had received. Under these conditions the five-day oxygen demand of the more highly oxidized sample was apparently twice as great as that of the same sample in a less highly purified state. This discrepancy may be due to the fact that one set of values were selected from the flat portion of the curve. Thus, a five-day oxygen demand of, say, 20 parts per million is not of much value unless a great deal is known concerning the state of oxidation of the sample.

The conclusions as given by the writer of the report, Emery J. Theriault are as follows:

1. "The Phelps formula holds with reasonable accuracy when applied to samples recently polluted with organic matter."

2. "For periods of incubation of less than 10 days it is possible to refer the results obtained under standardized laboratory conditions to the actual times of flow and temperatures of a stream."

3. "Under aerobic conditions the stabilization of organic matter apparently proceeds in two distinct stages."

4. "The rate at which a polluted water is deoxygenated depends largely on the condition of the sample with respect to its state of oxidation."

5. "It is necessary to exercise considerable caution in interpreting the results of analyses when the nitrification stage has almost been reached."

Diluting Water

An 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. Usually 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 another stream. As a result of the lack of uniformity in the use of various diluting waters, there has been much experimentation and discussion on the subject in order to obtain a standard diluting water. The factors affecting the water are the influence of pH on oxygen demand and bacterial count, and the influence of the mineral constituents of the diluting water on the oxygen demand and bacterial count under constant pH conditions.

A study of various diluting waters was made by Messrs. E. F. Eldridge and W. L. Mallmann (2). In this study, the college tap water was used as a standard to measure the other waters, because it gave the highest B.O.D. reading in the five-day period of incubation. The diluting water, according to the above writers, 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 bacteria.

Five diluting waters with different pH values were used in these studies. "The first was tap-water with a pH of 7.3; the second, distilled water, pH 6.2; the third, distilled water with 500 parts per million, $Na_2 CO_3$, pH 8.5; the fourth, distilled water with 500 parts per million, Na HCO_3, pH 7.5; and the fifth, distilled water with 500 parts per million, $Na_2 HPO_4$, pH 7.6." These waters were first drawn into five gallon bottles, one c.c. of sewage being added to each bottle, and the contents were then aerated for five hours. This was then allowed to stand two weeks to satisfy the natural demand of the water.

The sewage used was obtained from the East Lansing Imhoff tank. Dilutions of 2 per cent and 5 per cent were made of this sewage in 4 liter quantities. These dilutions were then siphoned into 250 c.c. bottles which were sealed with a water seal to prevent aeration during the determination.

These samples were incubated at 20 deg. C. for a definite period of time. The pH was determined at the start of the incubation, half way through the series and at the end. The B.O.D. were calculated and the results shown in graphical form in Chart 1.

The tap water curve is smooth as compared to the other waters

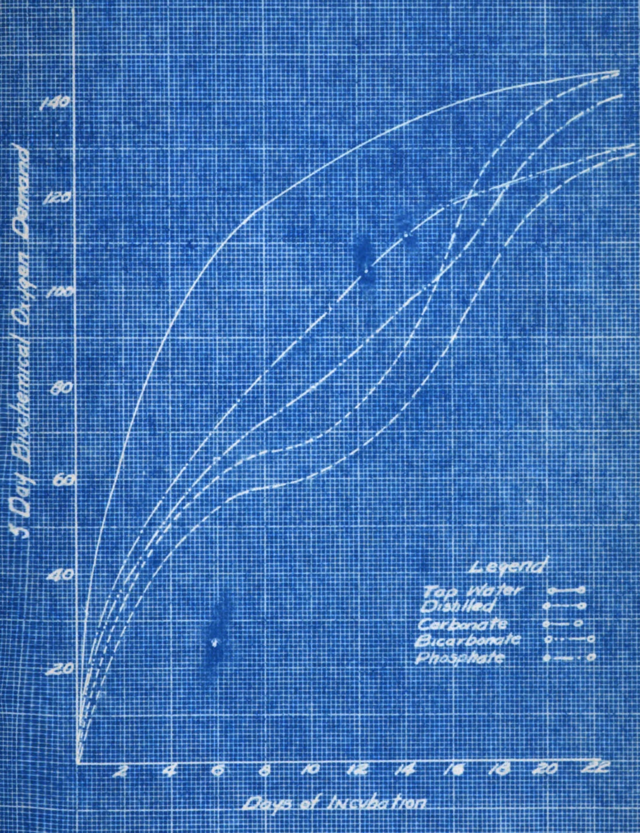

5 Day Biochemical Oxygen Demand
140
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
20
22
Days of Incubation
Legend
Tap Water
Distilled
Carbonate
Bicarbonate
Phosphate

Chart I

which had a considerable lay in the oxidation. This was primarily due to the effect of the hydrogen ion concentration on the growth and activity of the bacteria.

The pH determinations which were made at varying intervals showed very little change in the pH. The pH of the 5 per cent tap water dilution dropped from 7.3 to 7.1 at the end, and the distilled water from 6.2 to 5.7. The others remained approximately the same.

The results of the determination showed that a 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. Henkelehian and N. S. Chamberlin(3). 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 Deleware, 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 following composition, as suggested by Mohlman."

$NaHCO_3$ - 65 parts per million

$CaCl_2$ - 10 " " "

$CaSO_4$ - 15 " " "

$MgSO_4$ - 10 " " "

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, the water then being 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, sulphates, and iron.

The biochemical oxygen demand determination was made according to the "Standard Methods." The period of incubation was 5 days at 20 deg. C. for each type of water.

A few conclusions derived from the results of this experiment are as follows:

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. When the artificial waters were used, the B.O.D. values were similar.
4. The B.O.D. value of distilled water was low.

The results of this study were somewhat similar to those made by Messrs. Eldridge and Mallmann.

<u>Objections to Present Method</u>

The biochemical oxygen demand determination is a valuable test for measuring degree of pollution of streams and for the comparison of the relative strength of wastes. The test has some objections, the main one being the length of time required to complete the test. Twenty days usually is adopted as the period required for the almost complete oxidation of sewage dilutions when incubated at 20 deg. C. Since this is too long a time to await for results, it is common practice to use the five-day demand for calculating the B.O.D. of given sample. Even the five-day period is too long a time to complete the test.

EXPERIMENTAL

Procedure

The original procedure planned was to run three series of tests, each series to be composed of 5 per cent and 10 per cent dilutions incubated at 20 deg. C. However, it was found that the 10 per cent dilutions were not giving desired results. In order to have comparable results, two series were set up having dilutions of 5 per cent and 2 per cent.

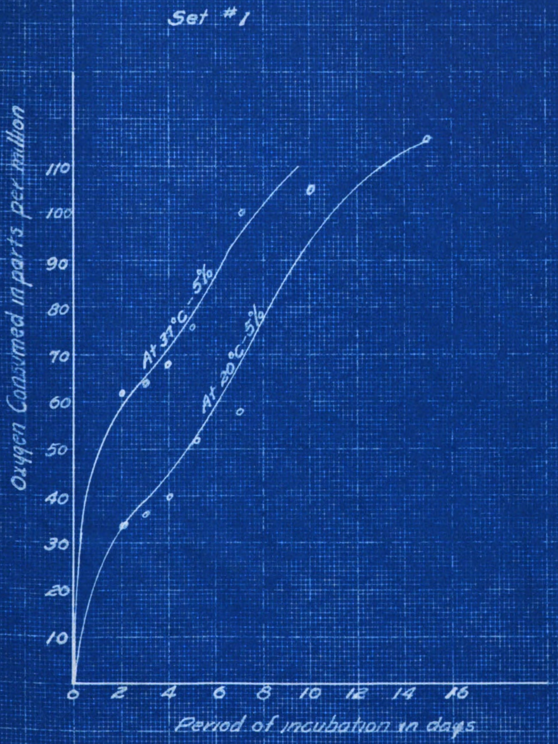
Each series was made up of forty bottles, eight bottles of each dilution incubated at 37 deg. C., and eight at 20 deg. C. These were tested for B.O.D. at one, two, three, four, five, seven and fifteen days. Included in the series was a set of eight diluting waters. The B.O.D. was computed by taking the difference between the dissolved oxygen of the diluting water and the dilution.

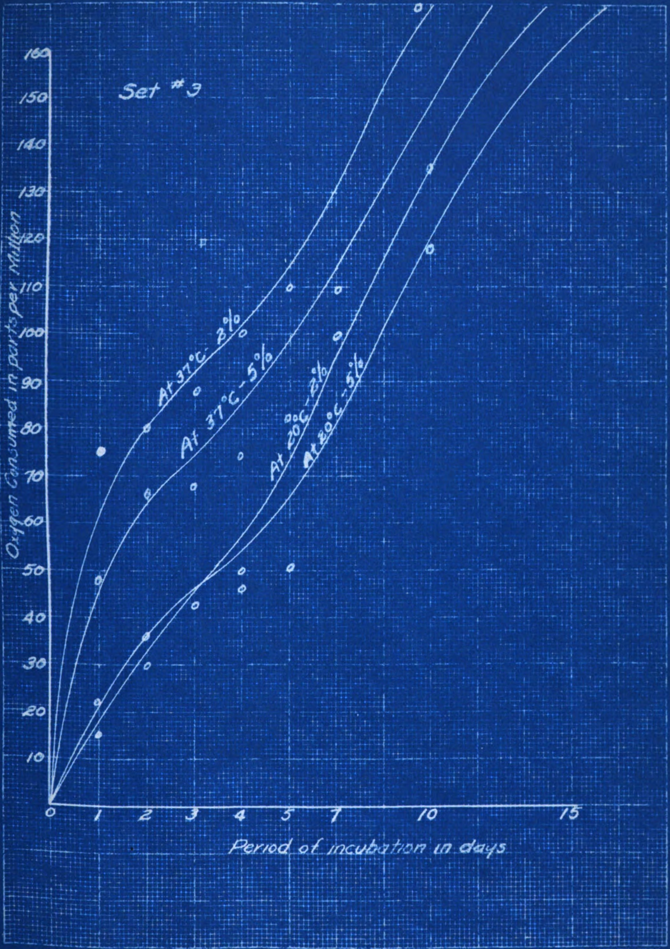
The dilutions were made up as explained in the introduction, the effluent from the East Lansing Imhoff tank being used.

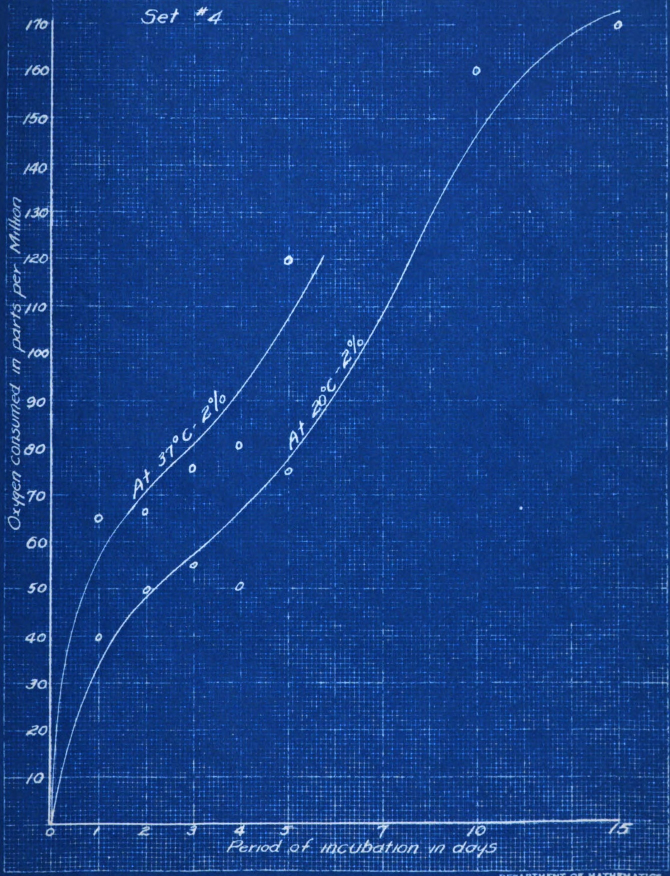
Results

The results of each series compared favorably with one another, the only series not giving a desirable outcome being No. 2. No reason can be given for this unless there was some carelessness in making up the dilutions or the sewage used had a greater oxygen demand.

The values of the observed oxygen demand were plotted against the period of incubation. The curves for each set are illustrated by charts shown below. Each chart has the set number designated. It may be


Set #1
Oxygen Consumed in parts per million
110
100
90
80
70
60
50
40
30
20
10
At 37°C.-5%
At 20°C-5%
0
2
4
6
8
10
12
14
16
Period of incubation in days


Set #3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Oxygen Consumed in parts per Million
At 37°C - 2%
At 37°C - 5%
At 20°C - 2%
At 20°C - 5%
0
1
2
3
4
5
7
10
15
Period of incubation in days


Set #4
Oxygen consumed in parts per Million
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
At 37°C - 2%
At 20°C - 2%
0
1
2
3
4
5
7
10
15
Period of incubation in days

noted that there is a certain amount of parallelism between the results of different dilutions obtained at different temperatures.

In order to obtain average values of all the sets, the results of each one were changed from parts per million of oxygen consumed to a percentage of the oxygen consumed in ten days. Only the 5 per cent dilutions were used, because the results on these were more consistent.

The percentages are shown in the following tables:

SET NO. I

	20 deg. C.	37 deg. C
1st Day	27.2 per cent	44.8 per cent
2nd "	32.0 " "	53.5 " "
3rd "	34.0 " "	55.0 " "
4th "	37.8 " "	58.4 " "
5th "	49.0 " "	65.5 " "
7th "	54.7 " "	86.0 " "
10th "	100 " "	100 " "

SET NO. III

	20 deg. C.	37 deg. C.
1st Day	18.7 per cent	48.9 per cent
2nd "	30.5 " "	67.3 " "
3rd "	36.4 " "	68.4 " "
4th "	39.0 " "	75.5 " "
5th "	42.4 " "	83.5 " "
7th "	86.5 " "	92.0 " "
10th "	100 " "	100 " "

SET NO. IV

	20 deg. C.	37 deg. C.
1st Day	35.7 per cent	60.7 per cent
2nd "	42.9 " "	83.0 " "
3rd "	57.2 " "	69.0 " "
4th "	57.2 " "	76.0 " "
5th "	76.0 " "	88.0 " "
7th "	78.6 " "	95.0 " "
10th "	100 " "	100 " "

The average values of the three sets are:

	20 deg. C.	37 deg. C.
1st Day	27.2 per cent	51.4 per cent
2nd "	35.1 " "	67.9 " "
3rd "	42.5 " "	64.1 " "
4th "	43.0 " "	69.9 " "
5th "	55.8 " "	79.0 " "
7th "	73.2 " "	91.0 " "
10th "	100 " "	100 " "

There is some discrepancy in these results. The second day under 37 deg. C., has a higher percentage of oxygen consumed than the third day. The cause of this is not known as there are so many times during the test where an error may arise due to technique, etc.

From these average values a curve, as shown by Fig. 3, was plotted. If you take the five-day period of incubation and trace up to the 20 deg. C., curve and then over to the 37 deg. C. curve, the time period is decreased from five days to approximately two days. This ratio is fairly constant as can be seen from the curves after a time period of two days has passed. Thus, by incubating the samples at 37 deg. C., the time period is decreased. Just how this may be applied to the standard biochemical oxygen demand determination so as to make a reasonably accurate and consistent procedure, it is hard to say. There are so many other factors which enter in during the test.

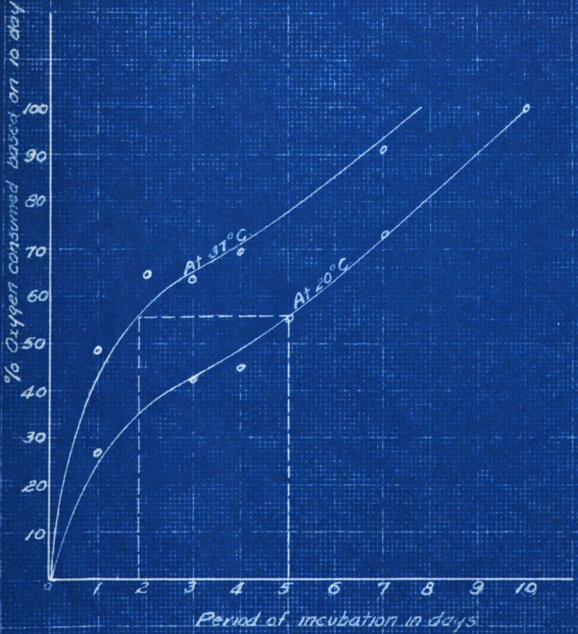

% Oxygen consumed based on 10 day
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
At 37°C.
At 20°C.
Period of incubation in days

Fig. 3

Conclusions

1. The time period is shortened considerably using 37 deg. C. incubation as compared to 20 deg. C. incubation.
2. The determination must be carefully run in order to obtain consistent results.
3. Much depends on the condition of the sample with respect to its rate of deoxygenation.

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