STUDY OF THE VELOCITY OF EXPRESSION OF BIOCHEMICAL OXYGEN DEMAND

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STUDY OF THE VELOCITY OF EXPRESSION OF

BIOCHEMICAL OXYGEN DEMAND

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CHAPTER I

INTRODUCTION

In water polution control and wastewater work, the "strength" of organic wastes is generally measured by the amount of oxygen required to stabilize them biologically. Micro-organisms utilize oxygen during aerobic metabolism of the organic constituents of waste. The amount of oxygen utilized is termed Biochemical Oxygen Demand (BOD) of the waste; and it is determined through a standardized procedure by seeding appropriatly diluted samples and incubating them at 20°C for five days along with systems containing only the seed organisms. The oxygen depletion due to seed alone is then subtracted from that recorded for the systems containing seed-plus-sample, and the BOD of the waste is computed by applying dilution factors (1).

The BOD test has been used for many decades as a basis for design and control of waste water works. It was a widely accepted assumption that the progression rate of the daily BOD in an incubated sample follows first-order Kinetics; in other words, the velocity of the biochemical oxidation of organic matter, has been assumed to be proportional to the remaining concentration of oxidizable material. This concept implies that the reaction follows an exponential function and that its proportionality factor is constant and not dependent on the amount of oxygen available. The mathematical relationship of the BOD process can be expressed as a differential equation:

$$-dL/dt = K L$$

This equation has the solution of:

$$Y = L(1-10^{-Kt})$$

where: Y = BOD at any time

L = Ultimate BOD

K = Rate constant

t = Time

The rate constant, K, was originally considered to have a value of 0.10 which corresponds to a stabilization rate of 21% of the remaining organic matter per day.

The above concept was proposed by early investigators who experimented with domestic sewage, and it had no theoretical basis (2, 3, 4, 5). Gradually, the validity of this idea was questioned as evidence of variations in K value accumulated especially after the waste waters began to vary considerably in their nature due to increased industrial activity. Ruchhoft (6) obtained value of K from 0.04 to 0.29 when he tested 50 sewage samples. In 660 individual analyses of samples of a highly reproducible soluble substrate, Busch (7) found that K varied from 0.109 to 0.539 with 44% variation. Sawyer (8) stated that "it was found that K values for sewage varied considerably from day to day". Many others (9, 10, 11, 12, 13, 14) observed the same inconsistency in K value.

Now it is realized that the 5-day BOD test value as an absolute measure of the final 20-day BOD (La) of the waste is questionable, and that it serves only as a means for "comparing wastes on a relative basis or for evaluating the effectiveness of a treatment plant" (7). The inadequacy of the test and the suggested formula in predicting the ultimate BOD value is illustrated in Figure 1. Although the 5-day BOD is fixed and the rates were considered constant, different values of K lead to significantly different L's.

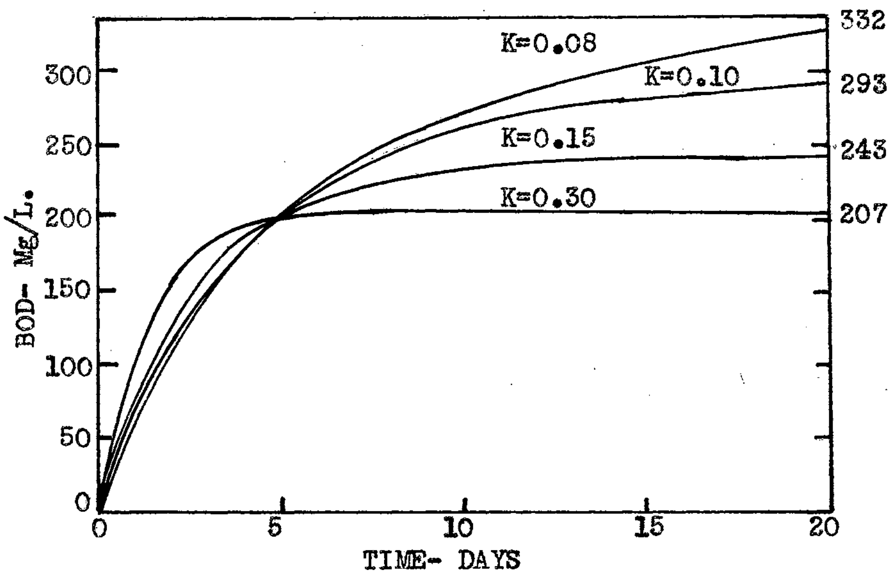

Figure 1. Ultimate BOD Computed From Different K's And One Fixed 5-Day BOD (After Sawyer, Ref. 8)

Also, it has been shown that the stabilization process does not necessarily follow one first-order Kinetic formulation (15, 16, 17, 18, 19, 20). This aspect will be discussed in more detail in a later part of this report.

Most of the technical literature cited recognized the over-simplification implied in the BOD formula as it has been used. Many experiments since the development of the equation revealed a "hump" in the BOD curve. This is commonly taken as a sign of nitrification (6, 10, 16, 21, 22). Recently Busch and Myrick (23) challenged this idea and attributed the "hump" to the activity of predators (protozoa), feeding on the bacteria. Both theories will be discussed more fully in the "Discussion" Section.

At present, there is much controversy over the exact shape, the significance, and the causation of the phasic nature of the BOD curve. The study reported herein is undertaken in an attempt to give an insight into three points regarding the problem:

1. Nature of BOD reaction Kinetics
2. Occurrence of a "hump" in the BOD curve
3. Population dynamics during expression of BOD

CHAPTER II

MATERIALS AND METHODS

The objectives of this work were to define the shape and kinetics of the Bod curve, and to examine bacterial growth during exertion of the biochemical oxygen demend under conditions of the Standard BOD Test. Accordingly, extended experiments were undertaken in which samples for measurement of viable cell counts were taken each time dissolved oxygen was measured for the determination of BOD.

In this study six separate experiments were conducted in which the above mentioned parameterswere examined. The experiments are identified in TABLE I. Throughout this text they will be referred to by their appropriate numerical designation. Each experiment is described more fully in a later portion of this section.

TABLE I

DESIGNATION OF BOD EXPERIMENTS

Exp. No.	Starting Date	Substrate	Initial Population
I	Nov. 28, 61	Glucose	2.6×10^4
II	Dec. 15, 61	Glucose	5.9×10^5
III	Mar. 8, 62	Glucose	7.9×10^4
IV	April 5, 62	Nutrient Broth	--
V	April 17, 62	Glucose	8.5×10^4
VI	April 25, 62	Nutrient Broth	23.8×10^4

Materials

Substrate: DIFCO Anhydrous d-Glucose was used in experiments I, II, III, and V. The concentrations used were 8 mg/l for experiments I and III; 5 mg/l for experiment II, and 9 mg/l for experiment V. The substrate was varied in nature and concentration in order to study the effect of this parameter on kinetics of the oxygen uptake process. In experiment IV and VI, nutrient broth was used. The BOD of the nutrient broth was assumed to be 40,000 mg/l when experiment IV was run. This proved to be a very high estimate when experiment IV failed due to the very slow oxygen utilization (1.12 mg/ml in 210.5 hrs.). When a separate 4-day BOD test was made, the approximate ultimate BOD of the nutrient broth was found to be 8500 mg/l.

The result of this test is shown in TABLE XX in the Appendix.

Seed: The seed used in the first two experiments was obtained from the effluent of the primary settling tank in the sewage treatment plant of Stillwater, Oklahoma. In both cases the liquid was allowed to settle for approximately one-half hour before using the supernatant as a seeding suspension. In all the other experiments except IV, the seed was obtained from the mixed liquor in a laboratory activated sludge unit operating on a synthetic waste in which glucose was the sole source of carbon.

The activated sludge in this unit was developed from an initial seed taken from the above mentioned treatment plant. Therefore, the population used in all of the experiments except IV, was of a heterogeneous nature. Although in sanitary engineering research pure culture studies are often of value, the advisability of using heterogeneous populations has been emphasized in the literature (24). Experiment IV was designed to study changes in bacterial predominance during the BOD process. Three types of organisms were used in this experiment in approximately equal concentrations. They were: Micrococcus, Lysodeikticus, Serratia Marcescens, and Pseudomonas Fluorescens.

Nutrient Medium

The medium used for plating organisms was BACTO nutrient agar, which was rehydrated by suspending 23 gm. in 1000 ml. of cold freshly distilled water and heating it to boiling. After boiling, the liquid was sterilized in a steam autoclave for 15 minutes at 15 lbs. pressure. Then it was allowed to cool to about 45°C after which it was poured in sterilized Petri dishes and allowed to solidify. The dishes were stored in an incubator at 37°C ± 1°C for a period which varied up to two weeks.[1]

Dilution Water

Deionized water was used. It was prepared by passing Stillwater tap water through a mixed bed deionizer (Barnstead Cartridge, type 0802). Stock nutrient solutions (Phosphate buffer, Magnesium Sulfate, Calcium Chloride, and Ferric Chloride) were prepared according to instructions in Standard Methods (1) p. 319, and 1 ml. of each was added for each liter of dilution water.

Apparatus

Incubating bottles of 300 ml. capacity with ground-glass stoppers were used. Incubators for BOD bottles and agar plates were air incubators thermostatically controlled.

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[1]This time length was justified by the author in a separate study of the spot plate method for counting bacterial cells (25).

Dissolved Oxygen Reagents

Reagents used for dissolved oxygen determination were prepared in accordance to Standard Methods (1), p. 309.

EXPERIMENTAL PROCEDURE

Preparation of Seed and Sample Dilutions

The necessary quantities of distilled water for the seed systems and the sample-plus-seed systems was kept in separate 20 liter glass bottles in a 20°C constant temperature room for approximately 24 hours. Just before the experiment was started, air was bubbled at the same rate into both stock bottles for a period of 15 minutes. Then the four nutrient solutions and the appropriate volume of seed suspension were added to each stock bottle. At this point in the experiment, both 20 liter jugs contained identical materials. The amount of seed used was decided upon by visual judgment in these experiments. In later experiments, in order to have better control over the number of organisms in the seed, trial counts for viable cells was made. The number of organisms per ml. of water was calculated from these trial runs. The results of the trials and the calculations made are shown in TABLE XXI in the Appendix. In all cases equal amounts of seed suspensions per liter of dilution water were used for the sample as well as for the seed bottle.

After introducing the solutions and the seed, stock bottles were shaken vigorously to mix the liquid well and to release any supersaturation of dissolved oxygen. The incubation bottles were then filled from the seed dilution stock bottle. Utmost care was exercised so that all bottles were filled in the same manner and with a minimum of liquid agitation. The first and the last incubation bottles to be filled were kept for the initial DO determination; and the rest were sealed with water and stored in the air incubator at 20°C.

The desired amount of substrate was then introduced into the sample stock bottle and the bottle was vigorously shaken. The sample-plus-seed incubation bottles were filled and stored in the same manner as described above. Initial dissolved oxygen was determined on this system in the same manner as for the seed.

Dissolved Oxygen (DO) Determination - Alsterberg (Azide) Modification of Winkler Method

The procedure followed in determining the DO of the seed and sample-plus-seed dilutions was as described in Standard Methods (1) p. 311.

For experiments I and II, duplicate bottles for the seed and the sample-plus-seed were used at each sampling period. During the rest of the experiments this was done only for the initial samples; for subsequent samples, two bottles of the sample-plus-seed and one of the seed were tested. However, whenever the DO values obtained were doubted additional bottles were used.

The average of the DO values of each set of bottles was corrected for the actual normality of the Sodium Thiosulfate as determined by standardizing it with biniodate according to the standard procedure. This corrected value of the average which is recorded in TABLES IX to XIV in the Appendix.

The Sodium Thiosulfate which was used for titration was standardized frequently. A smooth curve was plotted to connect the actual points obtained. This is shown in Figure 10 . The correction factor for the values of any run made between each two standardizations was computed from these curves.

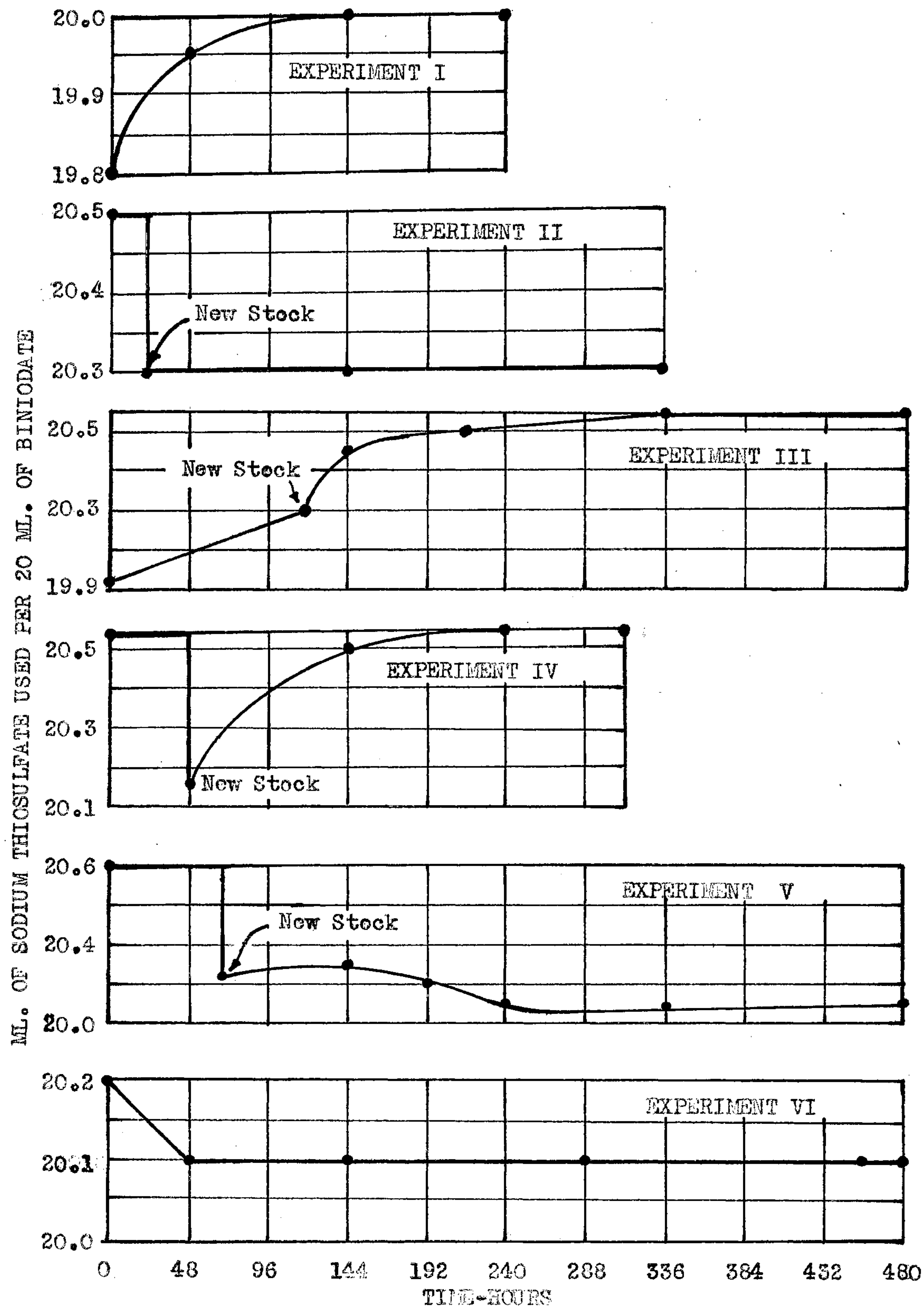

FIGURE 2- SODIUM THIOSULFATE STANDARIZATION CURVES

It was observed in experiments IV, V, and VI that the thiosulfate was becoming "stronger" instead of weakening as its storage time increased. This was suspected to be due to storing the solution in a plastic container instead of a glass bottle. To confirm this and to establish the reason for this unusual variation, a new stock was made and standardized twice. Then 60 ml. were stored in a clean glass bottle, and another 60 ml. were stored in a plastic container. After two weeks, the solutions in each bottle were standardized again in duplicate. The results as tabulated below showed a definite effect of the plastic container on the strength of the thiosulphate.

Ave. initial volume of thiosulphate used per 20 ml. of Biniodate = 20.1 ml.
Ave. volume of the thiofulphate when stored in glass for 14 days = 20.18 ml.
Average volume of the thiosulphate when stored in plastic bottle for 14 days = 19.88 ml.

Both containers were thoroughly and carefully cleaned. No explanation for this result can be offered other than to suggest the possibility of interferring substances contained in the plastic. In any event, the differences were not of serious magnitude and since the thiosulfate was frequently standardized, the slight fluctuations observed did not seriously effect the dissolved oxygen determinations.

The time for each experiment varied from 10 days to 20 days. Also, the intervals between two consecutive sampling periods was shortened from 24 hrs. in early experiments to 6 hrs. in the early phase for later experiments. This was done because it was found necessary to have more frequent samples early in the experiments in order to define the oxygen utilization curves more accurately. Throughout all of the experiments the BOD bottles were shaken daily to assure mixing and were sealed with water to prevent reaeration.

Bacterial Counts Determination

Before determining the DO of the incubation bottles used in each run, samples were taken out for plating. If two bottles were used in the same run, 1/2 ml. was drawn out of each bottle and introduced into the cell count dilution bottle. If only one incubation bottle was used, 1 ml. was taken out of it. When the sample was drawn out, the bottle stopper was removed very carefully to avoid any possibility of introducing air into the bottle.

Before plating each dilution, the dilution bottle was shaken vigorously about 25 times. Then the suspension was plated using the spot plate method. This method consists of adding, with a calibrated pipette, eight drops (4 per 1/2 plate) of 0.02 ml. volume each, from the properly diluted suspension of the bacterial cells, to the surface of the prepared nutrient agar. The validity of this method was established by the author in a separate study conducted especially for that purpose (25). The spots on the agar surface during the first three experiments were arranged as shown in Figure 3A, but later it was found more convenient for plating and counting ease to use the arrangment shown in Figure 3B.

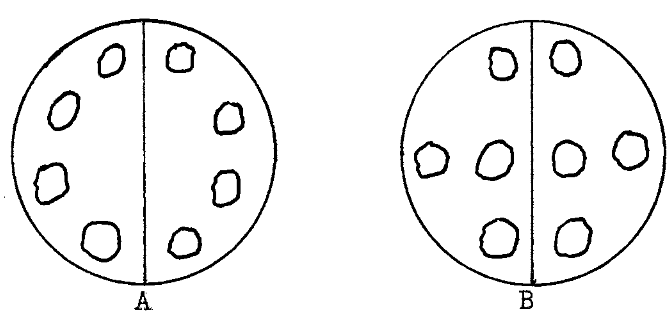

Figure 3- Arrangments of Spots on Agar Surface in Spot Plate Method

After the incubation period, which was as short as 24 hrs. in some experiments and as long as 48 hrs. in others, colonies formed by the viable cells were counted with the aid of a Darkfield Quebec Colony Counter. The necessary period of incubation was determined by observing the growth of the sample from the first run, till the colonies were large enough to count. Throughout the rest of the runs, this period was maintained.

From the earlier spot plate method study (25), it was concluded that the higher the number of colonies per 1/2 plate, the smaller is the coefficient of variation. Accordingly, if more than one dilution was made in each run, the dilution which yielded more colonies per spot was used for calculating the cell count. Figure 4 is taken from the report of the above mentioned study. It can be seen from this Figure that if 20% is the highest acceptable coefficient of variation, any number of colonies less than 17 per 1/2 plate should not be considered reliable. This was the criteria herein employed for obtaining the cell count.

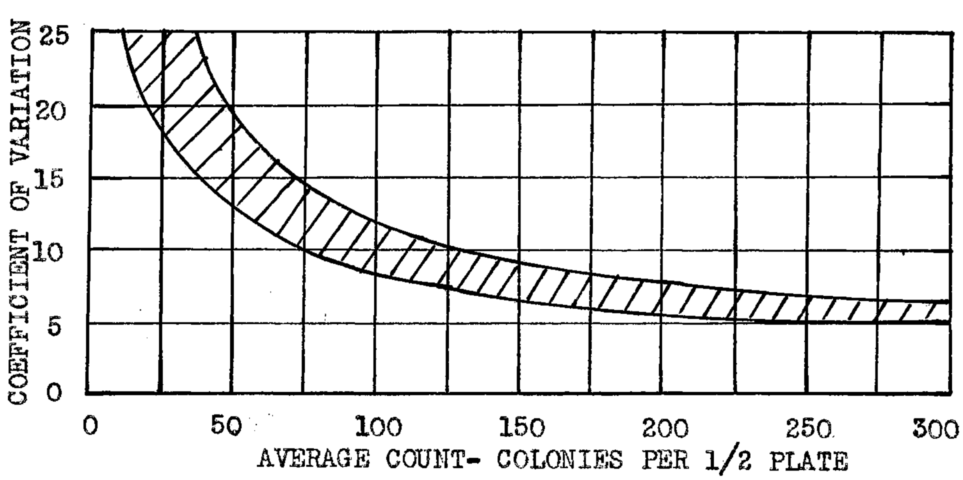

FIGURE 4 - AVERAGE NUMBER OF COLONIES PER 1/2 PLATE AND THE CORRESPONDING COEFFICIENT OF VARIATIONS IN SPOT PLATE METHOD
(Ref. 25)

CHAPTER III

EXPERIMENTAL RESULTS

The data for oxygen utilization in each experiment are presented in TABLES IX to XIV in the Appendix. The results for all experiments are shown graphically in Figures 5 to 10. It is noted that for all glucose experiments, except experiment III, all BOD curves have almost the same shape. There is a definite "hump" in all of them. The nutrient broth experiment curve behaved in the same manner as that of experiment III in being free of the "hump". In experiment IV, oxygen utilization was very slow, and the experiment was discontinued and disregarded after 210 hrs.

When the same data was plotted on semi-logarithmic graph paper, with time as the abscissa and BOD remaining[1] as the ordinate, it was possible in each case, except in experiments III and VI, to show three distinct reaction phases; which divided the BOD process into three first-order stages. Properties of each stage are tabulated in TABLE II; and the results are shown in Figures 11 to 15. Also, in the upper portion of these figures, the bacterial counts for both the seed and the sample during the BOD exertion are shown.

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[1]BOD remaining (L_t) equals La - Y_t where La is the 20-day BOD as observed or extrapolated (Fig. 5 and 6) and Y_t is the BOD exerted at time t.

Colony counts and the computed number of organisms per ml., which are plotted in Figures 11 to 15, are also given in tabular form in the Appendix (TABLES XV to XIX).

TABLE II

PROPERTIES OF BOD PROCESS STAGES

	Stage I				Stage II				Stage III			
Exp.	Oxygen Uptake		Length	K*	Oxygen Uptake		Length	K*	Oxygen Uptake		Length	K*
	mg/l	% of La	Days		mg/l	% of La	Days		mg/l	% of La	Days	
I	2.59	43.2	1	0.245	0.75	12.5	2	0.054	2.66	44.3	17	0.121
II	2.09	51.0	1	0.310	0.16	3.9	2	0.018	1.85	45.2	17	0.096
III	2.9	52.7	1.25	0.26	-	-	-	-	2.6	47.3	17.75	0.032
V.	3.3	48.5	1.5	0.193	0.5	7.4	1.67	0.040	3.0	44.2	14.5	0.159
VI	1.0	19.6	1.0	0.095	-	-	-	-	4.1	80.4	19.0	0.178

*K is the first-order kinetic constant

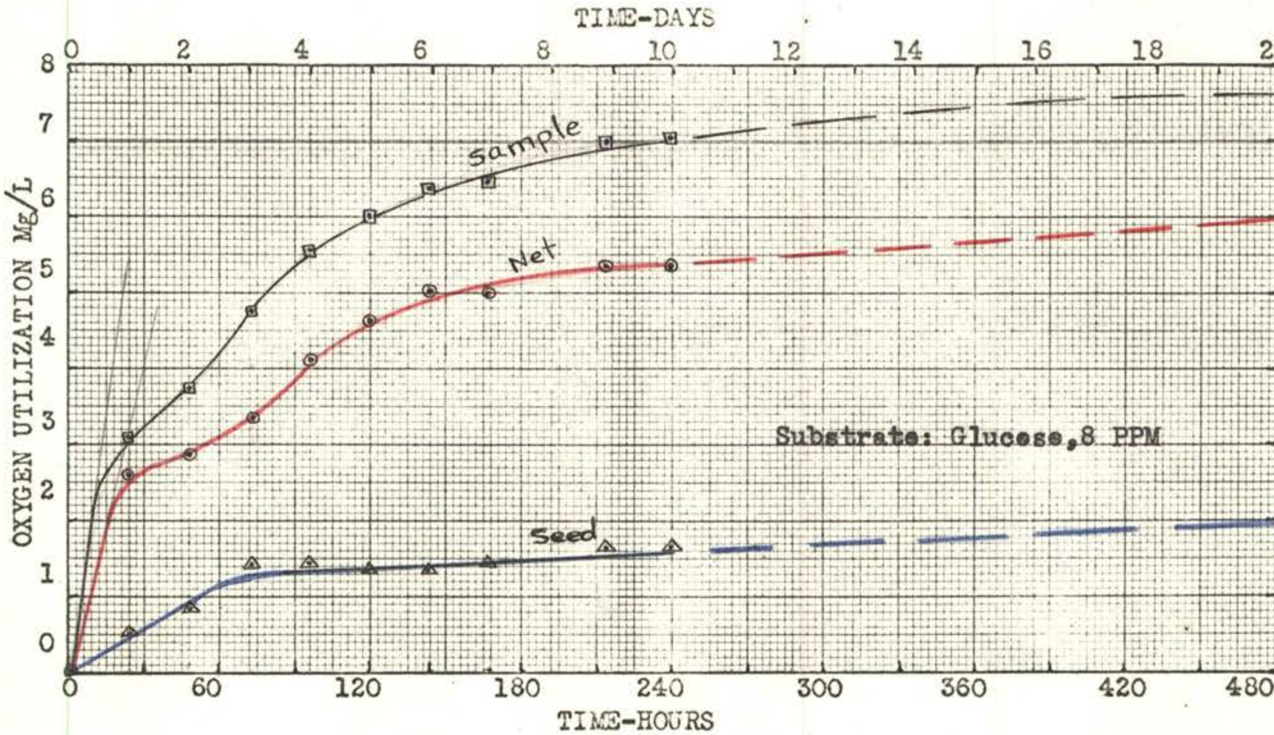

FIGURE 5 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT I

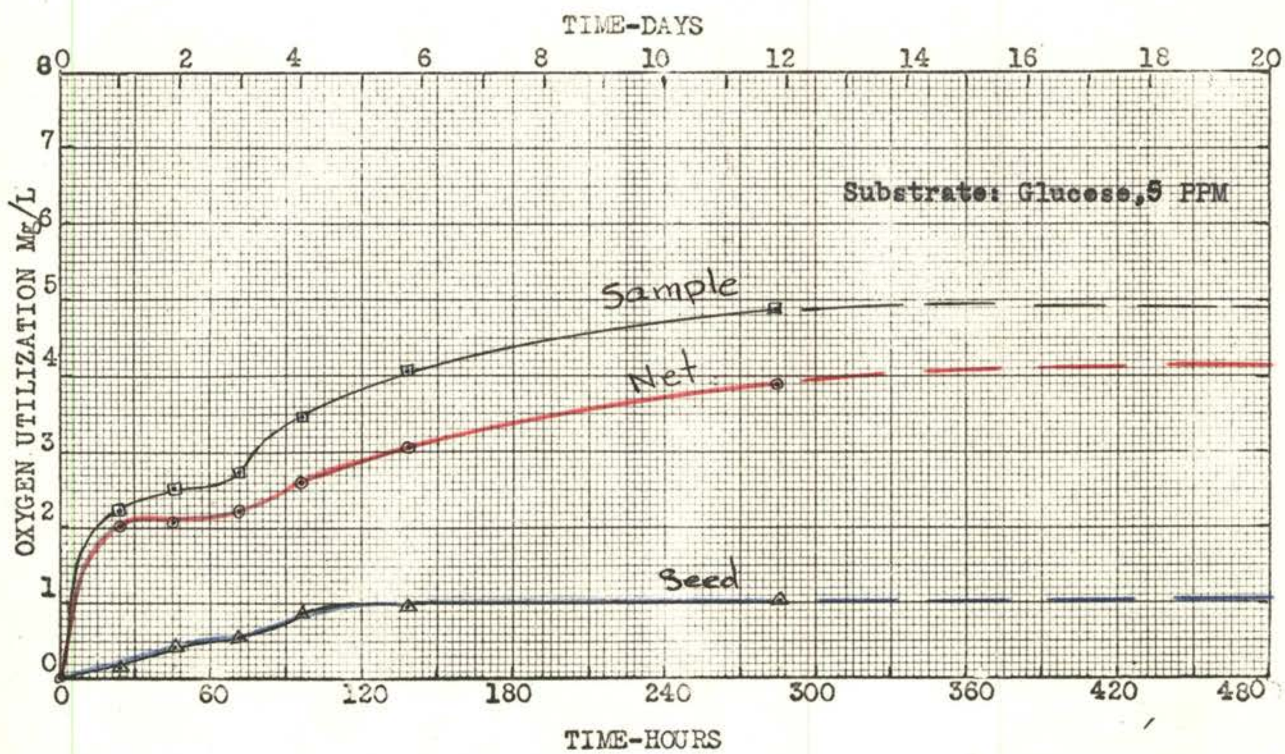

FIGURE 6 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT II

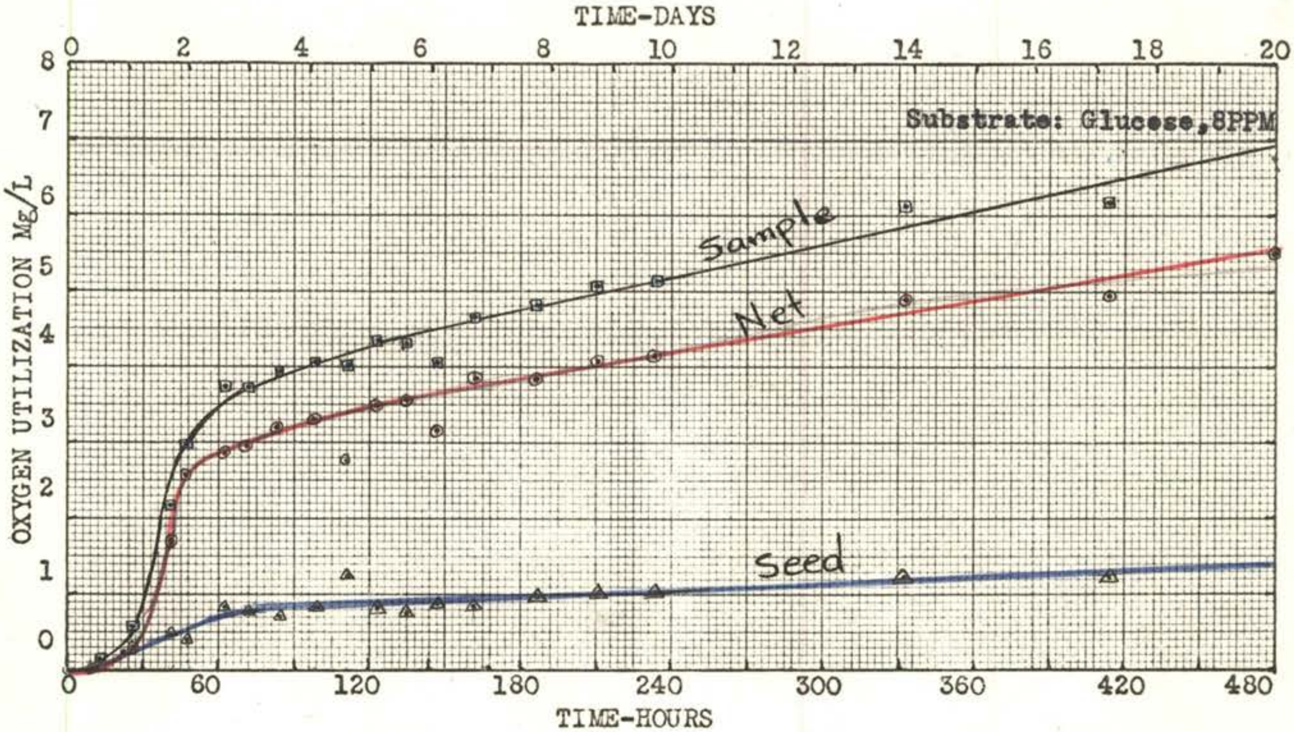

FIGURE 7 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT III

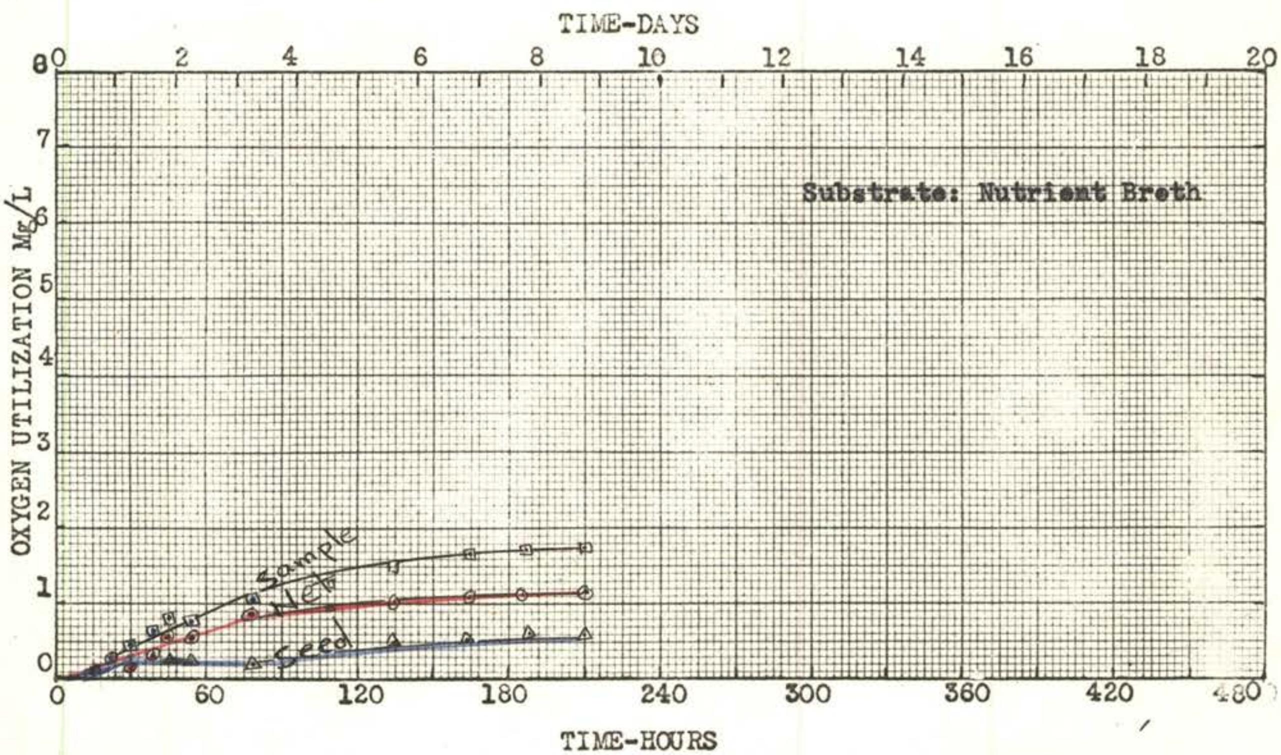

FIGURE 8 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT IV

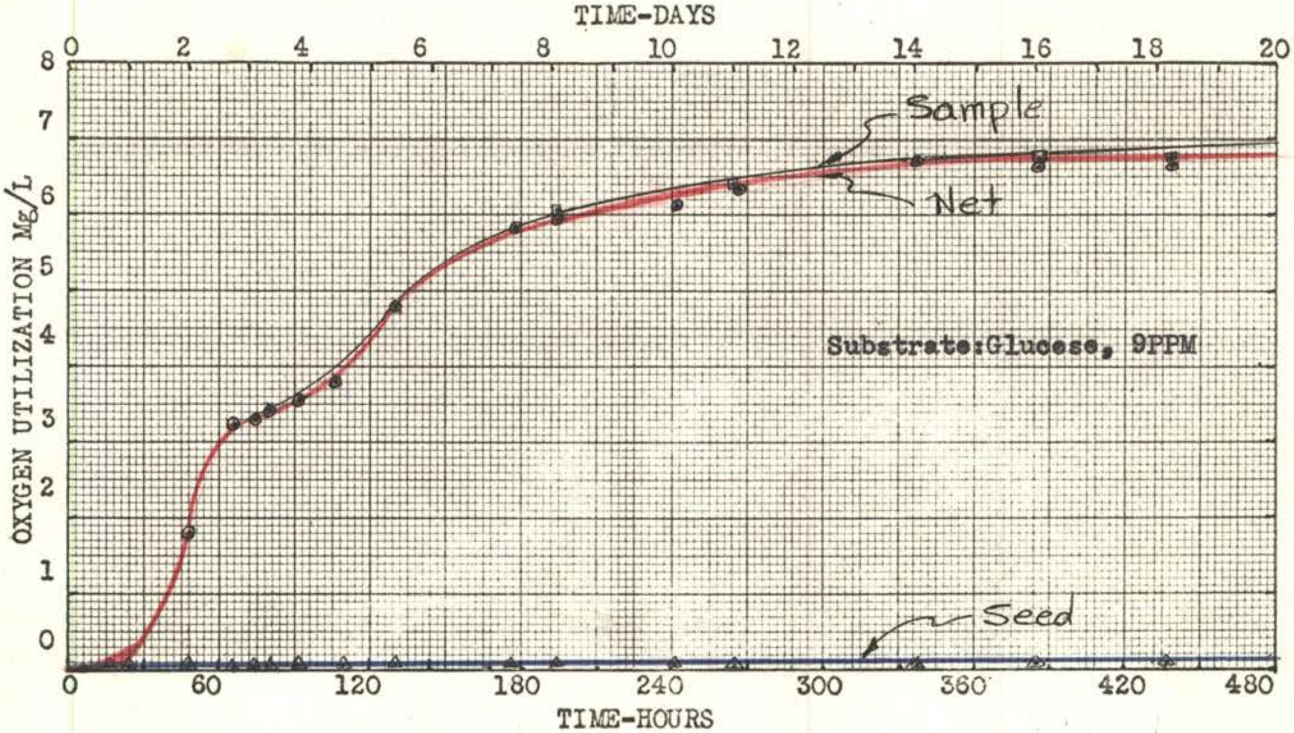

FIGURE 9 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT V

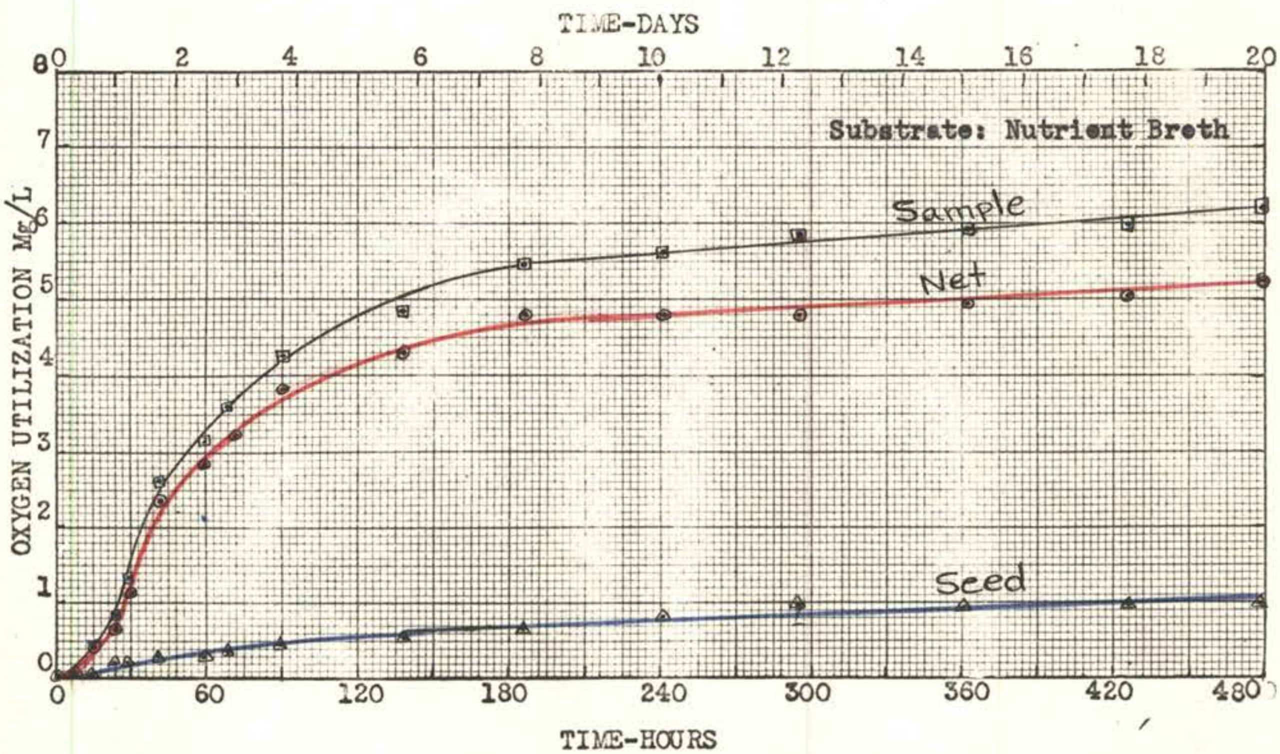

FIGURE 10 - OXYGEN UTILIZATION CURVES FOR EXPERIMENT VI

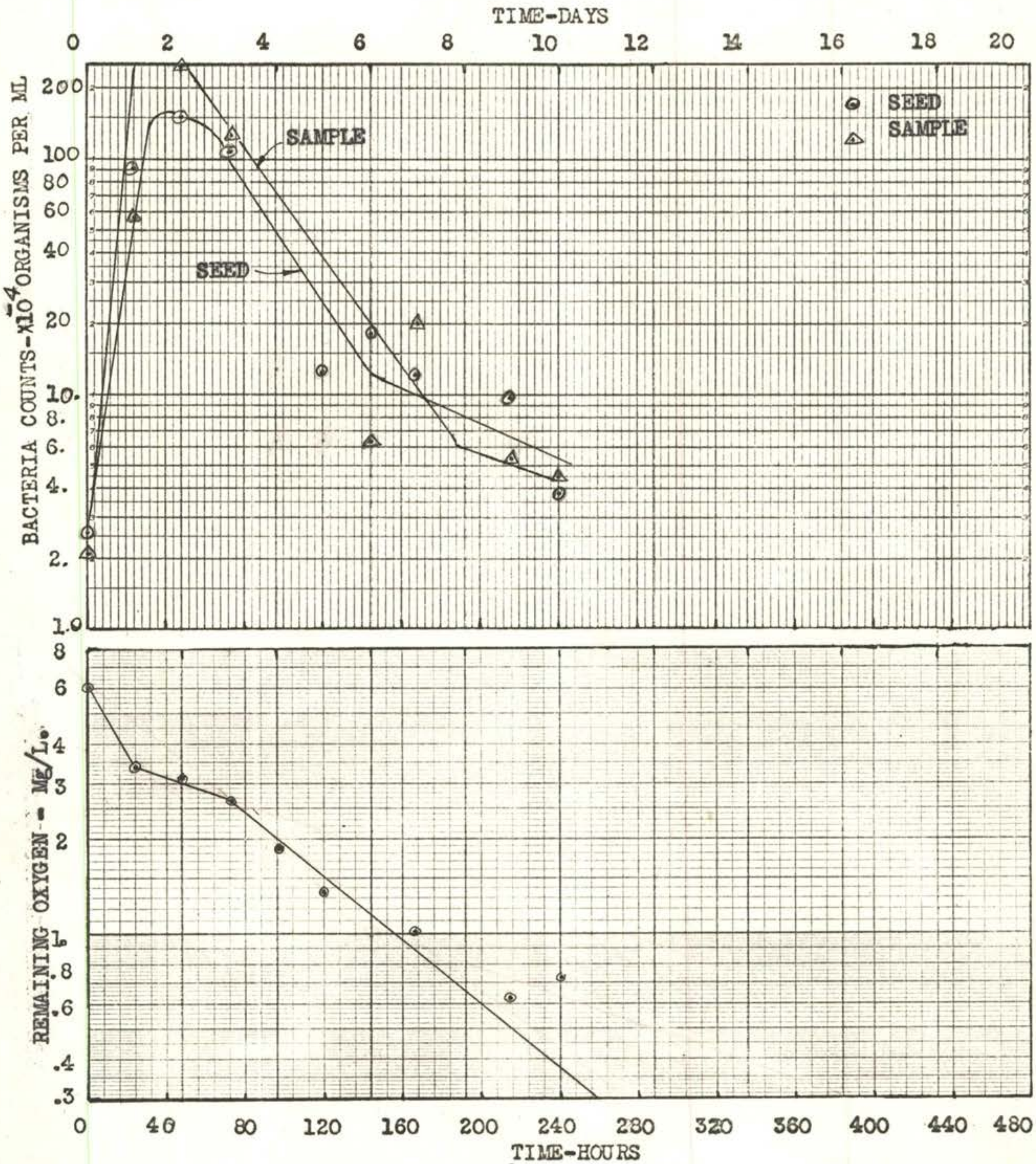

FIGURE 11- BACTERIA COUNTS AND NET OXYGEN UTILIZATION FOR EXPERIMENT I

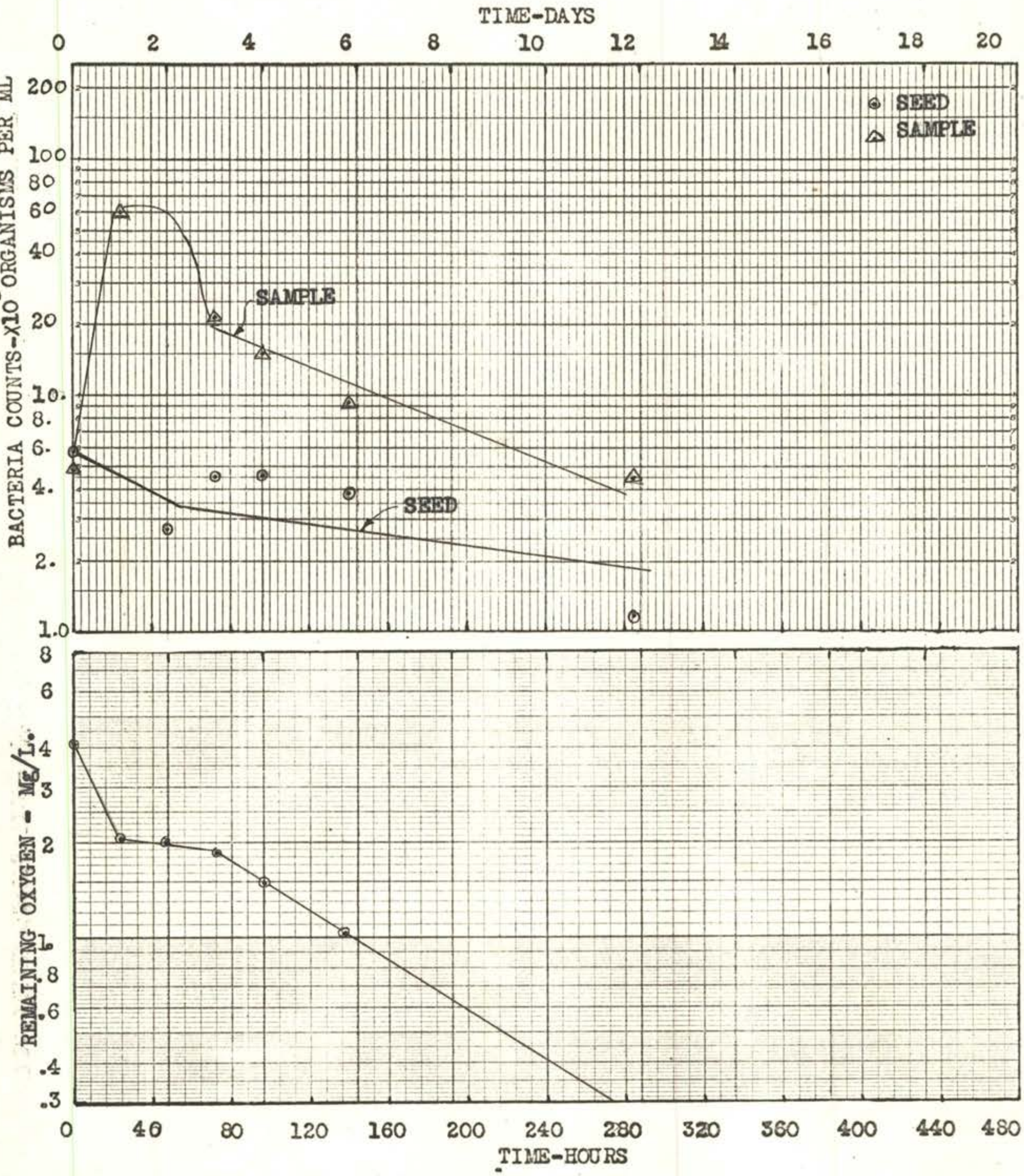

FIGURE 12 - BACTERIA COUNTS AND NET OXYGEN UTILIZATION FOR EXPERIMENT II

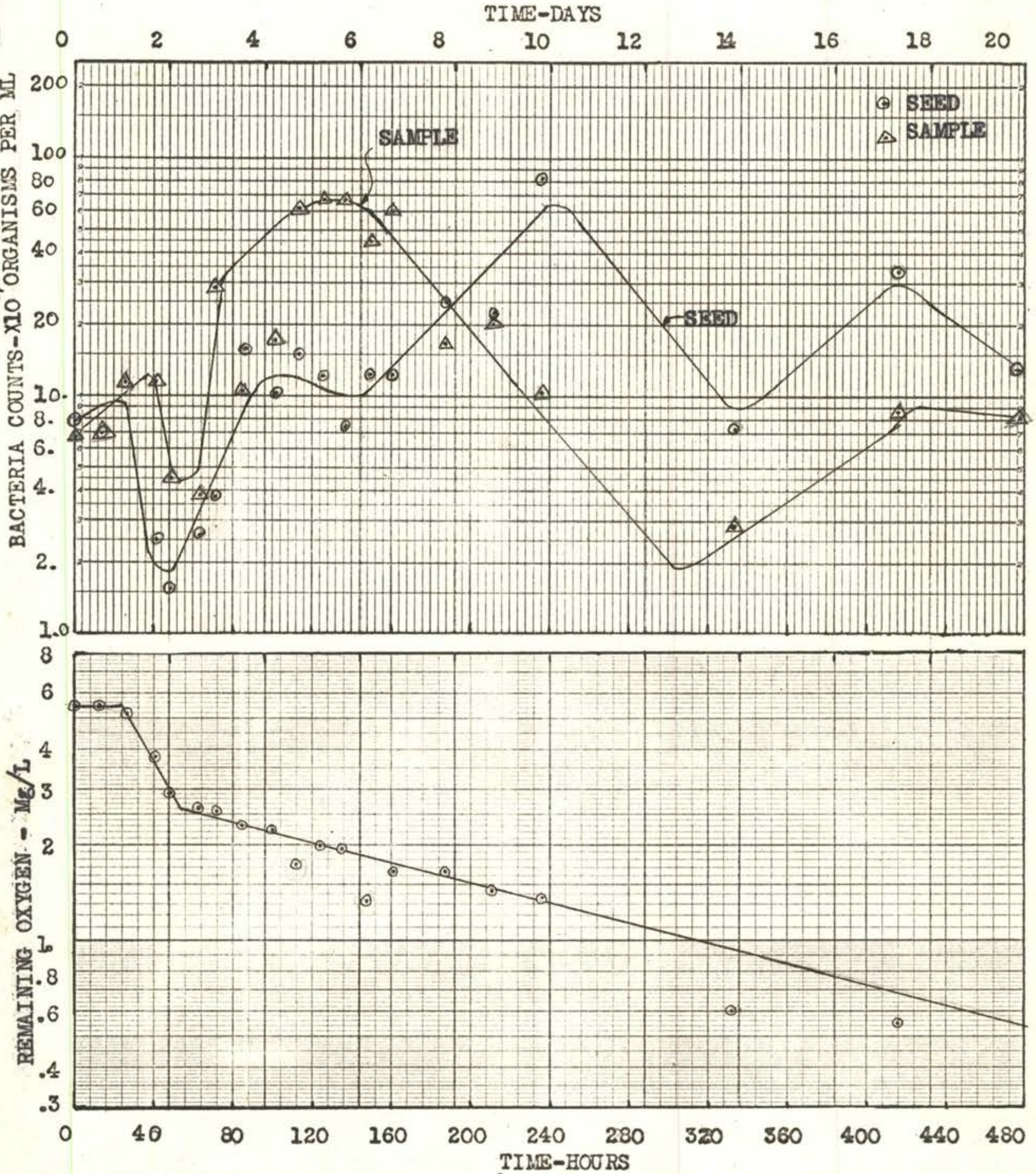

FIGURE 13- BACTERIA COUNTS AND NET OXYGEN UTILIZATION FOR EXPERIMENT III

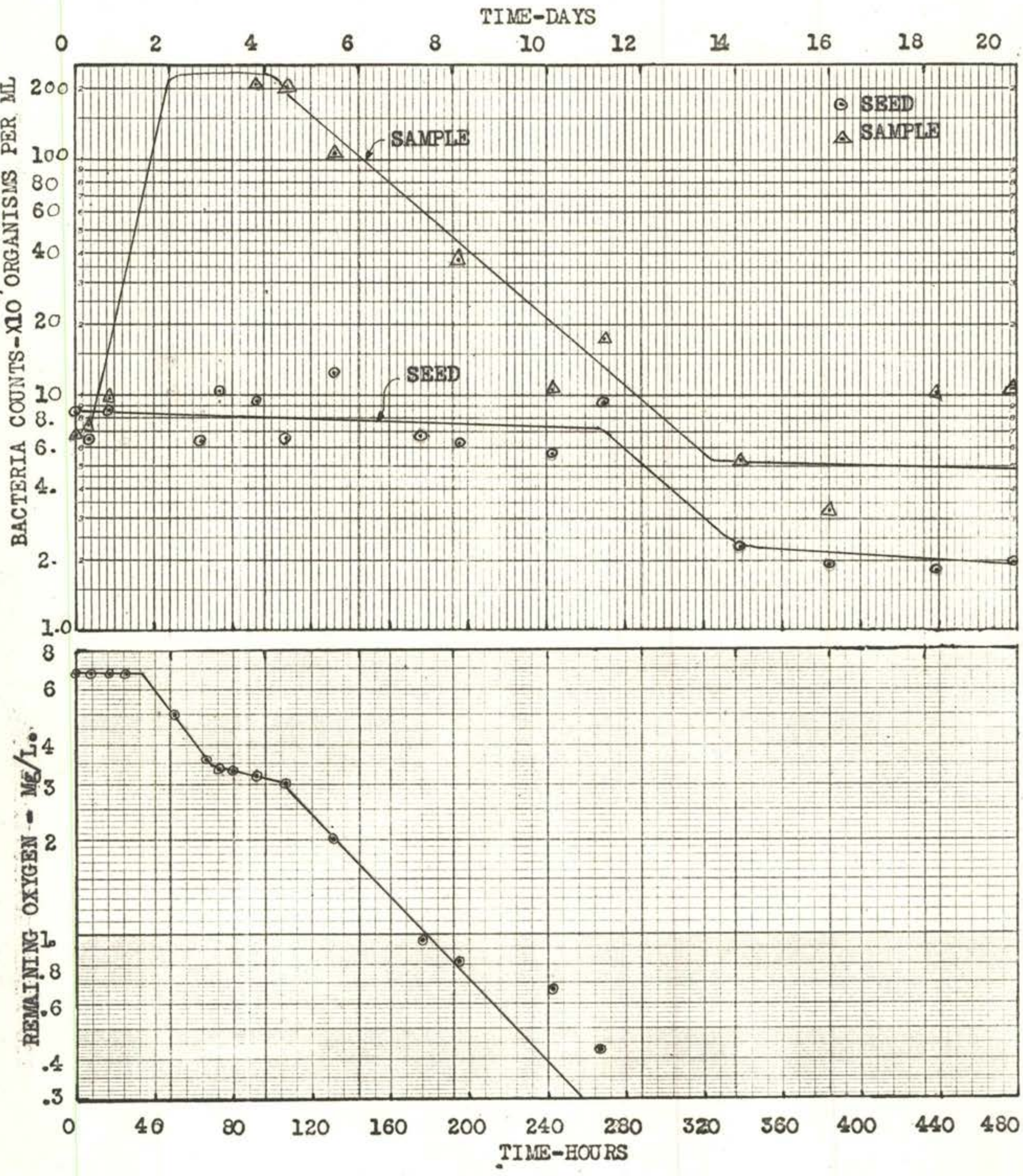

FIGURE 14- BACTERIA COUNTS AND NET OXYGEN UTILIZATION FOR EXPERIMENT V

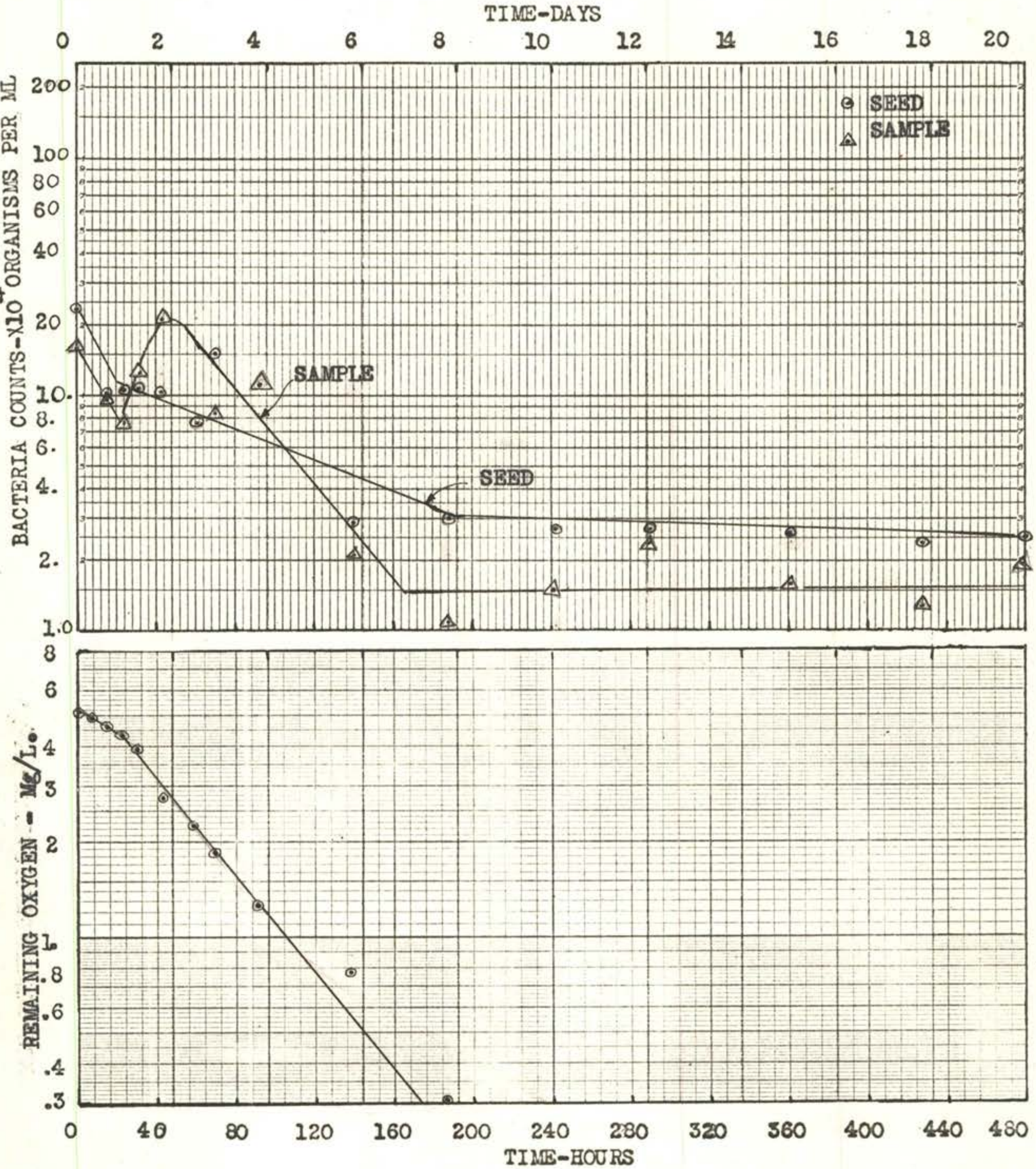

FIGURE 15- BACTERIA COUNTS AND NET OXYGEN UTILIZATION FOR EXPERIMENT VI

CHAPTER IV

DISCUSSION

There are two major points of discussion concerning the results of the experiments: (1) The diphasic nature of the curve, i.e. the existence of the "hump" in the curve. (2) The kinetics of BOD progression. Both of these points may be correlated with population dynamics.

Diphasic Nature of the Curve

From Fig.5,6,9, it can be seen that the BOD process does not follow one set of first-order kinetics throughout its progress. There is a "hump" in every curve for the glucose dilution experiments except in that of experiment III where there was no "hump" observed.

This "hump" had always been attributed to nitrification. Mohlman (15) reported that the increase in the measured BOD is roughly proportional to the concentration of amonia nitrogen and to the activity of the nitrifying bacteria. Sawyer and Bradney (26) also favored this theory of nitrification and stated that "after the carbonaceous oxidation is nearly completed, conditions allow the development of a nitrifying flora which oxidizes the amonia to nitrites and the nitrites to nitrate". Other investigators (6, 9, 11, 15) had the same point of view on this matter. Orford (17) hypothesized that since nitrification is a two phase process,[1] and the BOD curve when plotted on semi-log graphs has two distinct

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[1] $$2NH_3 + 3O_2 \xrightarrow[\text{Bacteria}]{\text{nitrite forming}} 2NO_2^- + 2H^+ + 2H_2O$$

$$2NO_2^- + O_2 + 2H^+ \xrightarrow[\text{Bacteria}]{\text{nitrate forming}} 2NO_3^- + 2H^+$$

straight line portions, then the "hump" in the BOD curve is caused by nitrification. An examination of the figures (Fig. 5, 6, & 7) he presented in his article showed that his curves do not have two but three straight line portions just as do those herein presented. His explanation for the possible existence of a third phase was that "it might only represent the respiration requirements of the remaining bacteria".

The "hump" in the curves of this study started after 70 to 90 hours of incubation. It is known that nitrifying organisms develop very slowly, and that it "usually takes at least 8 days for nitrification to become significant" (17). Buswell, et. al. (16) found that while X cells of hetrotropic Escherichia Coli becomes 98 X cells in 6 hrs. in milk at 37°C, X nitrifying cells increased only to 2 X cells in 31 hrs. in the BOD bottles. These findings indicate clearly that if nitrification is responsible for the "hump" in the BOD curve, it would have developed at a much later time than observed in this study. Since this did not occur, it is doubtful that nitrification caused the "hump". Also, in the experiments herein reported, the "hump" in the oxygen curve occurs before 50% of the theoretical total BOD was exerted; if nitrification does not begin until most of the carbonaccous matter is removed, it is difficult to explain the "hump" on the basis of nitrification.

Busch (7, 23) proposed that the variation in BOD value is ascribed to the "effect of varying ratios of bacteria to higher organisms in the seed population". To validate his proposition, he tested different samples with supposingly different bacteria to predator ratios. He concluded that "all samples agreed through 60 hrs. and, following the initiation of predator activity, the sample containing the seed which has not been altered physically yielded the highest 5-day BOD value" (7). From examining the curves (Fig. 6) presented in that paper, it can be seen that the sample which contained the unaltered seed had a higher oxygen utilization values from the start. Also, the effect of the different procedures followed in his seed preparation (centrifuging, filtration, homogenization, and ultra-sonoration) on the bacterial population was not ivestigated in order to reject that possibility. Besides, the nitrifying bacteria effect was not isolated or eliminated in those tests. If the second stage in the BOD curve is caused by the higher organisms activity while feeding on the bacteria, how can the third stage, which was obtained in this study as well as others, be explained? From this analysis it can be concluded that this propounded theory has as yet not sufficient proof to make it valid.

By observing the BOD curves and bacterial count curves in the Results section, it can be seen that the patterns of growth and die off of organisms are different; but, the "hump" always corresponded to the peak plateau in the bacterial population curve. The start of the third stage corresponds to the start of the organism die off process. Also, it is important to notice the similarity between the seed and the sample population progress. All these observations suggest that the changes in the BOD kinetics can be correlated to the organism growth and die off pattern; and since the pattern of population progression in both the seed and the sample is similar except for a higher rate of growth and faster decline; it can be suggested that the availability of substrate serves only to increase the magnitude of each population phase. This point deserves further study and consideration.

Kinetics

As it can be seen from the oxygen utilization curves (Figures 11, 12, and 14), oxidation progressed in three stages each of which could be plotted in accordance with first-order kientics. Orford and Ingram (17) presented curves of the same general shape in their critical review of the BOD monomolecular formula. At the end of each stage, the percentages of net oxygen utilized to the ultimate BOD, La (20-day BOD as observed or extrapolated from the curves) were approximately the same for all glucose dilutions tested. The average values were 48.8% at the end of the first stage and 55.5% at the end of the second stage. The values for each experiment are listed in TABLE III. Net oxygen utilization in the first stage in glucose dilution experiments expressed as percentage of theoretical ultimate oxygen demand[2] is shown in TABLE IV The average value of 34.7% arrived at in these experiments differs from that of 41.0% which was computed by Busch (7). He attempted to check this value by assuming the following reaction for a combination of respiration and growth of organisms, $8C_6H_{12}O_6 + 18O_2 + 4NH_3 \rightarrow 10CH_2O + 4C_5H_7NO_2$[3] $+ 18CO_2 + 30H_2O$.

The difference in results of Busch and those herein reported may be taken as indication that the equation assumed by Busch is not necessarily applicable to different systems even when the same substrate is used.

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[2]Theoretical ultimate oxygen demand is computed from the following equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

The molecular weight of $C_6H_{12}O_6 = 180$

The molecular weight of $6O_2 = 192$
Therefore theoretical ultimate demand is (192/180) x p.p.m. glucose.

[3]$C_5H_7NO_2$ is an empirical formula for bacterial cells.

TABLE III

OXYGEN UTILIZED AT THE END OF EACH STAGE EXPRESSED AS PERCENTAGE OF OBSERVED OR EXTRAPOLATED La

Exp.	At End of Stage I	At End of Stage II
I	43.2	55.7
II	51.0	54.9
III	52.7	52.7
V	48.5	55.8
VI	19.6	81.0
Ave. (except VI) =	48.8	55.5

TABLE IV

COMPARISON OF THEORETICAL ULTIMATE OXYGEN DEMAND AND FIRST STAGE OXYGEN UTILIZATION FOR GLUCOSE EXPERIMENTS

Exp.	Theoretical Ultimate Oxygen Demand	Stage I mg/l	Oxygen Demand,% of Theor. Ultimate
I	8.53	2.59	30.4
II	5.33	2.09	39.3
III	8.53	2.90	34.6
V	9.60	3.3	34.4
		Ave. =	34.7

La as obtained from the graphs of the glucose experiments and its percentage of ultimate theoretical oxygen demand for each experiment is presented in TABLE V. As the average of those percentages indicate, only 72.1% of the ultimate theoretical oxygen demand is exerted in a 20-day BOD test.

TABLE V

PERCENTAGE OF THEORETICAL ULTIMATE OXYGEN DEMAND
USED IN 20-DAY BOD

Exp.	Glucose p.p.m.	Ultimate Theor. BOD	Observed Ultimate BOD	% of Theor. Utilized in 20 Days
I	8	8.53	6.0	70.2%
II	5	5.53	4.1	80.7%
III	8	8.53	5.5	64.5%
V	9	9.60	6.8	77.0%
				Ave.= 72.1%

La was computed from the monomolecular equation using the 5-days BOD value for Y and 0.10 as it is usually assumed for K. The results were found to be different from the observed La: This is another illustration of the inaccuracy of the use of one set of kinetics and the usually assumed K value of 0.10. The results of these computations are shown below in TABLE VI.

If a value of K of 0.17, as favored by Sawyer (8), was used in computing La for experiment VI, because of the closer resemblance of nutrient broth to domestic sewage, the La value would be 4.9 mg/l which is 3.9% different from the measured value. These differences although small appear to militate against any generalization concerning K values.

TABLE VI

La AS COMPUTED FROM THE MONOMOLECULAR EQUATION
(K = 0.10) VS. OBSERVED La

Exp.	5-Day BOD	La Computed	La Measured	Percent Difference
I	4.6	6.23	6.0	3.8
II	2.9	4.24	6.1	3.4
III	3.5	5.12	5.5	6.9
V	4.4	6.43	6.8	5.5
VI	4.2	6.15	5.1	20.6

Determination of K and La From Observed BOD Values

A number of methods for finding the magnitude of La and K from a series of observations of Y and t have been proposed: The "Rapid Ratio Method", as proposed by Sheely (27), "The Moment Method" which was developed by Moore, Thomas, and Snow (28), and "The Daily Difference Method" as suggested by Tsvigolou (29) were herein used to evaluate K for the first 5 days of the BOD process in experiment VI. The BOD curve as plotted on semi-log graph paper fits the data very well for this period. The calculations for each method are shown herein and a summary of the results compared with the measured value of K are presented in TABLE VIII.

The Rapid Ratio Method

The reaction is assumed to be monomolecular in this method of calculation. Special graphs were devised for solving the BOD equation (27). A portion of one graph is reproduced here in Figure 17.

t	Y_t/Y_5*	K_1+
1	0.63/4.1 = 0.15	0.01
2	2.5/4.1 = 0.61	0.17
3	3.4/4.1 = 0.83	0.20
4	3.8/4.1 = 0.93	0.20
		Ave. 0.15

* Values obtained from arithmetic plot ofdata. Y_t = BOD at time t, Y_5 = 5-day BOD

+ K obtained from Figure 17.

The Moment Method

A specially prepared nomograph has been developed to be used for this method of calculation (28). A portion of this nomograph is reproduced in Figure 16.

t	Y	txY
1	0.63	0.6
2	2.5	5.0
3	3.4	10.2
4	3.8	15.2
5	4.1	20.5
	14.4	51.5

$\Sigma Y / \Sigma Yxt = 14.4/51.5 = 0.28$

From Figure 16, K = 0.06

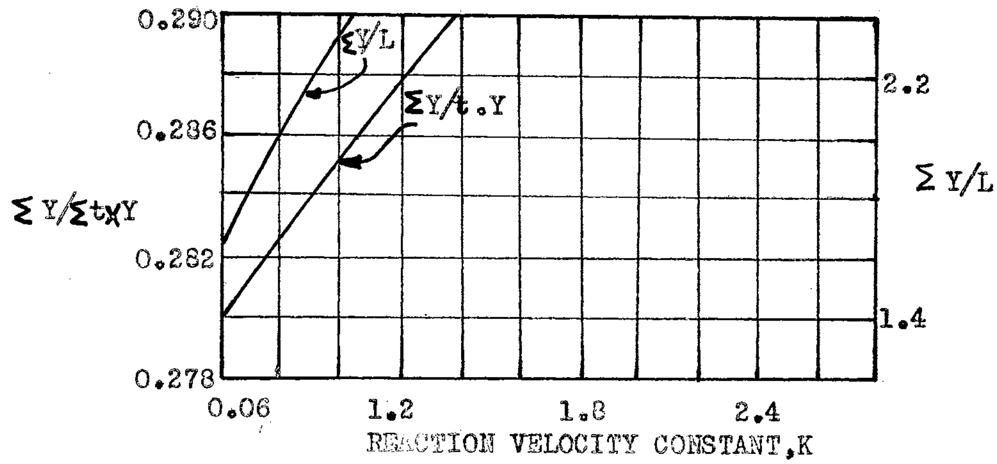

FIGURE 16 - NOMOGRAPH FOR K EVALUATION BY MOMENTS METHOD
(1-5 DAYS SEQUENCE)
(Ref. 29)

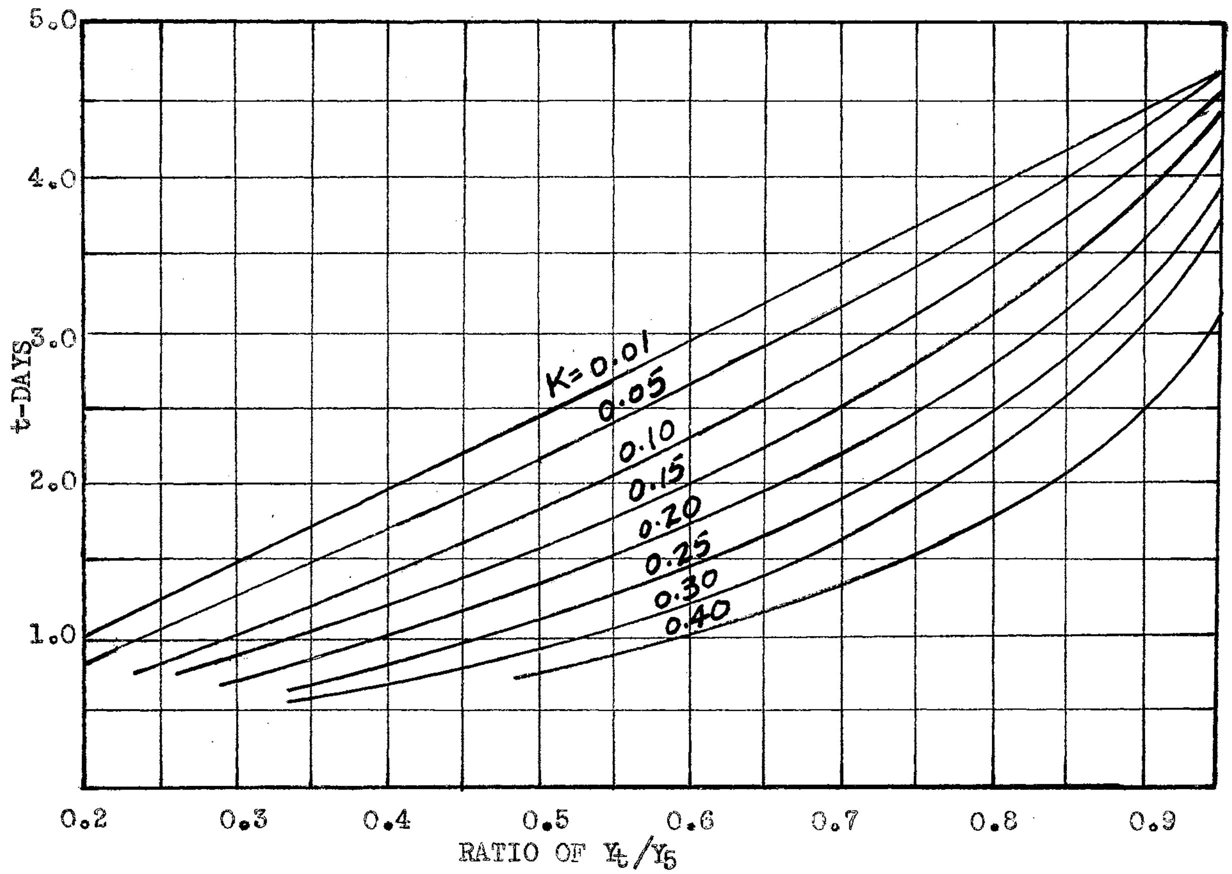

FIGURE 17 - NOMOGRAPH FOR K EVALUATION BY RAPID RATIO METHOD (Ref. 29)

Daily Difference Method

This method was devised by Tsviglou and it does not require special curves. The method takes into consideration the fact that exertion of the BOD is not a simple monomolecular reaction. It is considered by Tsviglou to be a "somewhat more complex reaction having a monomolecular base" (29). In this analysis and presentation of this method no BOD tests were made in the very early phase of the reaction where t is one day or less. Using data obtained during this interval in this study, the smooth fitting of the daily differences in BOD to a straight line on a semi-log graph paper fails. Figure 18 and TABLE VII show the data for experiment VI plotted as suggested by Tsviglou. The plots for the other experiments fall in almost the same pattern.

In this method, daily differences in BOD values are plotted on semi-log graphs as shown in Figure 18 and a straight line is fitted to the data obtained during the later days and extrapolated to time zero. Then the differences between this line and the observed data are plotted as a separate process (Fig. 18 B). Slope of the line in the first curve (A) gives K for the period of time involved and the slope of the line in the second curve (B) gives K for the early days.

When the daily differences as computed in TABLE VII were plotted it was impossible to draw a curve which will fit the values for the first day difference. Ignoring that value, K for the period of 3 to 5 days, was found to be 0.056 and for the period 0 to 3 days, 0.425. The weighted average of both is 0.278.

The actual K's for this period, 0-5 days, as observed from the semi-log plot of the BOD curve: K_1 for the first day = 0.095

K_2 for the next 4 days = 0.178

TABLE VII

DAILY BOD DIFFERENCES FOR EXPERIMENT VI

Time Interval Days	BOD Difference Mg/L.
0-1	0.63
1-2	1.87
2-3	0.90
3-4	0.40
4-5	0.30
5-6	0.30
6-7	0.20
7-8	0.20
8-9	0.10

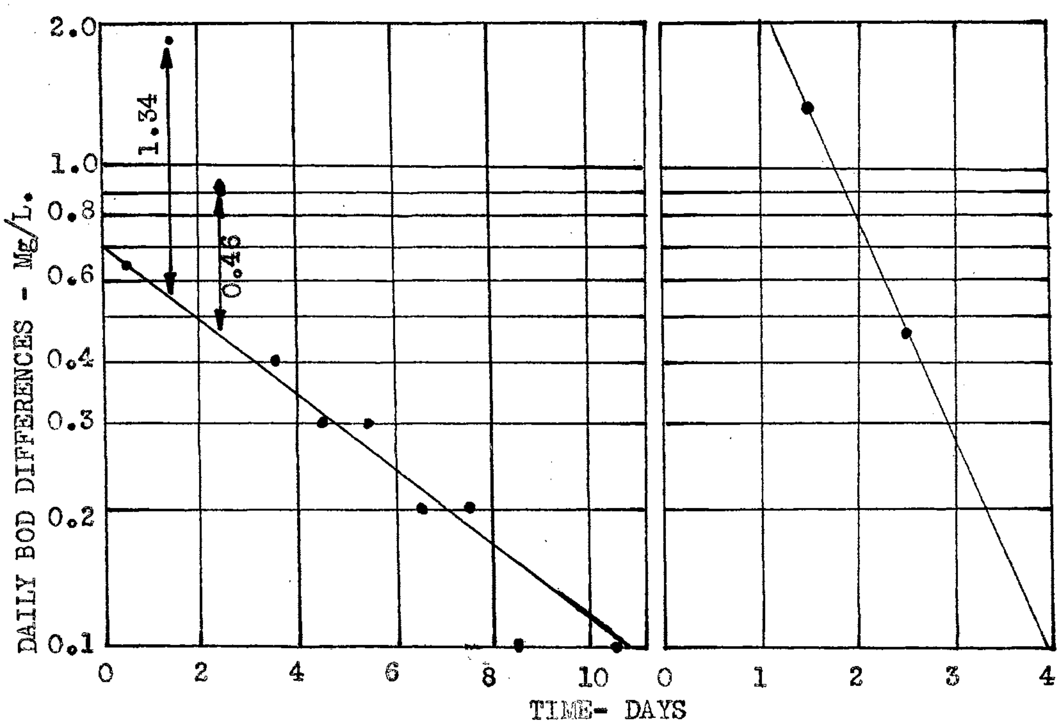

Figure 18- BOD Daily Differences Semi-Log. Plot for the First Five Days of Experiment VI

which yields a weighted average of 0.141 as shown below:

$$0.095 \times 1 = 0.095$$
$$0.178 \times 4 = \underline{0.712}$$
$$0.807$$

$K_w = 0.807 / 5 = 0.141$

The summary of the results of these calculations is shown in TABLE VII below, with the value of La as computed (using the monomolecular equation for t = 5 days and the observed 5-day BOD for Y).

TABLE VIII

VALUES OF K AND La COMPUTED BY DIFFERENT METHODS

		K	La
Rapid Ratio Method	=	0.15	4.98
Moment Method	=	0.06	8.2
Daily Difference Method	=	0.278	4.30
As Observed	=	0.141	5.10

These differences show that the formula and the methods used so far to calculate K do not give satisfactorily accurate results. More investigations are needed in order to arrive at a more concrete method and more valid equation to describe and evaluate the BOD reaction.

Calculations of actual K values as obtained from the tests made in these experiments showed no agreement in values, but the percentage of BOD used at the end of the first two stages of all the glucose dilutions were in closer agreement.

CHAPTER V

CONCLUSIONS

For the Glucose substrate herein used, and except for experiment III, the BOD reaction progressed in three stages each of which can be plotted as a first-order reaction. Oxidation rates vary from one stage and one experiment to the other.

The ratio of oxygen utilized up till the end of the second stage and during the third stage to the 20-day oxygen demand appears to be, on the average, fairly consistent; it is 55.5% for the first two stages and 45.3 for the third.

The length of each stage varied from one experiment to another and it does not appear to have a definite relationship with the number of bacteria in the seed material.

It was verified that the present BOD equation and those of the commonly used methods to compute K values do not adequately describe the BOD reaction. Therefore the use of one specific incubation time in employing the BOD test as a measure of pollution is not an adequate test. However, it should be noted that only two types of synthetic wastes were used in this study.

It is felt that the stages of the BOD reaction are related to the pattern of growth and die off of the bacterial population. This pattern is believed to cause the "hump" in the arithmetic plot of BOD tests. The peak plateau in the bacterial population curve appears to be involved in the causation of the "hump".

CHAPTER VI

SUGGESTION FOR FUTURE WORK

Diphasic Nature of the Curve

More investigations are needed to establish the characteristics and causation of the "hump" occurrence. This can be done by isolating the effect of nitrifying bacteria or that of the predators on the BOD progression. The following steps can be suggested for that purpose:

1. Run BOD tests using seed free of nitrifyers and of Protozoa, i.e. either pure culture or mixture of known cultures.
2. Run BOD tests using seeds free of Protozoa and containing nitrifying Bacteria. Then, vary the amount of nitrogen available in the dilution to study the effect on the "hump", i.e. its starting time and its size.
3. Run BOD tests using seed free of nitrifying bacteria or using a nitrogen source which cannot be used as an energy source for nitrifying bacteria.

Substrate - Organism Ratio

In each of the above suggested steps substrate concentration can be held constant while seed concentration is varied. It will also be useful to vary seed concentration while holding substrate concentration constant. It will be advisable to run similar experiments using substrates other than glucose and nutrient broth, since the only time in which a "hump" was observed was on glucose substrate.

Bacteria Population

A study of predominance effect on the shape of BOD curves can be undertaken by using two or more equal numbers of pure cultures as seed. The predominance pattern can then be correlated with the shape of the BOD curve.

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APPENDIX A

TABLE IX

OXYGEN UTILIZATION DATA FOR EXPERIMENT I

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	7.66	0	7.56	0	0
2	24	7.15	0.51	4.46	3.10	2.59
3	48	6.82	0.84	3.84	3.72	2.88
4	73	6.23	1.43	2.79	4.77	3.34
5	96	6.22	1.44	2.01	5.55	4.11
6	119	6.29	1.37	1.55	6.01	4.64
7	143	6.29	1.37	1.17	6.39	5.02
8	166	6.19	1.47	1.10	6.46	4.99
9	214	6.03	1.63	0.56	7.00	5.37
10	240	5.99	1.67	0.62	6.94	5.27

TABLE X

OXYGEN UTILIZATION DATA FOR EXPERIMENT II

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	7.55	0	7.60	0	0
2	24	7.37	0.18	5.39	2.21	2.03
3	46	7.12	0.43	5.08	2.52	2.09
4	72	7.02	0.53	4.65	2.75	2.22
5	96	6.67	0.88	4.11	3.49	2.61
6	138	6.60	0.95	3.57	4.03	3.08
7	285	6.53	1.02	2.72	4.88	3.86

TABLE XI

OXYGEN UTILIZATION DATA FOR EXPERIMENT III

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	8.62	0	8.18	0	0
2	13	8.43	0.19	8.03	0.15	0
3	26	8.33	0.29	7.59	0.59	0.30
4	41	8.13	0.49	5.99	2.19	1.70
5	48	8.22	0.40	5.20	2.98	2.58
6	62	7.78	0.84	4.47	3.71	2.87
7	72	7.85	0.77	4.47	3.71	2.94
8	85	7.90	0.72	4.25	3.98	3.21
9	99	7.80	0.82	4.10	4.08	3.26
10	111	7.38	1.24	4.17	4.01	2.77
11	123	7.79	0.83	3.84	4.34	3.51
12	135	7.86	0.76	3.88	4.30	3.54
13	147	7.83	0.89	4.11	4.07	3.18
14	161	7.77	0.85	3.49	4.69	3.84
15	187	7.63	0.99	3.37	4.81	3.82
16	211	7.59	1.03	3.07	5.11	4.08
17	236	7.59	1.03	3.02	5.16	4.13
18	333	7.48	1.24	2.04	6.14	4.90
19	415	7.38	1.24	1.99	6.19	4.95
20	491	7.43	1.19	1.44	6.74	5.55

TABLE XII

OXYGEN UTILIZATION DATA FOR EXPERIMENT IV

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	7.47	0	7.66	0	0
2	6.5	7.52	0	7.65	0.01	0
3	14.5	7.52	0	7.52	0.14	0.14
4	22.0	7.47	0	7.37	0.29	0.29
5	30.0	7.15	0.32	7.22	0.44	0.12
6	39.0	7.14	0.33	7.03	0.63	0.30
7	46.0	7.18	0.29	6.84	0.82	0.53
8	54.0	7.22	0.25	6.88	0.78	0.53
9	79.0	7.26	0.21	6.56	1.10	0.89
10	101.5	7.17	0.30	6.38	1.28	0.98
11	108.5	7.00	0.47	6.27	1.39	0.92
12	132.5	6.97	0.50	6.14	1.52	1.02
13	162.5	6.95	0.52	5.98	1.68	1.16
14	187.0	6.85	0.63	5.89	1.77	1.14
15	210.5	6.88	0.59	5.95	1.71	1.12

TABLE XIII

OXYGEN UTILIZATION DATA FOR EXPERIMENT V

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	7.90	0	7.82	0	0
2	7	7.92	0	7.82	0	0
3	16	7.86	0.04	7.79	0.03	0
4	25	7.85	0.05	7.81	0.01	0
5	49	7.87	0.03	6.02	1.80	1.80
6	66	7.90	0	4.57	3.25	3.25
7	74	7.86	0.04	4.45	3.37	3.33
8	80	7.86	0.04	4.32	3.50	3.46
9	92	7.86	0.04	4.23	3.59	3.55
10	106.5	7.90	0	4.02	3.80	3.80
11	130	7.90	0	3.02	4.80	4.80
12	176	7.85	0.05	1.94	5.88	5.83
13	194.5	7.82	0.08	1.76	6.06	5.98
14	241.5	7.82	0.08	1.63	6.19	6.11
15	265.5	7.86	0.04	1.42	6.40	6.36
16	337.5	7.86	0.04	1.07	6.75	6.71
17	385.5	7.82	0.08	1.10	6.72	6.64
18	437.5	7.81	0.09	1.10	6.72	6.63
19	485.5	7.81	0.09	0.75	7.07	6.98

TABLE XIV

OXYGEN UTILIZATION DATA FOR EXPERIMENT VI

RUN NO.	TIME HRS.	SEED D.O. Mg/L	SEED Δ D.O. Mg/L	SAMPLE D.O. Mg/L	SAMPLE Δ D.O. Mg/L	NET OXYGEN UTILIZATION Mg/L
1	0	8.15	0	8.10	0	0
2	7	8.13	0.02	8.06	0.04	0.02
3	15	8.13	0.02	7.61	0.49	0.47
4	23	7.93	0.22	7.15	0.85	0.63
5	29	7.93	0.22	6.75	1.35	1.13
6	43	7.85	0.30	5.45	2.65	2.35
7	58	7.84	0.31	4.95	3.15	2.84
8	69	7.77	0.38	4.48	3.62	3.24
9	90	7.68	0.47	3.89	4.29	3.82
10	138	7.58	0.57	3.22	4.88	4.31
11	186	7.48	0.67	2.64	5.46	4.79
12	241	7.33	0.82	2.49	5.61	4.79
13	289	7.18	0.97	2.24	5.86	4.89
14	361	7.18	0.97	2.19	5.91	4.94
15	426	7.18	0.97	2.12	5.98	5.01
16	480	7.18	0.97	1.89	6.21	5.24

TABLE XV

BACTERIA COUNTS (EXP. I)

RUN NO.	TIME HRS.	COLONIES PER 1/2 PLATE OF SEED: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{4}	COLONIES PER 1/2 PLATE OF SAMPLE: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{4}
1	0	21	0	0	2.6	17	0	0	2.1
2	24	TMC	73	8	91.3	TMC	TMC	47	58.7
3	48	-	120	11	150.0	-	200	22	250.0
4	73	-	88	0	110.0	-	100	11	125.0
5	96	-	11	0	13.7	-	11	0	13.7
6	119	100	11	-	12.5	?	11	0	13.7
7	143	150	15	-	18.7	85	5	-	6.3
8	166	100	14	-	12.5	96	16	-	20.0
9	214	78	7	-	9.8	43	4	-	5.4
10	240	30	4	-	3.8	36	4	-	4.5

TMC: Too many colonies to count

? : Counting was not possible

TABLE XVI

BACTERIA COUNTS (EXP.II)

RUN NO.	TIME HRS.	COLONIES PER 1/2 PLATE OF SEED: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{5}	COLONIES PER 1/2 PLATE OF SAMPLE: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{5}
1	0	-	46	6	5.9	-	39	3	4.9
2	24	120	12	1	1.5	TMC	TMC	48	60.0
3	46	220	24	3	2.7	-	130	13	16.3
4	72	TMC	36	4	4.5	-	170	26	21.3
5	96	TMC	37	-	4.6	-	120	10	15.0
6	138	TMC	31	-	3.9	TMC	74	-	9.3
7	285	96	21	-	1.2	TMC	36	-	4.5

TMC : Too many colonies to count

TABLE XVII

BACTERIA COUNTS (EXP.III)

RUN NO.	TIME HRS.	COLONIES PER 1/2 PLATE OF SEED: DILUTIONS 10^{-1}	10^{-2}	10^{-3}	ORG. PER ML. 10^{4}	COLONIES PER 1/2 PLATE OF SAMPLE: DILUTIONS 10^{-1}	10^{-2}	10^{-3}	ORG. PER ML. 10^{4}
1	0	-	63	8	7.9	-	55	5	6.9
2	13	-	56	5	7.0	-	56	9	7.0
3	26	TMC	95	-	11.9	TMC	95	-	11.9
4	41	-	20	0	2.5	-	98	0	12.3
5	48	130	2	0	1.6	TMC	36	4	4.5
6	62	210	3	-	2.6	TMC	31	5	3.9
7	72	-	60	30	3.8	TMC	TMC	23	28.7
8	85	TMC	130	-	16.3	-	TMC	87	10.9
9	99	-	81	5	10.1	-	TMC	140	17.5
10	111	-	120	19	15.0	-	TMC	49	61.2
11	123	-	100	10	12.5	-	TMC	55	68.7
12	135	-	58	1	7.3	-	TMC	55	68.7
13	147	-	97	33	12.1	-	TMC	36	45.0
14	161	-	100	34	12.5	-	TMC	48	60.0
15	187	-	200	20	25.0	-	140	17	17.0
16	211	-	180	51	22.5	-	170	25	21.4
17	236	-	-	66	82.5	-	82	14	10.3
18	333	-	58	11	7.3	-	23	5	2.9
19	415	-	270	31	33.8	-	70	6	8.8
20	491	-	110	10	13.8	-	66	8	8.3

TMC : Too many colonies to count

TABLE XVIII

BACTERIA COUNTS (EXP. V)

RUN NO.	TIME HRS.	COLONIES PER 1/2 PLATE OF SEED: DILUTIONS 10^{-1}	10^{-2}	10^{-3}	ORG. PER ML. 10^{4}	COLONIES PER 1/2 PLATE OF SAMPLE: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{4}
1	0	-	68	5	8.5	55	3	0	6.9
2	7	-	50	6	6.3	61	3	0	7.6
3	16	-	70	5	8.8	72	9	0	9.8
6	66	-	49	17	6.1	TMC	TMC	-	-
7	74	-	83	36	10.4	TMC	TMC	-	-
8	80	-	?	12	15.0	TMC	TMC	?	-
							10^{-4}	10^{-5}	
9	92	TMC	75	-	9.4	-	170	26	210.0
10	106.5	-	54	-	6.8	-	160	16	200.0
11	130	-	100	-	12.5	-	91	7	114.0
12	176	-	54	-	6.8	-	?	?	-
							10^{-3}	10^{-4}	
13	194.5	TMC	49	-	6.2	-	30	4	38.0
14	241.5	TMC	45	-	5.7	88	7	-	11.0
15	265.5	TMC	73	-	9.2	140	18	-	17.5
16	337.5	190	19	-	2.3	42	4	-	5.3
							10^{-1}		
17	385.5	150	14	-	1.9	38	260	-	3.3
18	437.5	140	5	-	1.8	84	TMC	-	10.5
19	485.4	170	92	-	2.1	92	-	-	11.5

TMC = Too many colonies to count
? = Counting was not possible

TABLE XIX

BACTERIA COUNTS (EXP. VI)

RUN NO.	TIME HRS.	COLONIES PER 1/2 PLATE OF SEED: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{4}	COLONIES PER 1/2 PLATE OF SAMPLE: DILUTIONS 10^{-2}	10^{-3}	10^{-4}	ORG. PER ML. 10^{4}
1	0	-	19	2	23.8	-	13	2	16.3
2	7	-	12	0	15.0	-	7	1	8.8
3	15	82	9	0	10.3	78	10	-	9.8
4	23	88	8	0	11.0	62	4	-	7.8
5	29	91	7	0	11.4	100	11	0	12.5
6	43	84	-	-	10.5	170	-	-	21.3
7	58	61	-	-	7.6	?	12	-	15.0
8	69	-	12	0	15.0	67	30	-	8.3
9	90	93	-	-	11.6	89	12	-	11.1
			10^{-1}				10^{-1}		
10	138	32	230	-	2.9	17	-	-	2.1
11	186	37	240	-	3.0	19	90	-	1.1
12	241	28	220	-	2.7	21	120	-	1.5
13	289	-	220	-	2.7	-	190	-	2.4
14	361	-	210	-	2.6	-	130	-	1.6
15	426	-	190	-	2.4	-	100	-	1.3
16	480	-	200	-	2.5	-	150	-	1.9

? = Counting was not possible

TABLE XX

DETERMINATION OF BOD OF NUTRIENT BROTH

Date: April 13, 62

Dilution Preparation: BACTO Nutrient Broth was rehydrated by dissolving 8 grams in 1000 ml. deionized water and then sterilizing it in the autoclave for 15 minutes at 15 pounds pressure (121°C)

Incubation Period: 4 days

Seed: 2 ml/l

Bottle No.	Ident.	Dilution Factor	Initial DO	Final DO	Depletion	BOD
40	Seed	-	8.17	7.57	0.60	-
8	Sample	625	8.17	-	-	-
13	Sample	1250	8.17	3.30	4.27	5330
88	Sample	1870	8.17	4.76	2.81	5270
23	Sample	2500	8.17	5.48	2.09	5230
104	Sample	3125	8.17	5.88	1.69	5280
						Ave.= 5278

From previous experiments 4-day BOD is approximately 92% of 5-day BOD. Therefore, Broth 5-day BOD is about 5270 ppm. and 5-day BOD is approximately 68% of 20-day BOD, therefore Total BOD of Broth is 8430 ppm.

TABLE XXI

TRIAL PLATING FOR ORGANISM COUNT DETERMINATION

Experiment III

Date: Mar. 8, 62

10^{-3}	10^{-4}	10^{-5}	10^{-6}
TMC	11	1	0
	14	0	0
	13	1	0
	26	0	
	64	2	

Therefore population is $64 \times 12.5 \times 10^4 = 8 \times 10^6$ org./ml.

Experiment IV

Date: Mar. 30, 62

SM = Serratia Marcesens
ML = Micrococcus Lysodeikticus
PF = Pseudomonas Fluorescens

SM (Optical density of 0.10 and wavelength of 600 mu)

10^{-3}	10^{-4}	10^{-5}
TMC	TMC	30
		37
		26
		32
		125

Therefore population is $125 \times 12.5 \times 10^5 = 16 \times 10^7$ org./ml.

ML (Optical density of 0.22 and wavelength of 600 mu)

Population is 3.8×10^7 org./ml. as computed in a trial on that date for a different experiment

PF (Optical density of 0.15, and wavelength of 600 mu)

10^{-3}	10^{-4}	10^{-5}
TMC	TMC	53
		61
		80
		50
		58
		302

Therefore population is $502 \times 10 \times 10^5 = 30 \times 10^7$

To have 1×10^5 org./ml. of each type of organisms, use:

0.67 ml/l of SM
2.67 ml/l of ML
0.33 ml/l of PF

Experiment VI

Date: April 23, 62 (CONTINUED)

TABLE (CONTINUED)

10^{-2}	10^{-3}	10^{-4}
59	6	3
60	10	3
76	9	1
	10	1
64	35	8

Therefore population is $253 \times 12.5 \times 10^2 = 3.5 \times 10^5$ org./ml.

VITA

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