STUDIES OF THE DIPHASIC NATURE OF BOD PROGRESSION

By

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CHAPTER I

INTRODUCTION

The biochemical oxygen demand (BOD) test is widely used for measuring the pollutional strength of sewage, industrial wastes or other polluted waters. This test is designed to measure the amount of molecular oxygen required to stabilize the decomposable matter present in a water by aerobic biochemical mechanisms. The oxygen demand is exerted by three classes of matter; carbonaceous matter, oxidizable nitrogen, and certain chemical reducing compounds. The organic material, which gives carbonaceous oxygen demand, and nitrogen in the form of NH_4 are subject to bacterial oxidation. Heterotrophic bacteria are responsible for the degradation of organic material while autotrophic bacteria oxidize ammonia. Reducing compounds generally represent a chemical demand rather than a biochemical demand. It is the carbonaceous BOD that is of primary interest.

Literature Review

A forerunner of the BOD test was the oxygen absorption test from which evolved the chemical oxygen demand test. This method failed to recognize that a biological process was the cause of oxidation of the organic matter present; however, it did attempt to determine the oxidizable matter present quantitatively. Forchamer first proposed the potassium permanganate technique for this determination in 1894 (1). Winkler in 1888 proposed his classic method for dissolved oxygen deter-

mination which with its modifications has become a standard technique in the biochemical oxygen demand test (2). Phelps (3) in 1909 first attempted to establish a mathematical relationship describing the observed rate of oxygen uptake of sewage. He used that principle of physical chemistry which states that the velocity of a chemical reaction is a function of some power of the concentration of the reacting substances. In the case of BOD, the reaction varied directly as the concentration of the food supply, measured in terms of its oxidizability. This relation assumes that a 10 percent dilution of carbon source has a reaction rate one tenth the reaction rate of a 100 percent sample. Theriault (2) in 1927 in his classical work found that Phelps' monomolecular or first order decreasing rate formula did describe the first or carbonaceous stage of deoxygenation. Furthermore Theriault found that the velocity constant (k) was 0.10 for all the various river waters he tested. In a latter work Phelps (4) stated that there is no reason to expect the kinetics of BOD exertion to be first order decreasing rate. This has been stated by others many times since Phelps.

It is interesting to note that in his studies measuring the BOD of river water Theriault used tap water to dilute his samples. Since that time many investigators have been concerned with defining optimum standard conditions under which the BOD test should be run. Many of these investigations have involved the effects of various BOD dilution water. Butterfield (5) states that the rate of biochemical oxidation is a function of the rate of growth and reproduction of viable organisms present. To obtain maximum BOD sufficient cell building elements, such as nitrogen, phosphorus, etc. must be present in the waste or in the dilution water. Heukelekian and Chamberlain (6) found that stream water with the

lowest concentration of salts gave the lowest BOD. They compared five artificial dilution waters but from their results they could not recommend any one of them. None of their dilution waters contained nitrogen. Holderby and Lea (7) investigated the effect of the pH of dilution water and found that within a pH range of 7.0 to 9.0 that the pH of bicarbonate dilution water had little or no effect on the BOD of domestic sewage or packing plant wastes. Lea and Nichols (8) found that the addition of four salts $MgSO_4$, $Ca_3(PO_4)_2$, KH_2PO_4, and $(NH_4)_2SO_4$ to bicarbonate dilution water made it a far superior medium for biochemical oxidation during the BOD test. Finally Ruchhoft (9) in a report to the Dilution Water Study Committee of the Federation of Sewage Works Associations recommended the use of the inorganic salts that are now described in the 11th edition of Standard Methods (10)

Many investigations have also been undertaken to describe the type kinetics and velocity constants observed by Phelps and Theriault in a more accurate manner. A departure, occuring at 8 to 10 days, from the "normal" rate of oxidation which could not be explained by the first order BOD equation was noted by many investigators. This departure was attributed to nitrification. It was generally observed that the nitrifying organisms did not begin to assert themselves until most of the carbonaceous oxygen demand had been exerted. Heukelekian (11) gave evidence indicating that oxidation of carbonaceous materials does not inhibit or retard nitrification which can take place simultaneously with carbonaceous BOD exertion provided that oxidation of carbonaceous materials does not produce a deficiency of oxygen or available nitrogen and there is an active nitrifying flora present. There usually is such a small number of nitrifying flora present that a long period of time is

needed to produce enough organisms so that their presence can be shown in oxygen uptake data.

Ruchhoft, Placak, and Ettinger (12) presented data using different dilutions of the same sewage. The dilution water used was that described by Lea and Nichols (13). This water contained $(NH_4)_2SO_4$ as nitrogen source. The data showed that oxygen utilization for the different dilutions was (approximately) directly proportional to the concentration of sewage present. Also their work indicated velocity constants of 0.15 rather than 0.10 as reported by Theriault (2). They found that the first order BOD equation could be fitted satisfactorily to the carbonaceous BOD exertion for any selected period of observation. They felt that the velocity constant (k) and ultimate oxygen demand (L) only applied to the specific time and conditions of the observation. " Consequently k and L values obtained for a short period of observation have little extrapolative value and cannot be used to estimate the expected BOD's for extended periods" (14). The Subcommittee on Sewage Treatment at Military Installations of the Committee on Sanitary Engineering of the National Research Council (15), headed by Mohlman, submitted a report in 1946. This report which included a comprehensive study on all phases of sewage treatment, presented the oxygen demand characteristics of a number of raw sewages from different military posts. When the first order BOD equation was applied to this data considerable variation in the rate constant (k) was found. The values ranged from 0.10 to 0.30 with an average k of 0.18. This variability in k invalidated, somewhat, the usual assumption that the 5 day BOD was directly proportional to the strength of a sewage.

Work using industrial waste as substrate has shown that the velocity

of BOD exertion is not always directly proportional to substrate concentration (16). This has been referred to as a "sliding scale" of BOD exertion (17). In such cases it is generally observed that the highest dilution yields the higher BOD values. Toxicity of the waste and acclimation time have been cited as the reasons for this departure from "normal" BOD exertion.

Mills and Stack (18) investigated the use of acclimated seed to determine the biochemical oxidation load of an industrial waste. They found significant differences in the reactiveness of acclimated and non-acclimated seed and they felt that the values obtained using acclimated seed were more representative of what is found in nature.

Butterfield (19) studied the growth of bacteria at substrate concentrations usually existing in sewage. He observed that for each concentration of substrate used, multiplication of cells takes place for a certain period (usually 48 hours) and then is checked. The number of bacteria remains fairly constant after the peak number has been reached. For each concentration of substrate there was a maximum bacteria population. Butterfield used dextrose and peptone as substrate. The growth of Bact. aerogenes and a small sewage coccus were studied in pure culture in his investigation.

Butterfield, Purdy, and Theriault (20) studied the relationship between protozoa, bacteria, and oxygen uptake. They found with pure cultures that cell multiplication ceased after a few days but cell numbers remained fairly constant after the maximum number was reached and that this reduction in activity was reflected in the oxygen uptake. Oxygen depletion produced by the growth of the protozoa Colpidum in the absence of bacteria was only a small portion of that observed in the presence of

bacteria and plankton. In a mixture of bacteria and Colpidum they observed a rapid increase in bacterial numbers to a limiting number of about 7×10^6 organism/ml during the first 24 hours. The Colpidum increased slowly requiring 3 to 6 days to reach their limiting number. As the Colpidum approached this limiting number the bacteria count began to decrease. This decrease continued even after the Colpidum reached their peak numbers and started to decrease. The oxygen uptake proceeded at a rapid rate while the bacteria were increasing in number and oxygen uptake continued not only after their limiting number had been reached but also after the limiting number of Colpidum was observed.

Butterfield and Wattie (21) found that a lag in oxygen uptake could be due to a small number of bacteria present at the beginning of the test. In their study an increase in initial cell numbers decreased the lag in oxygen uptake.

Heukelekian (22) investigated the effect of dilution on bacterial numbers. He used sewage as substrate and phosphate buffered dilution water. He found as did Butterfield (18) that there was a relationship between food supply and maximum cell numbers. He presented curves relating bacterial numbers, dissolved oxygen, substrate concentration, and time. At substrate concentrations of 2.0, 2.5, 5.0 and 10 percent sewage there was a change in the kinetics of the dissolved oxygen curve. Heukelekian did not discuss this occurrence.

In 1954 Tidwell and Sorrels (23) working on a short term BOD test gave data, using Ps. aeurginosa as seed material, which showed that the BOD of a given sewage varies not only with the amount of organic matter present, but also with the number of organisms capable of oxidizing that sewage and not with the total number of organisms present. Their BOD

curves indicate a plateau which corresponds to the apparent peak in cell population. The tests were of a short time period and cell numbers had not decreased much below the maximum number.

Hoover, Jasewicz, and Porges (24) observed diphasic oxygen uptake using milk waste as substrate. They described these two phases as; first, the rapid growth of cells with assimilation of available nutrients into cells; and second, slow endogenous respiration in which the cell oxidize their own protoplasm slowly in order to obtain energy for maintenance in absence of available exogenous nutrients.

Buswell, Mueller, and Van Meter (25) described the BOD exertion curve as having two parts, one corresponding to cell multiplication and the second associated with a resting or a dying culture. They did not think that either stage could be described by first order kinetics.

Zehnpfenning and Nichols (26) also felt there is diphasic oxygen uptake during BOD exertion. They state that the first phase is controlled by substrate concentration and the second stage is determined by the number of bacteria and by the reproduction rate of these bacteria rather than substrate concentration.

Lea and Nichols (27) have shown data relating oxygen uptake and population dynamics. Their experiments were done over a five day period. In all of their experiments the population count curves have the same shape as the BOD exertion curve. Both appear to follow decreasing rate first order kinetics.

Busch (28) and Busch and Myrick (29) (30) working on a short term BOD test observed a diphasic reaction separated by a lag or plateau. The first stage according to Busch represented the conversion of the available substrate into cell material and intermediate storage products.

The second stage consisted of the endogenous utilization of the stored decomposition products. Oxygen depleted in excess of that required for this activity was attributed to predator activity. It was felt that a large number of protozoa in the seed inoculum "masked" or covered up the plateau. The plateau was observed in synthetic and domestic sewage, glucose, glutamic acid, and a mixture of glucose and glutamic acid. He had no data on population dynamics.

Tisivoglou (31) reported the plateau in studies using Kanauka River water. This river receives both domestic and industrial wastes.

Lee and Oswald (32) compared the number and varieties of organisms in seeds and related these counts to the BOD exerted. Their oxygen uptake rate curves showed a rapid uptake followed by a sharp decrease. This was due to three phases in cell growth. The first phase consisted of a rapid increase in respiration, indicating rapid assimilation of nutrients and cell growth. Whether cell division took place could not be determined. It was considered that although cell division took place the cells remained in clumps and therefore appeared as single cells in colony counts. The second phase was the dispersion of the clumps resulting in high cell counts. The third phase was claimed to be one in which the cells having finally dispersed and failing to find a new source of nutrients rapidly died giving rise to the decrease in both cell numbers and respiration rate. Sterile sewage was used in their investigation.

McWhorter and Heukelekian(33) have obtained the plateau in the Warburg apparatus using various substrate concentrations (100,500, mg/l glucose) and 0.5 ml settled sewage seed. Potassium nitrate was used as the nitrogen source. No lag or plateau occurred in systems with substrate concentration of 1000 and 2000 mg/l glucose. They observed for the system

exhibiting the plateau that while the increase in substrate concentration increased oxygen uptake, the accumulated uptake at the plateau expressed as percent theoretical oxygen demand was the same for both systems. They also investigated the effect of predators. Oxygen uptake curves for sonicated and normal seeds did not diverge until 45 percent of the theoretical oxygen demand had been exerted. Since this was above the plateau value they concluded that the disappearence of the plateau with increasing substrate concentration could not be solely attributed to changes in the predator-bacteria ratio.

Wilson and Harrison (34) have observed a very slight plateau in BOD exertion curves for chemical plant liquors subjected to various degrees of treatment. Gaudy, Bhatla, Abu-Niaaj (35) (36) have expressed doubt that the plateau shown by Wilson and Harrison (34) is identifiable with the type of phenomenon demonstrated by Busch (28), by McWhorter and Heukelekian (33) and by Gaudy et al.(35) (36), Gaudy and Bhatla (37). However, Wilson and Harrison (34) have shown a plateau that is similar to the one shown in the works cited above. Using a pure culture and phthalic acid substrate they measured oxygen uptake in two systems containing different amounts of initial seed inoculum and concluded that the number of cells present determined the magnitude of the second stage.

Abu-Niaaj (38) observed the plateau in his research using glucose as substrate. In one experiment with nutrient broth substrate there was no plateau. A relationship between the plateau and cell population dynamics was found. Abu-Niaaj observed that the peak in population occurred at approximately the same time as the platuea.

Gaudy, Bhatla, and Abu-Niaaj (35) (36) and Gaudy and Bhatla (37) demonstrated the plateau using sewage seed, acclimated hetrogenous seed

acclimated hetrogenous cells harvested at the end of log growth phase, and acclimated Escherichia coli seed inoculum. Substrates used were glucose, sorbitol, ribose, and mixtures of glucose and ribose, and of glucose and sorbitol. They concluded that the plateau was independent of the type seed employed in their studies. Under normal seeding conditions and substrate concentrations the first phase of oxygen uptake corresponded to a rapid increase in cell numbers. The range of maximum numbers of cells corresponded to the plateau. The second stage of oxygen uptake corresponded to a decrease in cell numbers. The duration of the maximum cell density corresponded to the severity or length of the plateau. Four theories were presented explaining the existance of the plateau. The main idea in all four was similar, the plateau is caused by a change from the metabolism of the original exogenous food source to metabolism of the new food sources produced by the cells. This change may require an acclimation period which is mirrored in the oxygen uptake.

Observed BOD Kinetic Curves

The major BOD kinetics have been classified by Gaudy, Bhatla, and Abu-Niaaj (35) (36). These are shown in Figure 1. These are:

1. First order decreasing rate which was shown by Theriault (2).
2. The autocatalytic curve which has a lag in the early portion of oxygen uptake. This lag is not necessarily a lag in bacterial activity, but in order to use a first order decreasing rate mathematical expression it must be treated as a lag.
3. The multiphase curve which was shown by Hoover, Jasewicz, and Porges (24). This is a rapid first order decreasing rate curve followed by a

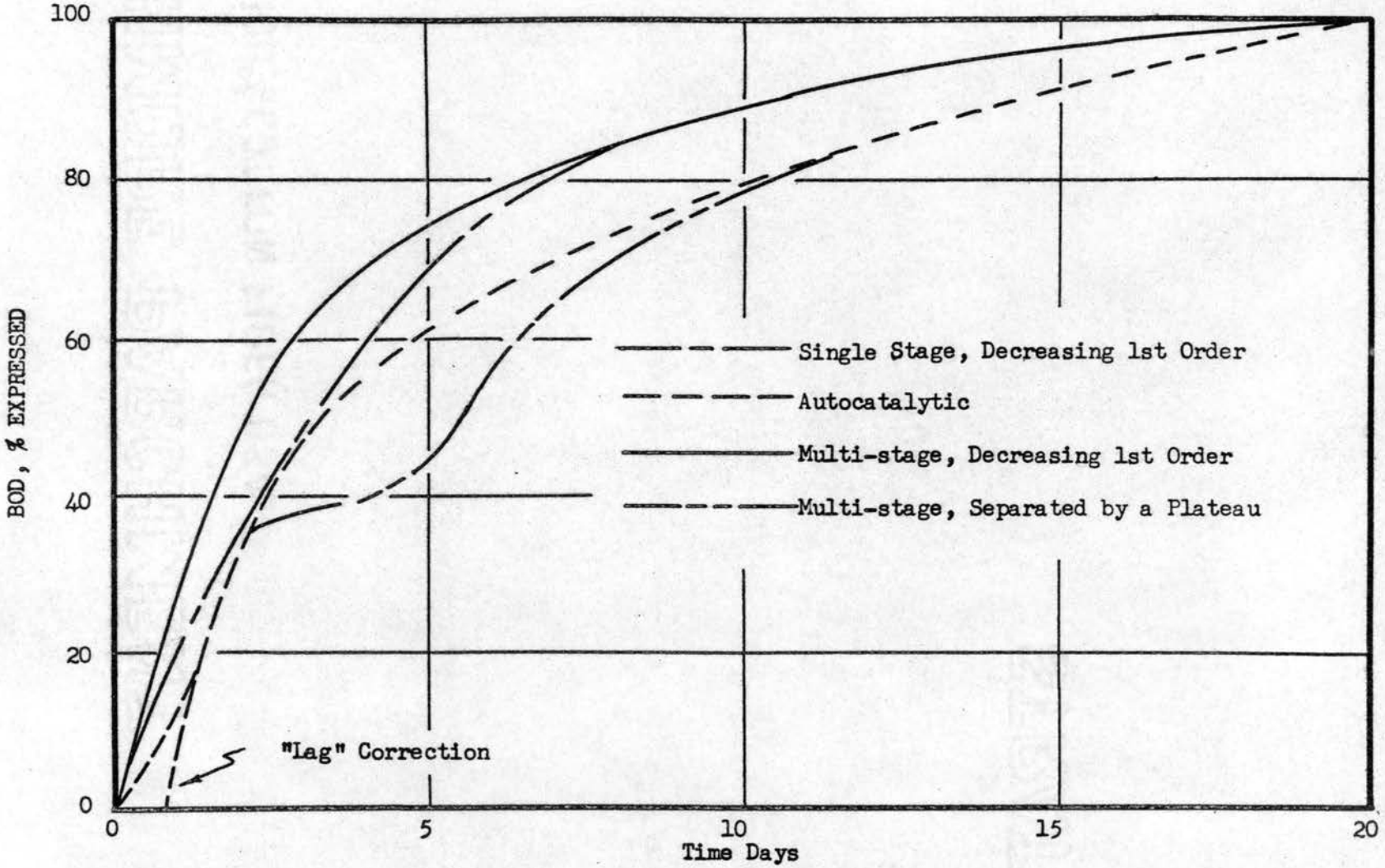

Fig. 1 VARIOUS KINETIC EXPRESSION OF CARBONACEOUS BOD

slower one.

4. A curve with a plateau separating two first order curves, which was shown by Busch (28).

Purpose and Scope of the Present Investigation

The purpose of the research herein reported was to investigate the effect of substrate concentration and the effect of initial seed inoculum on the diphasic nature of BOD progression and to study the occurrence of diphasic BOD exertion in whole wastes. This later aspect is of particular importance, since most of the research to date on this phenomenon has been accomplished using synthetic wastes. The experimental work was divided into three parts. These divisions covered: (1) effect of substrate concentration, (2) effect of initial seed inoculum, (3) type BOD exertion obtained using various complex substrates.

CHAPTER II

MATERIALS AND METHODS

BOD Determination

Reagents for determination of BOD and dissolved oxygen were those described in Standard Methods for the Examination of Water and Waste - water(10). The Alsterberg modification of the Winkler method was used for dissolved oxygen determination. The sodium thiosulfate was standardized with biniodate each day it was to be used. One ml of each dilution water reagent was added to each liter of deionized dilution water. This is the amount specified in Standard Methods. This gives 0.445 mg of nitrogen per liter of dilution water. According to Rao and Busch (39) there is a deficiency in nitrogen requirements if over 8.06 mg/l glucose is used. This will be discussed in a latter section of this thesis.

Determination of Viable Bacteria Population

Viable bacteria count estimates were obtained by the spot plate surface counting technique (40). A 0.08 ml sample was placed (four spots of 0.02 ml each) on the surface of a suitable growth medium containing agar. The bacterial numbers are determined by counting the number of colonies that grow from the sample. Duplicate plates were made of all samples. Nutrient agar (Difco) was used for all estimates of bacterial numbers. This method has been used by Gaudy, Abu-Niaaj,

and Gaudy (40) and McKinny, Langley, and Tomlinson (41) and has proved as reliable and slightly more accurate than the pour plate method for aerobic organisms. A recent statistical study has shown the distribution of counts to be Poissonian (40).

Seed Types

Seed A-Acclimated Seed

This source of seeding material was obtained from the effluent of a laboratory batch operated activated sludge unit. The system was fed once each 24 hours with the same material to be used in the BOD experiments. Before feeding, one-third of the mixed liquor was wasted, the remainer was allowed to settle for one hour at which time one-half of the remaining liquor was wasted. A portion of this wasted effluent was used as seeding material. The batch activated sludge was developed from sewage seed from the Stillwater, Oklahoma, sewage treatment plant.

Seed B-Washed Acclimated Seed

In some experiments the seeding material was obtained from the activated sludge batch unit as for seed A, however, the cells were harvested by centrifugation. These cells were washed once in 0.05 M phosphate buffer and used as seed material. This type seed was used in experiment 4 to observe the difference of oxygen exertion between washed seed and unwashed seed. In the seed concentration studies the high initial bacterial numbers inoculum was washed to reduce substrate carry over. This was also the reason the cells were washed in Part 3.

Seed C-Sewage Seed

This source of seeding material was obtained from the primary clarifier effluent at the Stillwater, Oklahoma, municipal sewage treatment

plant. A sample was obtained from the plant approximately one-half hour before use in any particular experiment. The effluent was allowed to stand under quiescent conditions during this time. The top most portion was drawn off and used to seed the dilution water.

Substrates

The exogenous carbon source for Part 1 and 2 of these studies consisted of glucose in varying concentrations. In Part 3 the following carbon sources were used, neutral sulfite semichemical pulping liquor, Kraft black liquor, Kraft mill effluent, sterile sewage, and nutrient broth. All seed types except the sewage seed were thoroughly acclimated to the carbon source to be used in the experiment. Batch acclimated seed was grown in a medium containing 1000 mg/l glucose or approximately 1000 mg/l BOD pulp mill waste. The amounts of inorganic salts used were (concentration in mg/l); $(NH_4)_2SO_4$, 500; $MgSO_4 \cdot 7H_2O$, 100; $FeCl_3 \cdot 6H_2O$, 0.5; $MnSO_4 \cdot H_2O$, 10; $CaCl_2$, 7.5. These components were dissolved in 0.01 M potassium phosphate buffer, pH 7.0 containing 100 ml of tap water per liter.

Experimental Protocol

Standard dilution water was equilibrated in a 20°C constant temperature room for 24 hours and aeration was begun 30 minutes prior to preparing BOD's for incubation. For each different system to be studied in a particular experiment, replicate portions of dilution water were taken. Inorganic salts, seeding material, and substrate in the required concentrations were added. Organic substrate was withheld from those portions used for the seed bottle. Both seed and seed plus sample flasks

were vigorously stirred and BOD bottles filled immediately. Samples of all systems were taken to determine initial dissolved oxygen and initial viable bacteria population. BOD bottles were incubated at 20°C. under a water seal. BOD bottles were removed periodically for determination of dissolved oxygen remaining and changes in cell numbers. Samples for measuring the viable cell population were obtained by carefully withdrawing 1.0 ml from BOD bottles immediately prior to adding Winkler's dissolved oxygen reagents. Appropriate dilutions were made using sterile distilled water. These dilutions were plated and incubated at 37°C. for 24 hours before counting. It had been shown by preliminary study that dissolved oxygen values were not affected by the extra manipulation required in withdrawing 1.0 ml before adding the dissolved oxygen reagents. A study was also made by Gaudy et al.(35) which obtained the same results. The experimental protocol used in these studies was the same as that previously used the O. S. U. Bio-engineering laboratory (35) (36) (37) (38).

CHAPTER III

RESULTS

Part 1 Effect of Substrate Concentration on BOD Kinetics and Bacterial Growth.

In this study glucose in various concentrations (2, 5, 8, 12 mg/l) was used and an attempt was made to maintain the same initial bacteria population in all experiments. Seed was obtained from the activated sludge batch unit. In general seed type A was used. Experiments 1 and 2 (Fig. 2, 3, 4) show results of studies using 2, 5, 8 mg/l glucose substrate. The intial bacteria population was approximately 1.3×10^4 organism/ml in experiment 1 and 2.0×10^3 in experiment 2. All the concentrations show plateaus or changes in kinetics before correction was made for the oxygen uptake in the seed system. The net 2 mg/l curve in experiment 1 did not exhibit the plateau since it was masked by the plateau in oxygen uptake in the seed system. The rapid rise in oxygen uptake in the seed at 60 hours caused an apparent decrease in net oxygen uptake in the 2 mg/l system in experiment 1 (see oxygen concentration at 72 and 84 hours). The oxygen values at 60 hours on the net 2 mg/l curve and at 84 hours on the net 5 mg/l curve in experiment 1 were not given the same weight as the others because in the gross curves (Fig. 4) these were clearly not representative of the BOD exertion.

BOD exertion curves in experiment 1 and 2 are similar. Comparing the BOD exertion curves to the viable bacteria counts, it is seen that

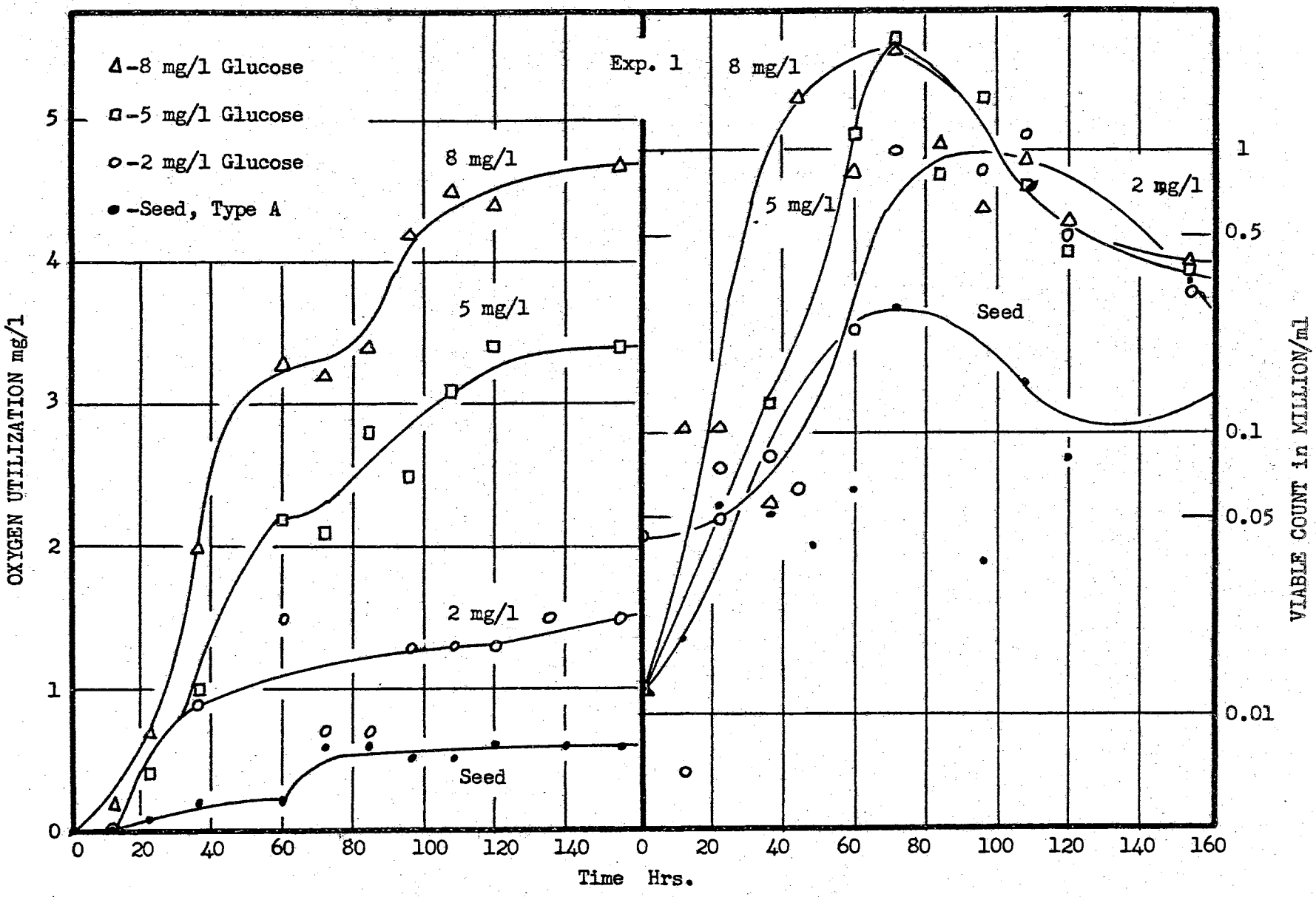

Fig. 2 NET BOD AND VIABLE BACTERIA COUNT, SEED TYPE A

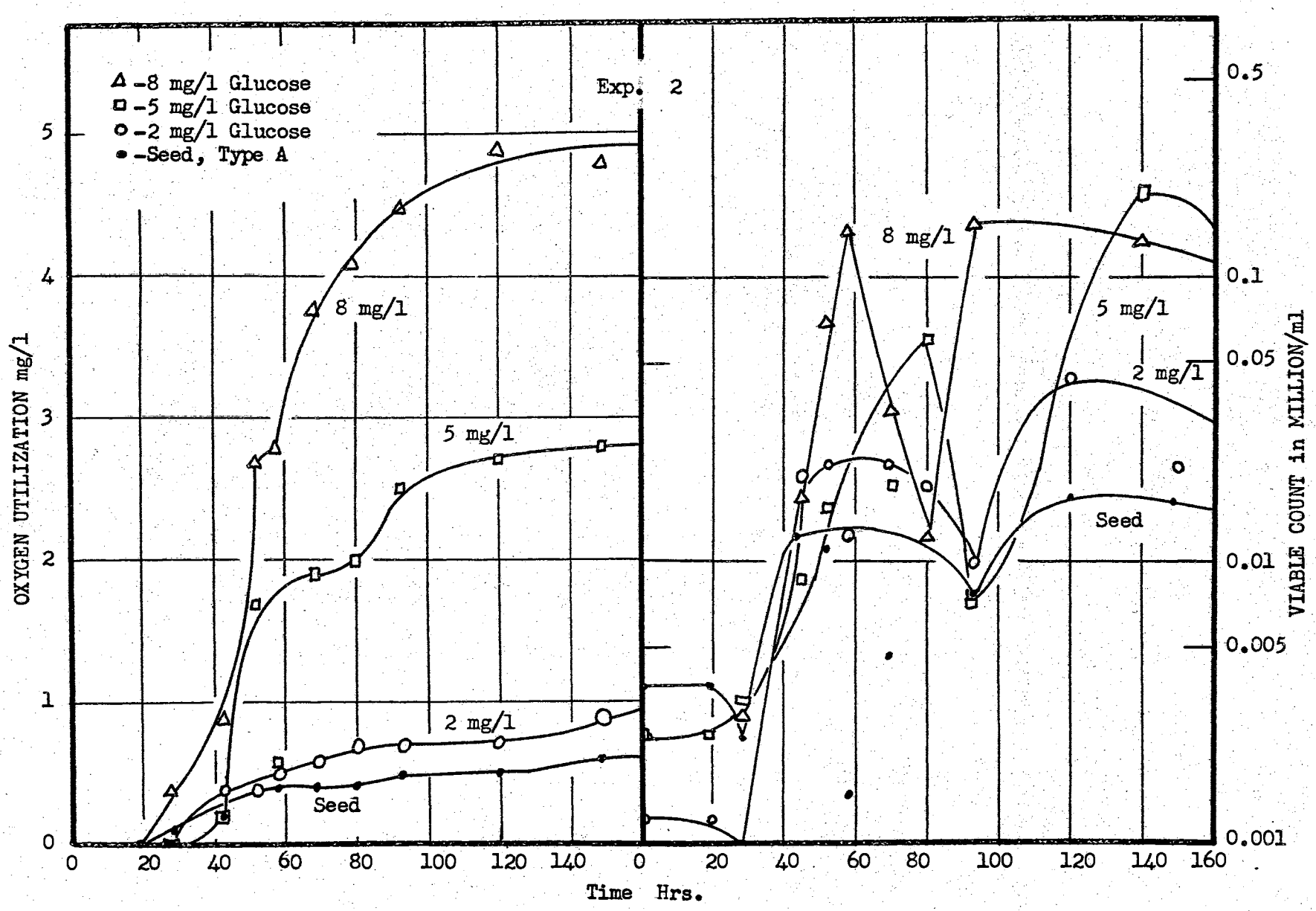

Fig. 3 NET BOD AND VIABLE BACTERIA COUNT, SEED TYPE A

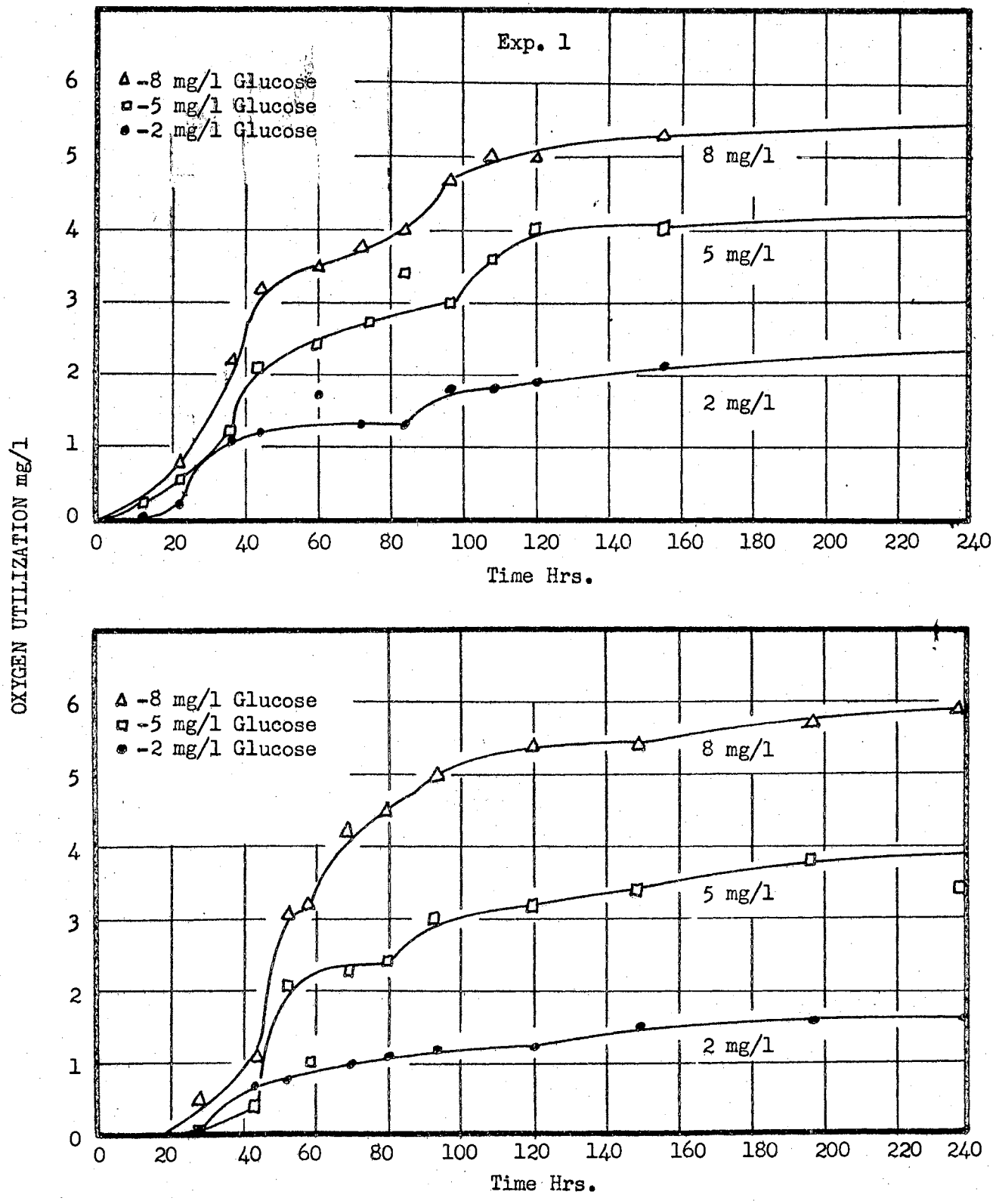

Fig. 4 Exp. 1 & 2 GROSS BOD CURVES

the peak or range of maximum bacterial numbers corresponds to the plateau or the break in oxygen uptake. The rapid increase in cell numbers corresponds to a rapid increase in oxygen uptake. The die off or decrease in cell numbers corresponds to a second rise in oxygen uptake.

Experiment 3 (Fig. 5) was run using 12 mg/l glucose substrate. The initial bacteria population was approximately the same as in the previous two experiments. There is little evidence of a plateau, however, there is a slight change in kinetics at 80 hours. This break corresponds to the point of maximum cell numbers. This curve could easily be drawn as a smooth autocatalytic curve which is usually cited as a typical "lag" BOD curve.

Experiment 4 (Fig. 6) was run using 8 mg/l glucose substrate. Type B seed was used. This seed was obtained from the activated sludge batch unit and washed once in 0.05 M phosphate buffer solution. The initial cell population was approximately 1.0×10^3. This seed produced an oxygen uptake curve similar to the seed used in the previous three experiments. The seed was obtained just prior to the time that substrate was added to the unit. Substrate was added once every 24 hours; therefore, a large amount of substrate carry over would not be expected either in the washed or unwashed sample and both should exhibit similar oxygen uptake curves, which they did. However, it is seen that there was some increase in viable population (approximately 150,000 organism/ml). This aspect will be discussed in a later section. The gross BOD curve exhibited a plateau which occurred at the same time that cell growth reached its maximum density. The seed showed the same characteristics. The second rise in oxygen uptake corresponded to the die off of bacteria. This second rise in the seed caused an apparent decrease in oxygen uptake

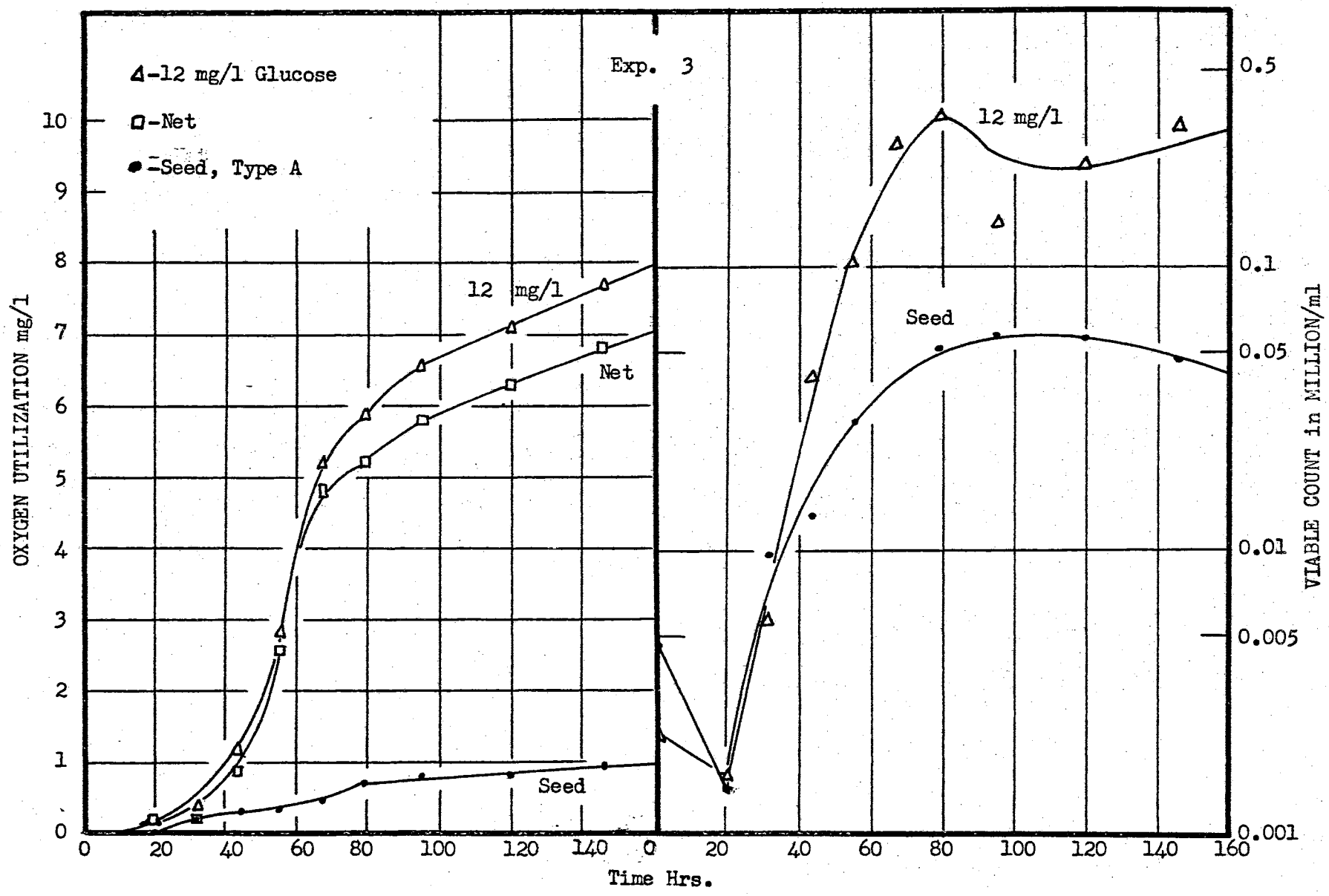

Fig. 5 BOD AND VIABLE BACTERIA COUNT, SEED TYPE A

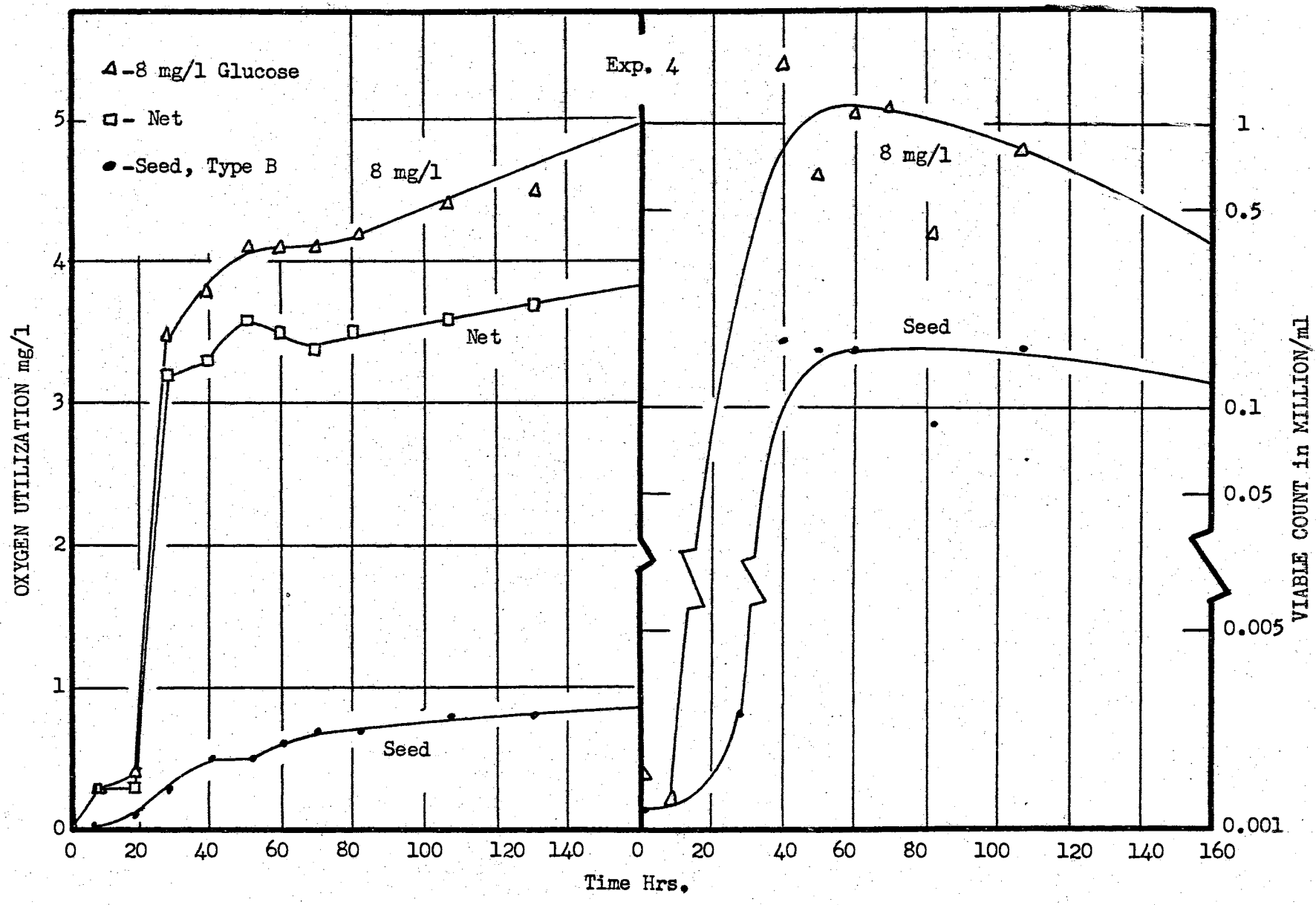

Fig. 6 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

in the net curve.

The major points observed in Part 1 were: (1) substrate concentration does not effect diphasic oxygen exertion; (2) the change in oxygen uptake kinetics or the presence of a plateau corresponds to the range of maximum bacteria density; (3) the rapid oxygen uptake corresponds to the rapid multiplication of bacterial numbers and the second rise in oxygen uptake corresponds to the die off of cells.

Part 2 Effect of Initial Seed Concentration on BOD Kinetics and Bacterial Growth.

This series of experiments (exper. 5 through 10, Fig. 7 through 12) deals with the effect of initial bacteria population in the seed inoculum on the BOD exertion. The initial bacteria population ranged from 5.0×10^2 to 1.0×10^7 organism/ml. In experiments 5 A and 5 B, 12 mg/l glucose substrate was used. The seed material (Type A) for these experiments was obtained at the same time. 0.5 ml/l of seed material was used in experiment 5 B. This yielded an initial population of 5.0×10^2 organixm/ml. In experiment 5 A, 2.5 ml/l seed material was used which yielded 1.9×10^4 organism/ml. Both the low seed and high seed system exhibited the plateau. The high seed system has a shorter lag period than the low seed system. The plateau is in the range of maximum cell numbers. The low initial inoculum systems (seed and seed plus sample) produced more cells than the corresponding high seed system. Reasons for such an occurrence will be discussed in a later section. In experiment 6 (Fig. 8), 12.5 mg/l glucose substrate was used. The initial bacteria population was 3.5×10^6. There was no plateau but a change in kinetics occurred at the point of maximum cell numbers. The large amount of cells in the seed inoculum caused an immediate and rapid up-

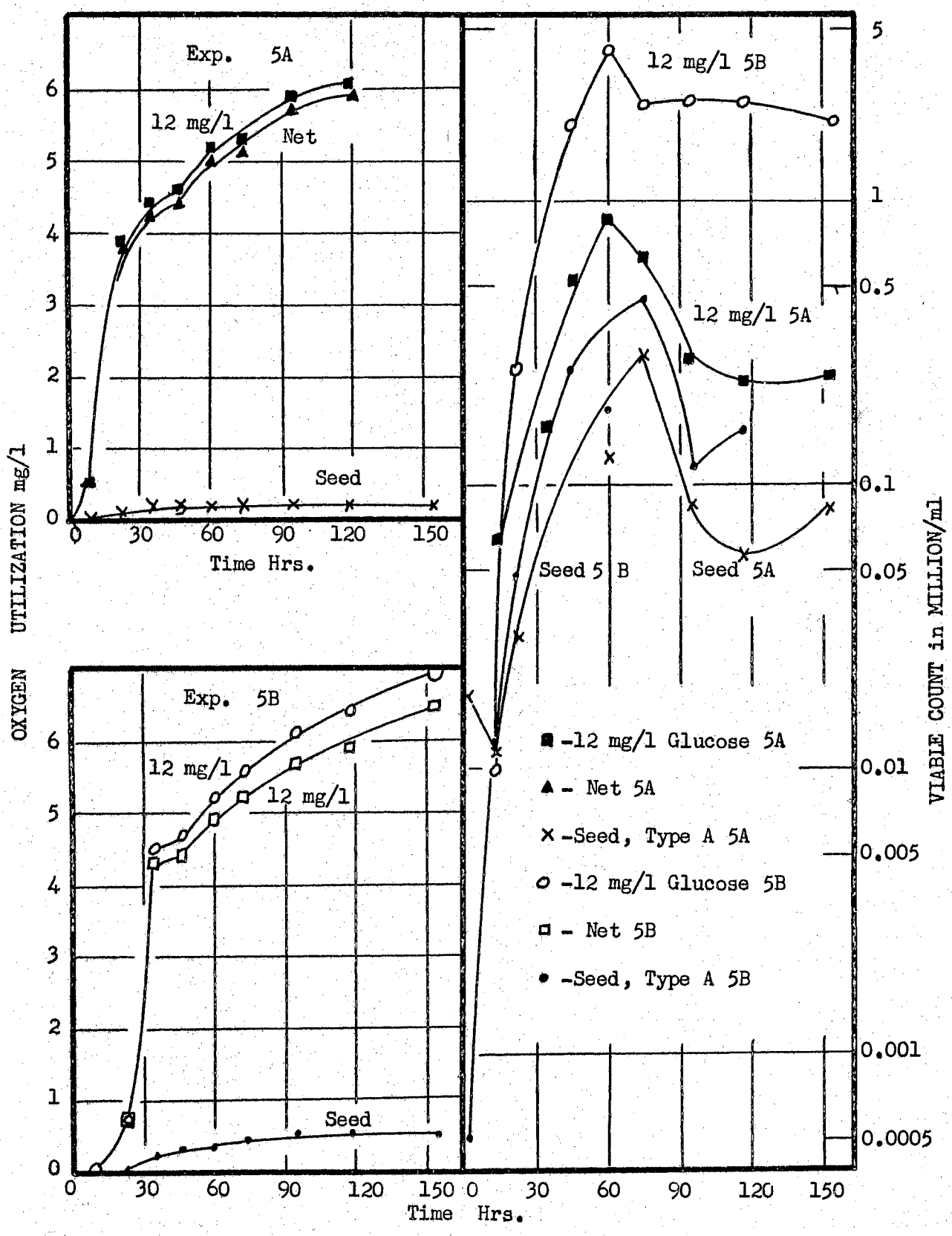

Fig. 7 BOD AND VIABLE BACTERIA COUNT, SEED TYPE A

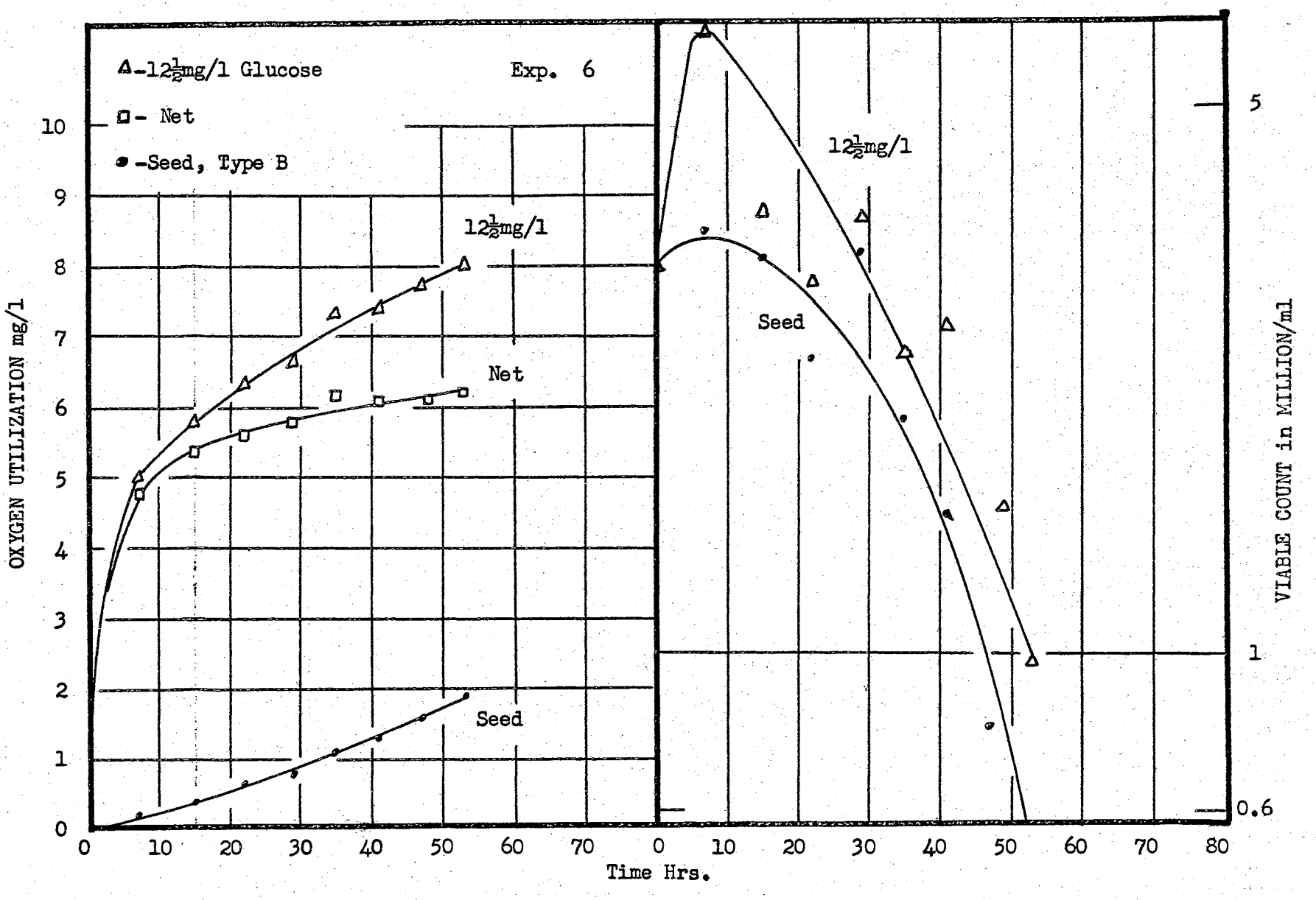

Fig. 8 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

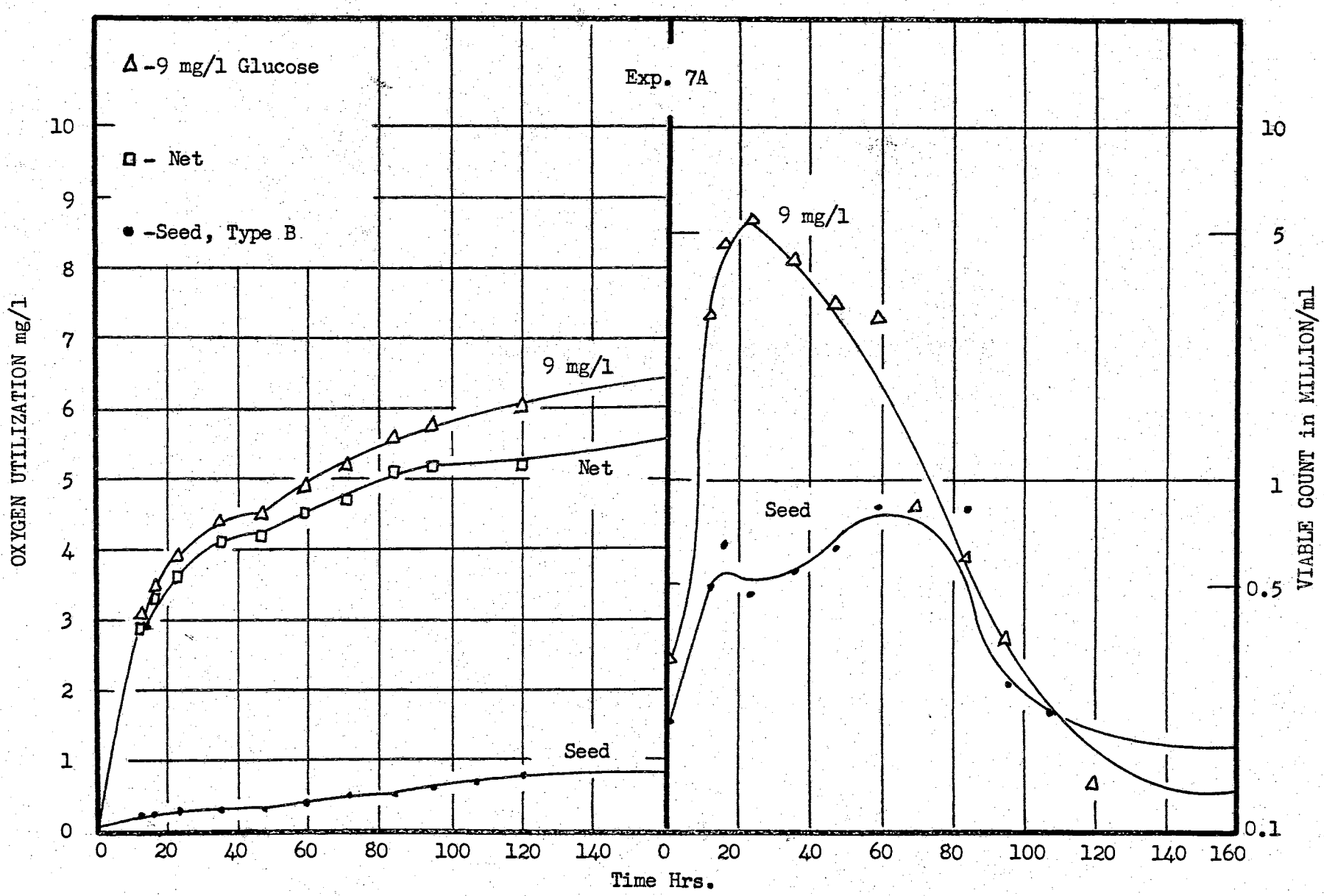

Fig. 9 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

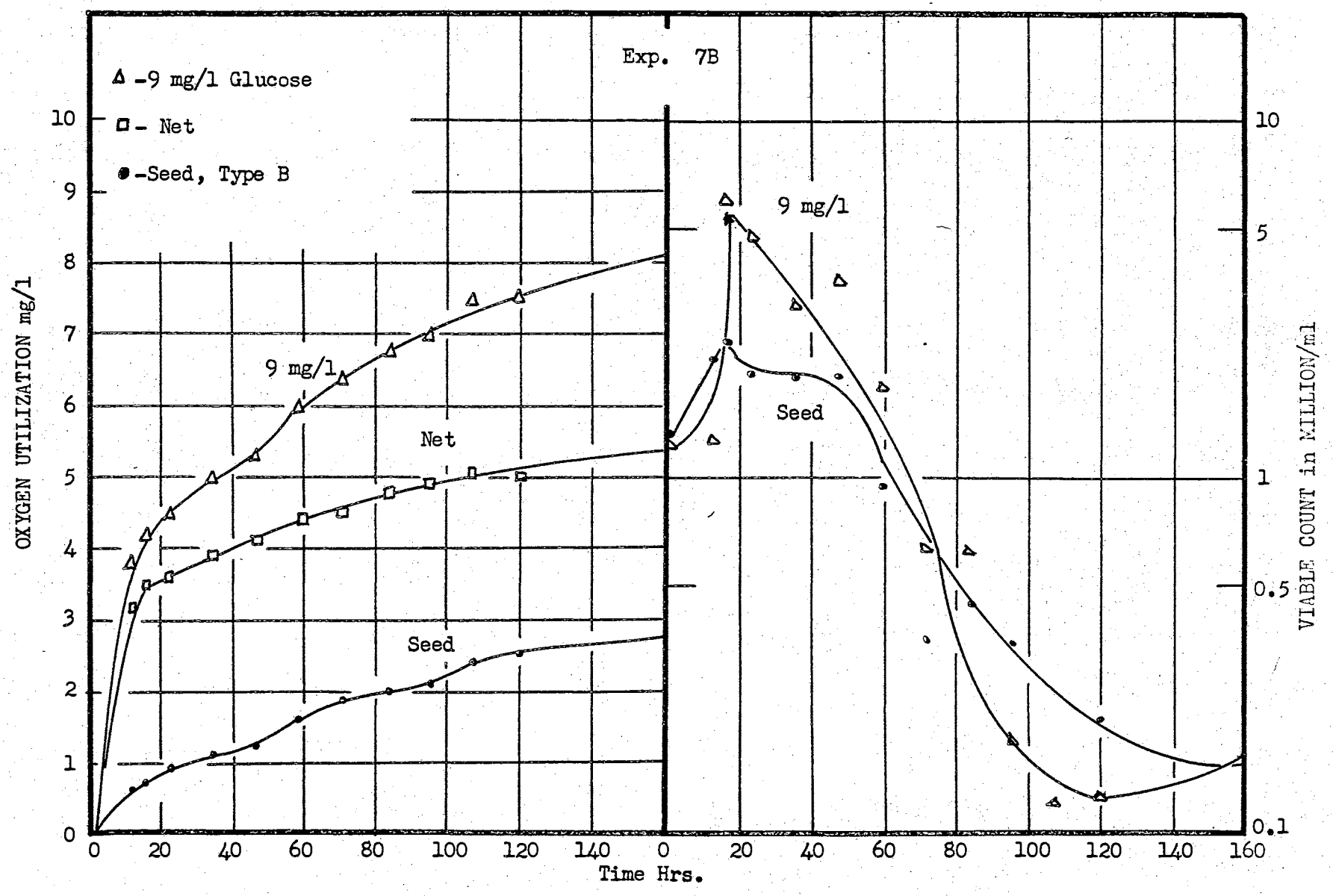

Fig. 10 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

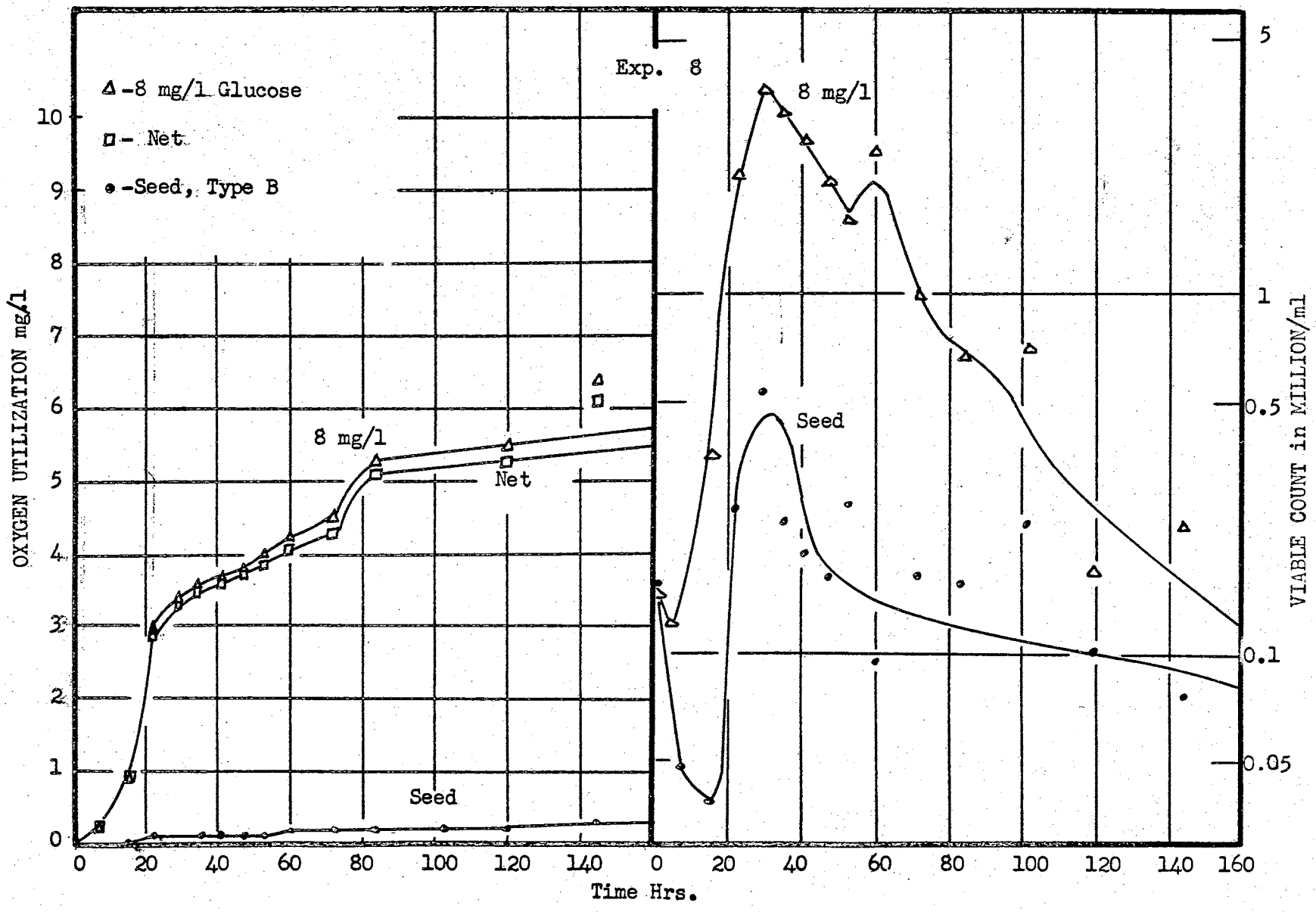

Fig. 11 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

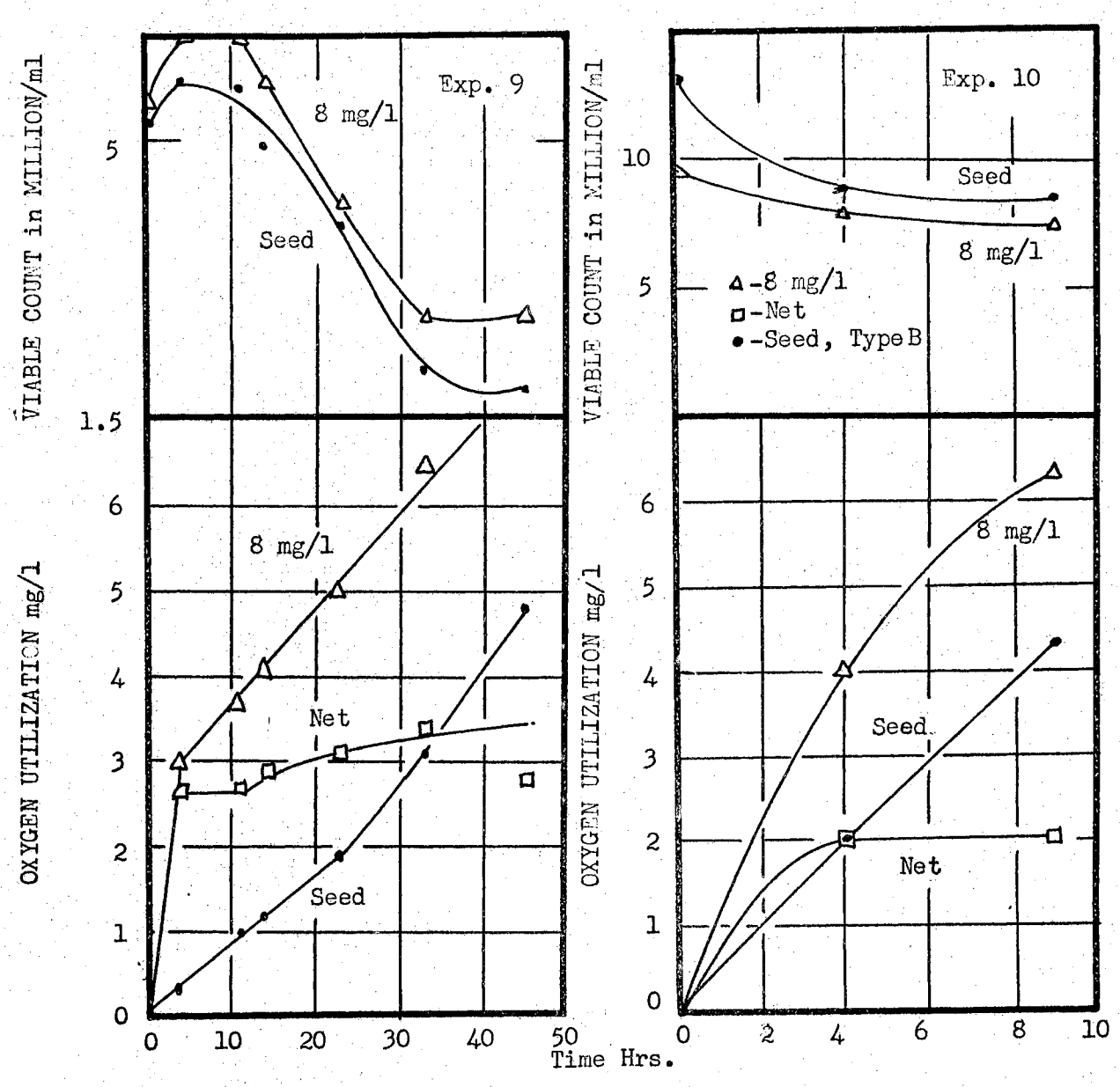

Fig. 12 BOD AND VIABLE BACTERIA COUNT, SEED TYPE C

take of oxygen.

In experiment 7 A and 7 B (Fig. 9 and 10), 9 mg/l glucose substrate was used. An inoculum of 0.5 ml/l seed material (Type B) yielded an initial population of 3.0×10^5 organism/ml in experiment 7 A. The same seed inoculum but at a concentration of 2.0 ml/l produced 1.3×10^6 organism/ml. The difference in cell numbers is shown in both the seed and gross BOD exertion curves, the more cells the greater the oxygen uptake. Both seed BOD curves show multi-phasic oxygen utilization. The gross BOD curves of both the high and low seed plus sample systems exhibited a plateau which corresponds to the range of maximum bacteria population density. The seed exertion characteristics in experiment 7 B masks out the plateau of the sample system. The net curve does exhibit diphasic oxygen uptake with the break at the point of maximum bacteria density.

Experiments 8, 9, and 10 (Fig. 11 and 12) and experiment 4, Part 1 (Fig. 6) have a substrate level of 8 mg/l glucose and cover a wide range of initial bacteria population. Seed type B was used in all four experiments. Initial cell numbers range from 1.0×10^3 to 1.0×10^7. Even though the seed was obtained at different times from the same unit there was a difference in seed activity which can be seen in experiment 4 (Fig. 6) and experiment 8 (Fig. 11). In this case the system with the lower amount of cells used more oxygen. Experiment 8 (Fig. 11) has three phases which correspond to the two peaks in the viable bacteria count curve. The oxygen values at 145 hours were not heavily weighed in drawing the curve since data taken beyond this time (see appendix) indicated that this particular oxygen value was probably in error.

In experiment 9 (Fig. 12) the initial bacteria population was 5.0×10^6 organism/ml. There is a rapid change in kinetics in the range of

maximum cell numbers with the second phase corresponding to a die off of cells. Experiment 10 (Fig. 12) had an extremely high initial seed population, approximately 1.0×10^7, and there was an immediate cell die off. Only three points were obtained before all oxygen in the BOD bottle was used. Using these three points the gross BOD exertion curve appears to be first order decreasing rate. It appears that the substrate present was used entirely for maintenance of the cells. The net oxygen utilization curves in experiments 9 and 10 (Fig. 12) are much below that expected with a substrate concentration of 8 mg/l glucose.

The majority of the glucose substrate experiments were carried out beyond the times shown in the figures. The data beyond that shown is given in the appendix.

The general occurrences observed in Part 2 were: (1) the initial population did not effect diphasic BOD progression as long as there was cell multiplication; (2) the change in kinetics or existance of a plateau occurs in the range of maximum bacterial population density; (3) the rapid oxygen uptake corresponds to the rapid increase of cell numbers and the second phase corresponds to the decrease or die off of cells.

Part 3 BOD Kinetics and Bacterial Growth Using Complex Substrate.

A standard and reproducable complex medium was used in the first studies of the effect of complex substrates. This medium was nutrient broth which consists of Bacto-beef extract and Bacto-peptone.

In experiment 11 (Fig. 13), 0.625 ml/l (5 ml in 8 liters) nutrient broth substrate was used. The nutrient broth solution had a 5 day BOD of 2,400 mg/l and a COD of 6,535 mg/l. In experiment 12 (Fig. 14), 1.875 ml/l (15 ml in 8 liters) nutrient broth was used. This was the

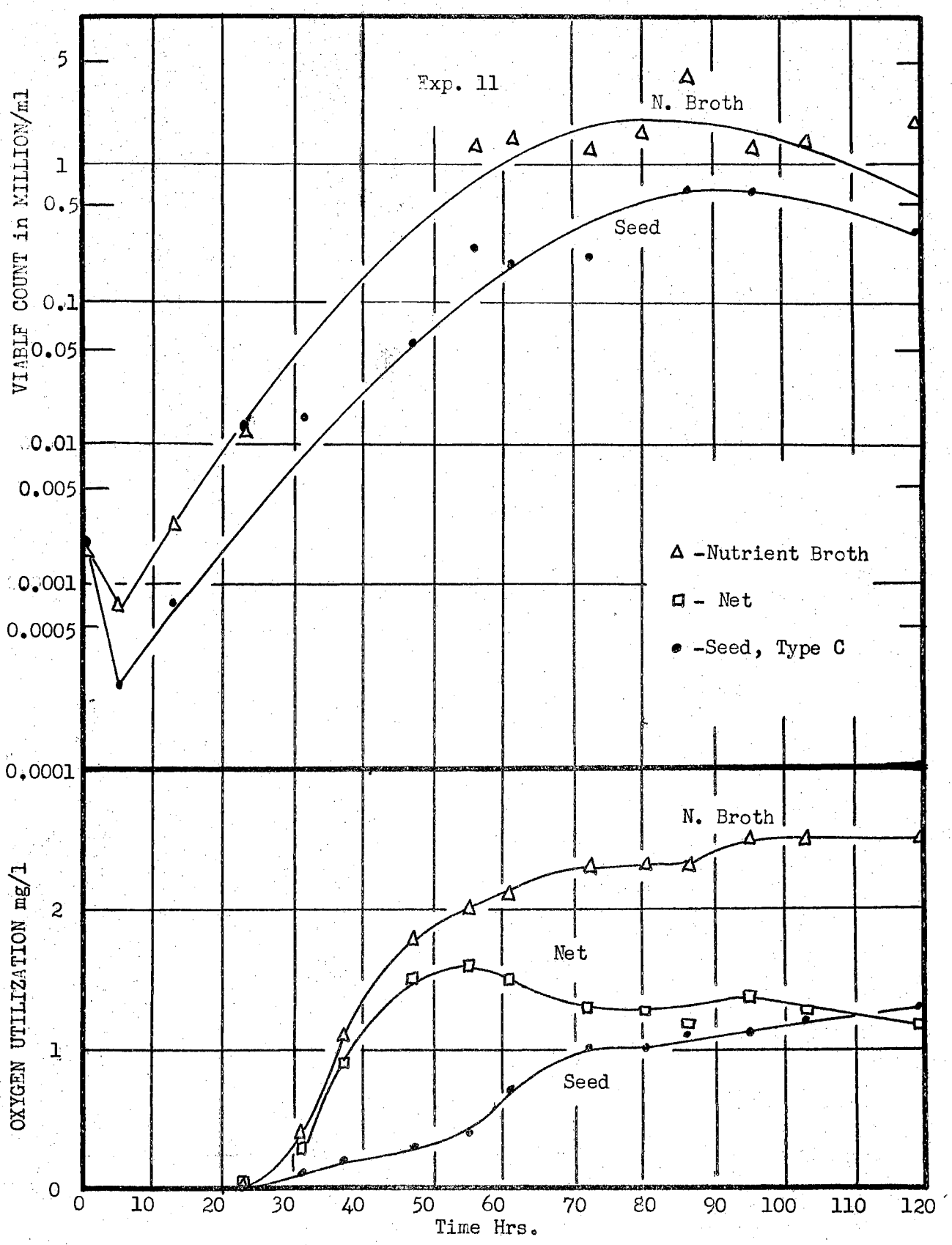

Fig. 13 BOD AND VIABLE BACTERIA COUNT, SEED TYPE C

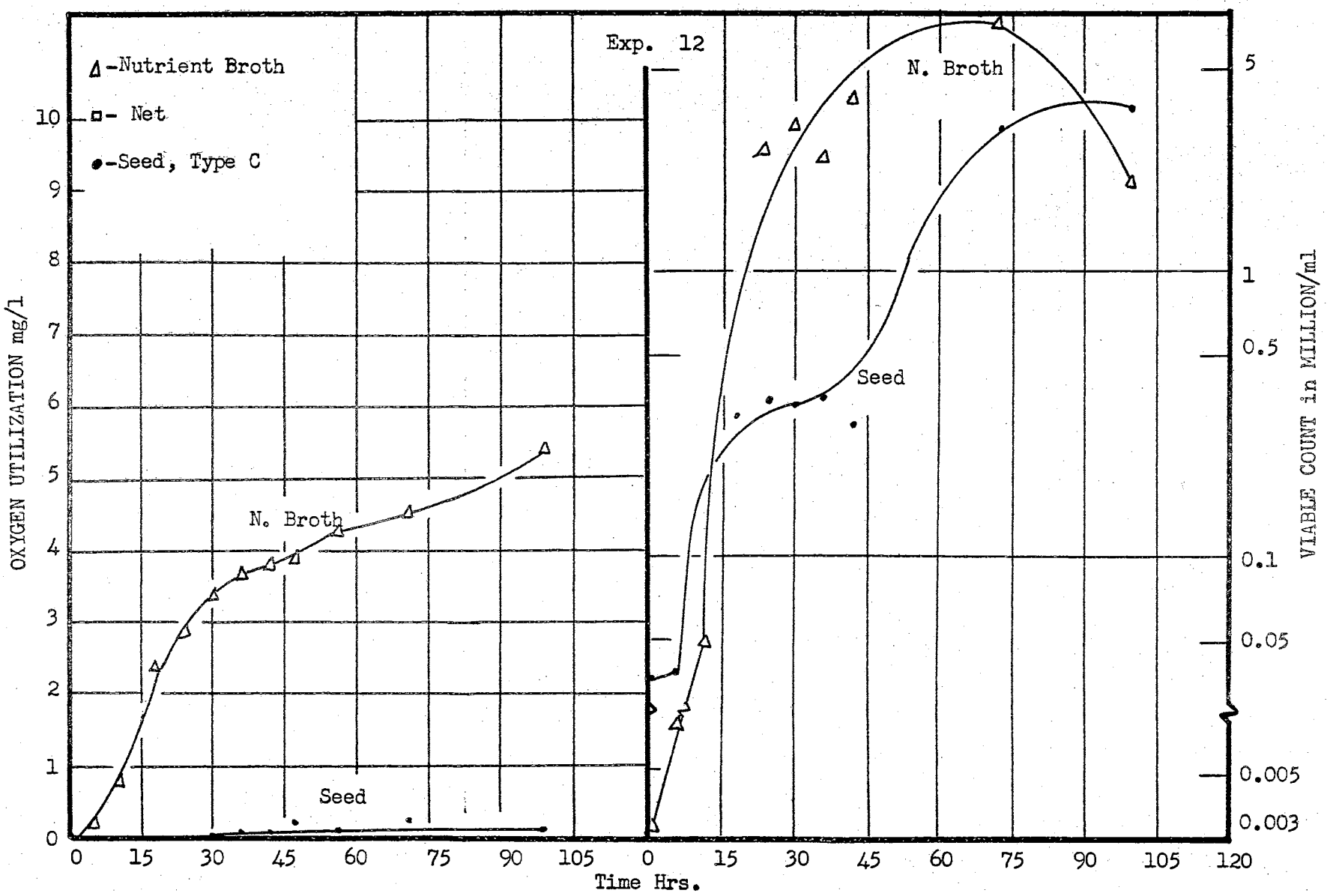

Fig. 14 BOD AND VIABLE BACTERIA COUNT, SEED TYPE C

same nutrient broth that was used in experiment 11. The 5 day BOD could not be obtained because oxygen was depleted by the end of 100 hours. The BOD at 100 hours was 4,270 mg/l. The seed material used was Type C (settled sewage). The initial inoculum in both experiments was 0.25 ml/l which gave a bacteria population in the range of 1×10^3 to 1×10^4 organism/ml. There was a considerable difference in the activity of the seeds. However, these seeds were obtained at different times from the municipal sewage treatment plant and the results obtained were typical of the wide variation in bacterial activity of a domestic sewage. Both experiments have a slight plateau, but the curves could be drawn as smooth autocatalytic curves. The slight plateaus correspond to the range of maximum cell numbers. A sharp rise in the seed curve of experiment 11 (Fig. 13) caused the net curve to have an apparent decrease in oxygen uptake. (This is the reason the 5 day BOD is low. A 5 day BOD test was made at the same concentration of nutrient broth that was used in experiment 11. This gave a 5 day BOD of 4500 mg/l).

In experiment 12 (Fig. 14), 347,000 cell/ml were produced in the seed flasks before any oxygen utilization was shown. However, it has been calculated that production of approximately 5×10^5 cells/ml may be required to yield an oxygen uptake of 0.2 mg/l (35). There is a change in kinetics in the bacterial growth curve of the seed (experiment 12) and possibly a change occurring at the same time in the seed plus sample system.

In experiment 13 (Fig. 15) sterile sewage substrate at a dilution of 40 ml/l (320 ml in 8 liters) was used and seed type C was added in the amount of 0.25 ml/l. The sewage had a 5 day BOD of 155 mg/l. Both the gross and the net oxygen utilization curves show the full gamut of oxygen

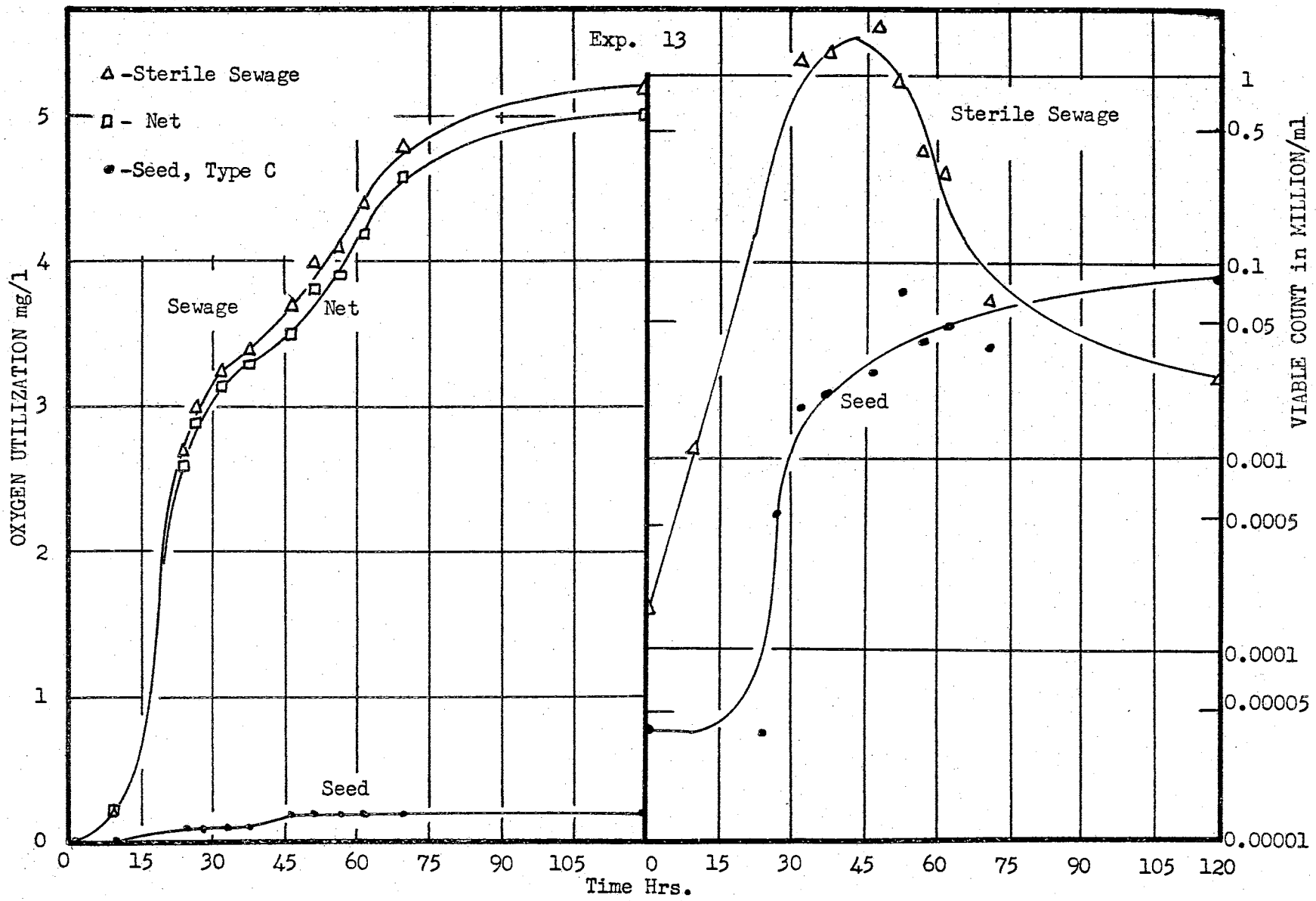

Fig. 15 BOD AND VIABLE BACTERIA COUNT, SEED TYPE C

uptake. There are two autocatalytic portions with the change occurring in the range of maximum cell density. The second phase corresponds to the decrease in cell numbers.

In experiment 14 (Fig. 16) and 15 (Fig. 17) neutral sulfite semichemical pulp liquor was used as the substrate. The neutral sulfite semichemical process is a fairly recent wood pulping process. The waste liquor used in these experiments had been employed to cook the wood pulp but had not undergone any recovery treatment process. This waste had a COD of 337,000 mg/l and a 5 day BOD of 29,360 mg/l. The amount of waste used in both experiments was 0.1875 ml/l (1.5 ml in 8 liters). The initial seed amount was 0.625 ml/l of seed type B. This seed was acclimated to the neutral sulfite semichemical waste liquor and washed once with 0.05 M phosphate buffer solution. The seeding material for both studies was taken form the same activated sludge batch unit but it was harvested at different times. In both experiments the initial inoculum was approximately equal but it is seen that there was a difference in seed activity shown in both the oxygen uptake and bacterial growth data. Both the net and gross oxygen utilization curves in the seed plus sample flasks for either experiment could be drawn as smooth curves although there are slight plateaus in oxygen uptake in both experiments. The plateau in experiment 14 (Fig. 16) is not as readily seen in the gross curve as it is in the net curve. This plateau occurs at the point of maximum cell numbers. The oxygen value at 62 hours was not given the same weight as the other points since it is not on the curve produced by the general trend of oxygen utilization. However, this oxygen value corresponded to the beginning of an increase in cell numbers and could indicate another plateau or change in kinetics. The plateau in experi-

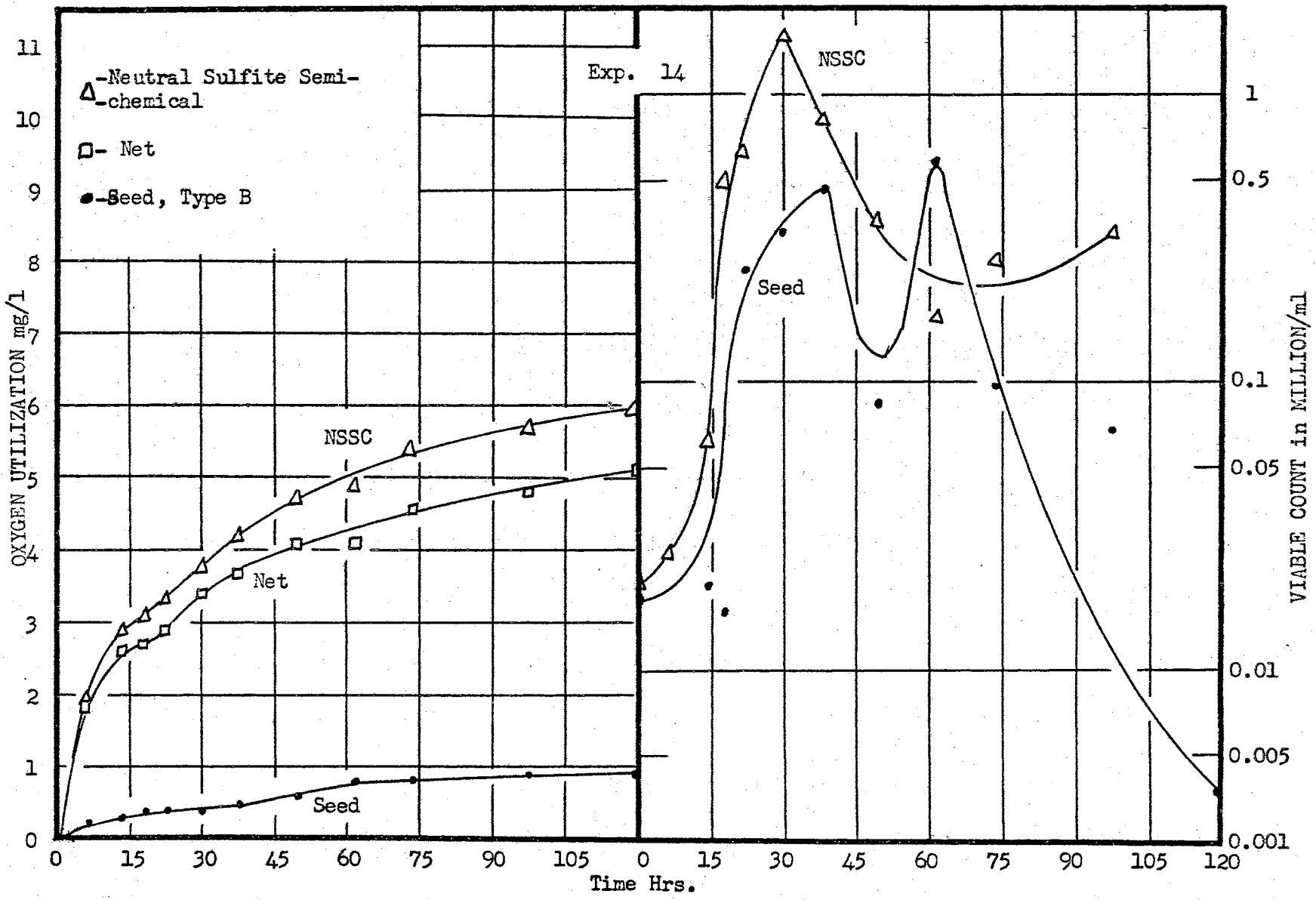

Fig. 16 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

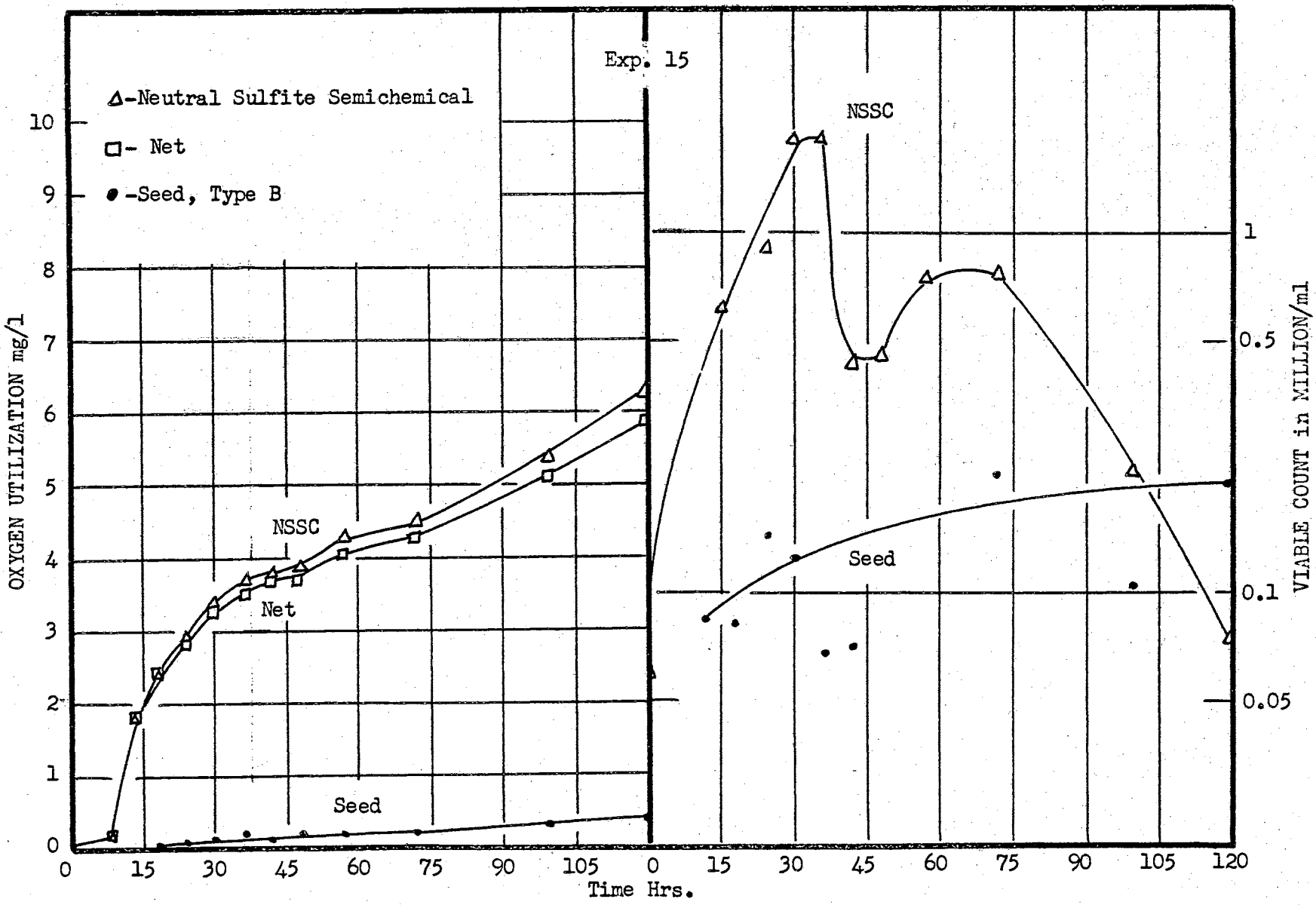

Fig. 17 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

ment 15 (Fig. 17) occurs at the point where cell regrowth begins. Although viable bacteria counts were not complete in experiment 14 an after growth was indicated as in experiment 15 where after growth was definitely shown.

In experiments 16 A (Fig. 18) and 16 B (Fig. 19) Kraft pulp mill waste was used as substrate. Black liquor and total mill effluent (dilute black liquor) were the two wastes used. Black liquor is liquid that is removed after the wood pulp has been digested. The total mill effluent is black liquor that has undergone a recovery process, and effluent from the recovery process is combined with washwater. The seed used in these two experiments was acclimated to the black liquor. It was harvested and washed with 0.05 M phosphate buffer solution. The concentration of the black liquor was 0.1775 ml/l (1.42 ml in 8 liters) which had a COD of 833,000 mg/l and a 5 day BOD of 34,340 mg/l. The oxygen utilization curve (Fig. 18) exhibits a plateau at about the range of maximum cell density. The seed shows very little oxygen uptake. There is a sharp peak in the bacterial count curve at 30 hours, at which time approximately 45,000 cells were produced. During this time there was no oxygen uptake.

Experiment 16 B (Fig. 19) was performed using the same seed material that was used in experiment 16 A. 26.66 ml/l (213 ml in 8 liters) of total mill effluent was added to each liter of dilution water in the sample plus seed system. The COD of this waste was 2520 mg/l and the 5 day BOD was 230 mg/l. The viable bacteria counts exhibit two peaks. The oxygen utilization curve of the sample system shows two autocatalytic curves separated by a plateau. The plateau occurs at the first peak in cell numbers. As bacteria counts increase another autocatalytic oxy-

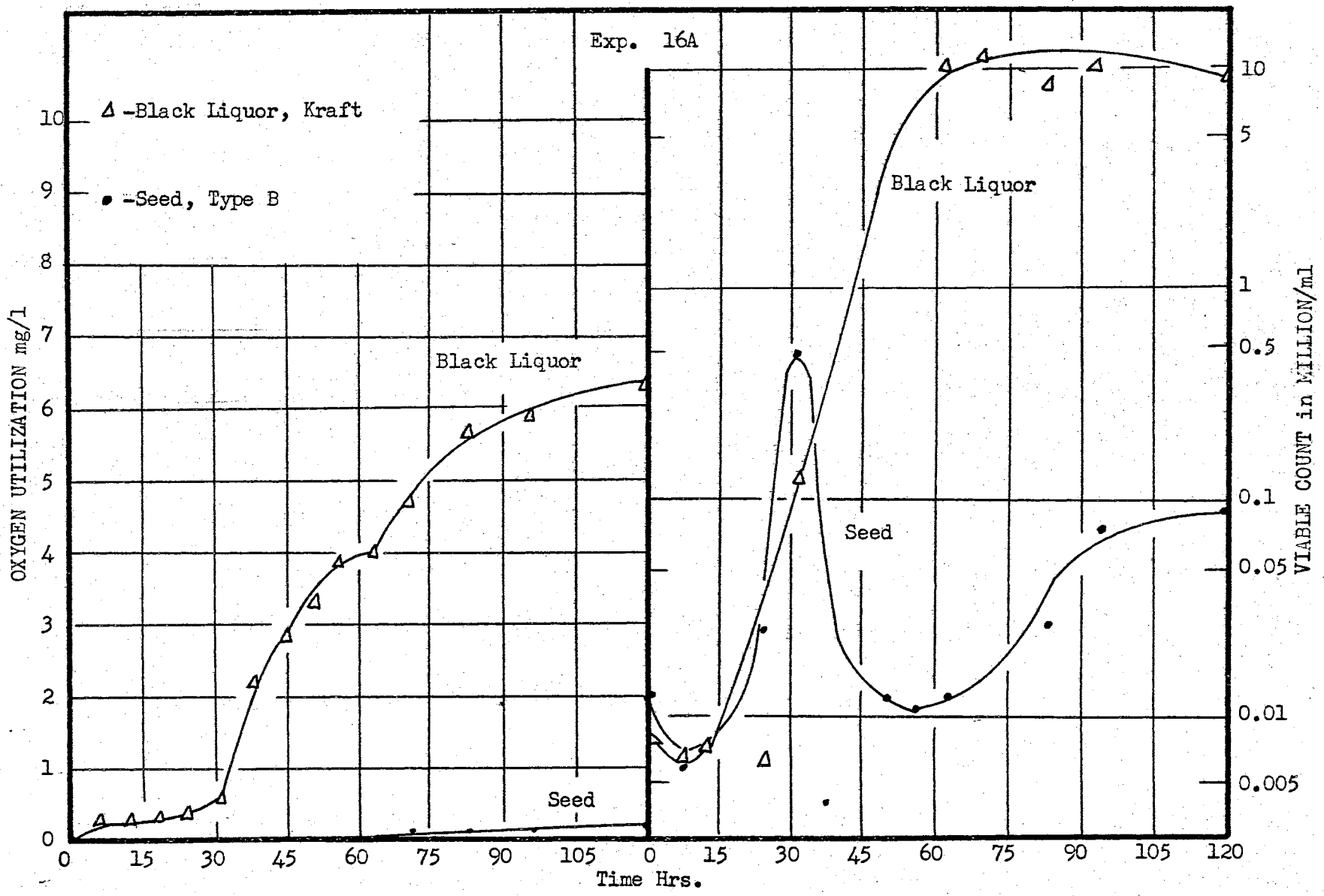

Fig. 18 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

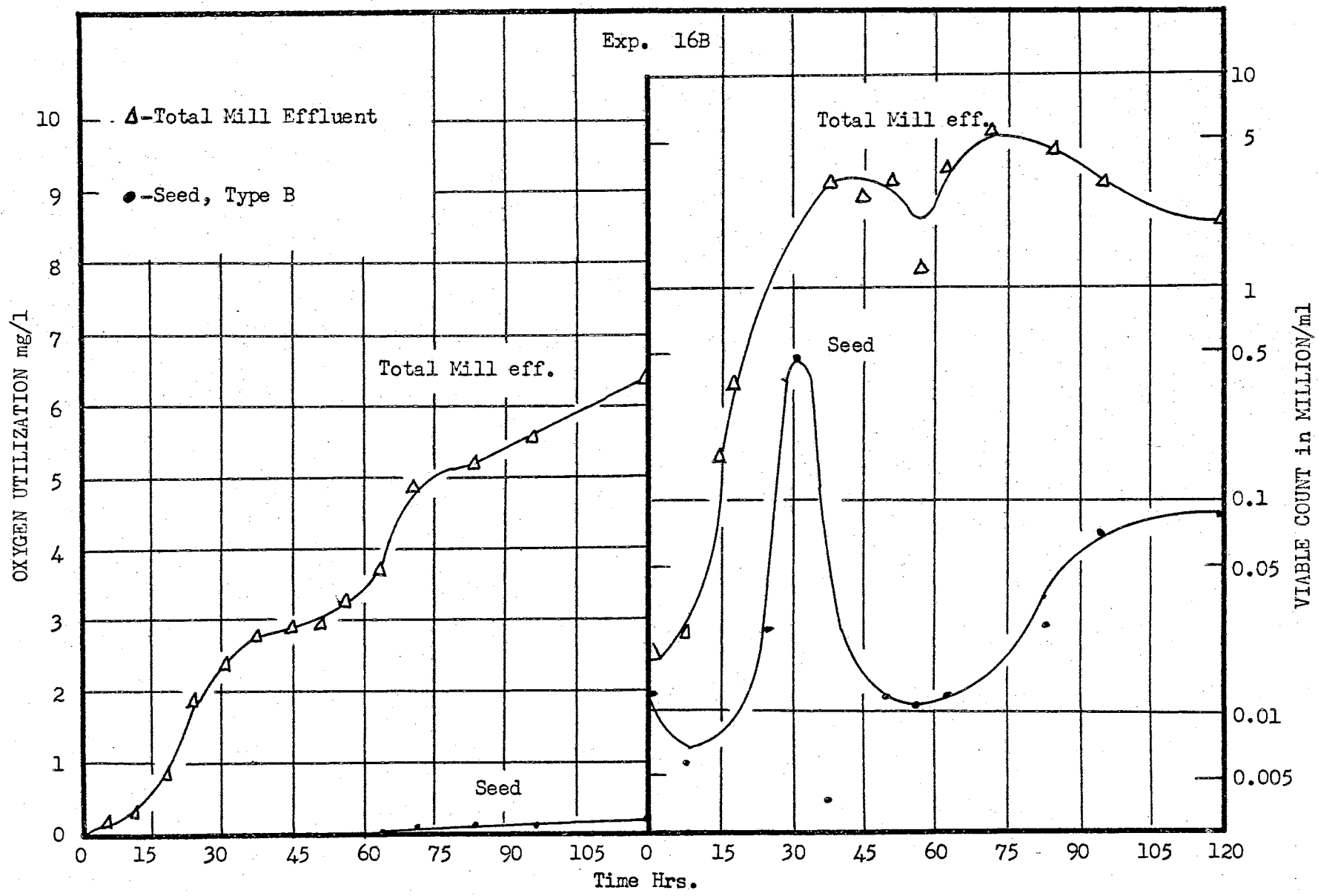

Fig. 19 BOD AND VIABLE BACTERIA COUNT, SEED TYPE B

gen uptake curve occurs. Oxygen uptake increased as the die off of cell progressed.

Experiments of Part 3 show that diphasic oxygen uptake can occur in whole waste which represent fairly complex media as well as with various single carbon sources and mixtures of two pure compounds.

Several investigators have reported that the plateau occurs at about 35 percent of theoretical oxygen demand (28) (33) (36). Table 1 shows the results of this type calculation for the present study. Such a calculation could not be made for the complex waste studies. However the twelve experiments for which such calculations was made the plateau occurred on the average of 37 percent of the theoretical oxygen uptake.

TABLE I

PERCENTAGE OF THEORETICAL OXYGEN DEMAND EXPRESSED AT THE PLATEAU OR CHANGE IN KINETICS OF THE NET BOD CURVE

EXPERIMENT NUMBER	THEORETICAL DEMAND	DEMAND AT CHANGE	PERCENT THEORETICAL	REMARKS
1	8.6	3.1	36	Fig. 2
1	5.4	2.8	42	Fig. 2
1	2.1	+	--	Fig. 2
2	8.6	3.2	37	Fig. 3
2	5.4	1.9	35	Fig. 3
2	2.1	+	--	Fig. 3
3	12.8	5.0	39	Fig. 5
4	8.6	3.2	37	Fig. 6
5A	12.8	4.4	34	Fig. 7
5B	12.8	4.3	33	Fig. 7
6	13.2	4.8	36	Fig. 8
7A	9.6	4.2	44	Fig. 9
7B	9.6	3.5	36	Fig. 10
8	8.6	3.0	35	Fig. 11
9	8.6	+	--	Fig. 12
10	8.6	+	--	Fig. 12
			Aver. 37	

+ No plateau.

CHAPTER IV

DISCUSSION

The results herein presented show that diphasic oxygen uptake, during exertion of carbonaceous BOD, exists in most cases. It was found in these studies that diphasic oxygen uptake occurs regardless of the type and amount of initial bacteria population (as long as cell multiplication occurs), initial substrate concentration, and type carbon source. Other workers have demonstrated the plateau or diphasic oxygen uptake using single carbon sources or mixtures of two carbon sources (28) (29) (34) (36) (37). Very little work has been accomplished using complex synthetic or whole wastes. The results of Part 3 show that the plateau or diphasic oxygen uptake does occur using complex substrates such as nutrient broth, domestic sewage, and pulp mill wastes.

The kinetics of oxygen utilization seem to depend upon bacterial population growth characteristics more than any other factor. No attempt was made to alter the type or concentration of nitrogen source from that recommended in the standard 5 day BOD test. Even though Rao and Busch (39) indicated a deficiency in nitrogen requirements at glucose substrate concentration above 8.06 mg/l, it was felt that the nitrogen level was sufficient in the systems herein studied even in cases where the initial glucose concentration was above 8.06 mg/l. Rao and Busch based their calculations on a 17:1 BOD to nitrogen ratio. Using standard dilution water in which 1 ml of buffer solution per liter of dilution water contains 1.7 mg/l NH_4Cl , the available nitrogen content is 0.445 mg/l.

This would allow a BOD up to 7.55 mg/l. Others have recommended a BOD to nitrogen ratio of 20:1 (42) (43). This ratio would give a BOD of 9.00 mg/l which is approximately the amount of oxygen that can be dissolved in water at 20°C. In making their calculation Rao and Busch used an empirical formula ($C_5H_7NO_2$) for protoplasm which they claim defines cell material "reasonably well". This empirical formula yields 11.4 percent nitrogen in the cell material. Assuming there is 16 percent nitrogen in protein, the cell material would be composed of 71.3 percent protein. Cell material may contain from 30 to 75 percent protein; this would place the protein value in the empirical formula considerably higher than the average (approximately 50%). Therefore, this empirical formula leaves some doubt as to how "reasonably well" it defines cell material. In most systems studied the maximum initial dissolved oxygen was appoximately 8.5 mg/l. This is 0.5 mg/l of useable dissolved oxygen below that which can be expressed as a BOD without causing nitrogen deficiency (using 20:1 BOD to nitrogen ratio). It is interesting to note the number of bacterial cells that could be produced from 0.445 mg/l nitrogen. The weight of a bacterial cell (coccus or a normal rod) may be estimated as 2×10^{-10} milligrams (35). If 50 percent of the cell is composed of protein and the nitrogen content in protein is 16 percent, the following calculations can be made:

2×10^{-10} mg/cell x 50% protein x 16% nitrogen = 0.16×10^{-10} wt. of nitrogen in cell.

0.445 mg/l nitrogen $\div$ 0.16×10^{-10} mg nitrogen/cell = 2.78×10^{10} cell/l = 2.78×10^{7} cell/ml.

The maximum number of cells produced or used in any of the glucose substrate experiments was 1×10^{7}. Based upon these considerations it was felt that nitrogen concentration and that of other salts did not serious-

ly affect the bacterial growth characteristics. With all factors held constant except the substrate concentration, seed material, and carbon source it was shown that the plateau or change in kinetics corresponds to the range of maximum bacteria population.

In some systems there was a lag in oxygen uptake even though the bacteria population was rapidly multiplying. Gaudy, Bhatla, and Abu-Niaaj (35) have calculated that it may take 5×10^5 organism/ml to yield an oxygen uptake of 0.2 mg/l. This is generally found to be true both in seed and sample plus seed systems. A rapid increase in cell numbers (log growth phase) corresponds to a rapid rise in oxygen uptake. A second rise in oxygen uptake corresponds to the die off of cells.

It appears that substrate was needed for maintenance in the systems with a high initial bacteria population. The systems with very high initial seed inoculum had a net oxygen utilization much below that of systems having the same amount of substrate but " normal " amount of initial seed material. This was shown in experiments 9 and 10 (Fig. 12). This could be the reason that more cells were produced in the sample plus seed system with a low initial seed inoculum (experiment 5 B, Fig. 7) than in the system with a higher initial seed inoculum (experiment 5 A, Fig. 7). More substrate was needed for maintenance in the more heavily seeded system, and therefore less substrate was available for the energy required to produce new cells. However this does not fully explain why the lower initial inoculum in the seed system grew to a higher popluation density than the seed in the higher initial population system.

The results of experiments 5 A and 5 B (Fig. 7) and 7 A (Fig. 9)

and 7 B (Fig. 10) in which lower initial inocula than that used for experiments 9 and 10 show that even though there were different amounts of growth in the seed dilution flasks the initial seed population had little affect on the net oxygen utilization curves. The net oxygen uptake curve in experiment 5 A is almost identical to the net oxygen uptake curve in experiment 5 B, and the same is true of experiments 7 A and 7 B. However seed oxygen uptake does not always exhibit the same kinetic characteristics as the oxygen uptake in the seed plus sample systems. In some cases the correction for seed oxygen depletion masks the plateau exhibited in the gross oxygen uptake curves. In other cases the correction for seed oxygen depletion produces a plateau or even produces an apparent decrease in oxygen utilization that is not exhibited in the seed plus sample system oxygen utilization curve. Standard Methods (10) recommends " setting up a series of seed dilutions and selecting those resulting in 40-70 percent oxygen depletions in 5 days." One of these is used to calculate the correction due to the small amount of seed in the dilution water. To obtain 40 to 70 percent oxygen depletion in 5 days without any substrate available would require a very large bacteria population. Therefore, if the Standard Methods recommendation was followed the seed oxygen depletion would be made for a large endogenously respiring bacteria population. Using this recommendation the ratio of amount of seed in the seed plus sample system to amount of seed in the seed control system is applied to the oxygen utilized by the seed control system. The figure obtained is subtracted from the oxygen utilized in the seed plus sample system to give the BOD of the waste. Considering the many varied types of kinetics that occur in the seed systems and the affect of unusually high initial seed inoculum, it would seem best

to start with a small number of cells which would use little or no oxygen in the seed system so that the oxygen utilization obtained from the seed plus sample system would be attributed only to the waste being studied and not be affected by oxygen utilized by an endogenously respiring bacteria system. More research needs to be done on endogenous respiration at low population densities in order to resolve various aspects of the seed subtraction procedure. In addition considerable research effort should be expended using natural populations in order to determine the consistency of endogenous respiration with and without the presence of an exogenous carbon source.

It was generally shown by the results of these studies that the plateau or change in kinetics of oxygen uptake occurs in the range of maximum bacteria density. It also was shown that the longer period of time that the bacterial numbers remained near the maximum population density the longer the duration or length of the plateau. Gaudy et al. (37) also have indicated this relationship.

It is interesting to note that the plateau was smaller in the high substrate concentration systems than in the lower substrate concentration systems. This trend was also observed by McWhorter and Heukelekian (33) for Warburg studies using very much higher substrate concentrations than those employed in the BOD test. The kinetics and mechanisms of high energy systems such as those used in the Warburg apparatus have always seemed to hold for low energy systems such as those in the BOD bottle. The results reported herein do not prove or disprove the above statement. However they do indicate than more research needs to be done to define the kinetic and possibly the mechanistic relationship between high and low energy systems.

The net oxygen uptake data (see appendix) from the 8 mg/l glucose substrate run of experiment 1 were plotted on semilogarithmic paper (Fig. 20) in order to show the various kinetic phases which may exist during exertion of BOD. There are five phases shown. Four of the phases were first order or approached first order and the other phase was the plateau. The first phase was first order increasing rate which corresponds to the log growth of bacteria. This phase was followed by a portion that approaches first order decreasing rate kinetics. The third phase was the plateau which was followed by two first order decreasing rate curves. This curve shows that the kinetics of BOD exertion could not be defined by just one mathematical relationship as is usually attempted. The relationship that defines the carbonaceous BOD as one continous first order decreasing rate curve needs to be reveiwed and changes made so that a relationship that truly expresses BOD exertion kinetics may be formed.

The results of the studies using complex substrates were in general similar to the studies using glucose substrate. The plateau or diphasic oxygen uptake has not often been observed in studies employing complex substrates. However, there have been enough such reports to warrant serious consideration of revised kinetic concepts of BOD exertion. Hoover, Jasewicz, and Porges (24) observed diphasic oxygen uptake using milk waste as substrate. Busch (28) observed the plateau using domestic and synthetic sewage. (More detail is given on these investigations in the Literature Reveiw section.) Eariler researchers such as Theriault (2) and Butterfield (19) used river water, sewage, and single carbon source compounds and did not observe diphasic oxygen uptake. This was probably due to many reasons. Among these the following may be cited:

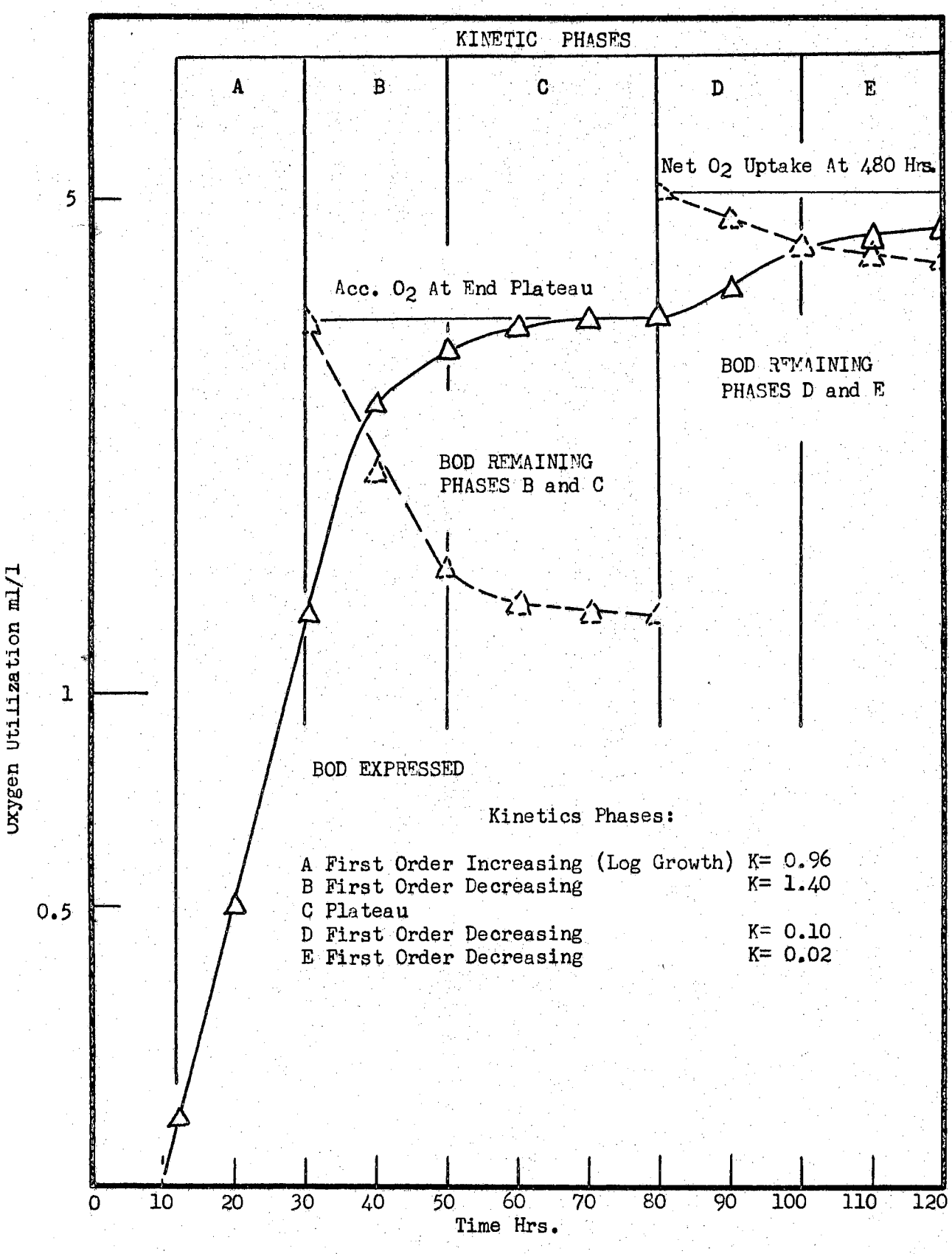

Fig. 20 Exp. 1 BOD EXERTION, 8 mg/l GLUCOSE SUBSTRATE, SEED TYPE A

waste characteristics have changed over the last sixty years; the use of dilution water that did not provide optimum bacterial growth condition because the needed inorganic salts were not available in the dilution waters used; dissolved oxygen determinations were made in most cases at 24 hour intervals which would make it possible to overlook the plateau because data were not taken when the plateau occurred.

The results of the studies on complex media (Part 3) can be divided into two groups: those with a well defined plateau and those with a slight plateau. It was shown that the range of maximum bacteria population corresponded either to a change in kinetics or the plateau. The nutrient broth and neutral sulfite semichemical pulp waste exhibit a slight plateau but for all practical purposes the curves could be drawn as smooth curves. The domestic sewage and two Kraft mill wastes exhibit well defined plateaus. The number of compounds in a waste seem to have little affect on the plateau. The domestic sewage which had a well defined plateau probably contained the most compounds of the four complex wastes studied. It certainly contained more than the Kraft mill wastes which exhibited a plateau. It would seem that the type of compounds present have the greatest effect on the occurrence of the plateau rather than the number of compounds present.

Gaudy and Bhatla (37) have given four related theories explaining the existence of the plateau. The first theory assigns one cause for the plateau to be due to the change in predominating organisms. However, they explained that this cannot be the general cause of the plateau because studies using pure cultures have also exhibited the plateau (34) (36).

The second theory cites the cause of the plateau to be due to an

acclimation period. Because of the different structural configuration of the original substrate and released cellular components such as lysis products an acclimation period may be required to synthesize new enzymes to metabolize the new substrates.

Another possibility which is given in the third theory is that an acclimation period is needed before the cells can use certain storage products for energy. The length of the plateau and its existence in a system in which cells do not die but lose their ability to replicate would depend upon the nature of the storage products and the induction time required for producing the necessary enzymes.

An acclimation period required for the induction of enzymes to initiate metabolism of new exogenous compounds is the explanation of the plateau given in the fourth theory. The new exogenous compounds were produced during rapid metabolism of the original carbon source. Some cells release intermediates into the medium rather than channeling all the substrate into storage products.

In evaluating their results the above authors felt that the change in kinetics is due to a period in which the cells acclimated to stored intracellular products or to exogenous by-products after the exogenous carbon source or sources have been utilized. Concerning the decrease in viable bacteria count during the second stage of oxygen uptake, the amount of by-products or stored intracellular products are probably not sufficient to maintain the large number of cells produced by the original substrate, therefore, a decrease in cell numbers occurs. This decrease in cell numbers does not necessarily indicate a decrease in cell respiration or activity. This is seen in the occurrence of a rapid rise in oxygen uptake after the plateau.

The present study was not concerned with the further deliniation of generalized theory of causation of the plateau; therefore, the biochemical mechanism or mechanisms responsible for the plateau or change in kinetics cannot be determined beyond that which can be postulated from observed oxygen uptake and population dynamics. And on this basis the author is in general accord with the theories of causation given by Gaudy and Bhatla (37).

CHAPTER V

SUMMARY AND CONCLUSIONS

The effect of substrate concentration, effect of initial bacteria population and effect of complex wastes on the diphasic nature of BOD progression and on bacterial growth were studied. From these studies the following conclusions were made.

1. The occurrence of diphasic oxygen uptake in BOD progression is independent of the type substrate used. The type compounds present rather than the number of compounds seem to have some effect on the length or occurrence of a plateau.

2. Under seeding conditions where there is an increase in cell numbers, the first phase of oxygen uptake corresponds in general to a log growth phase of cell multiplication, the change in kinetics of oxygen uptake corresponds to the period of maximum cell number, (the duration of the plateau or change in kinetics depends on the period of time the cell numbers remain near the maximum population), the second phase of oxygen uptake corresponds to the die off of cells.

3. It would seem best to use a low initial cell population inoculum in the BOD test so that the seed blank would have little or no oxygen uptake and all oxygen uptake could be attributed to that of the sample system.

4. The mechanism or mechanisms causing the plateau or change in kinetics could not be clearly delinated from the results reported herein.

CHAPTER VI

SUGGESTIONS FOR FURTHER WORK

To obtain more information from which a better understanding of BOD exertion can be developed, the following research suggestions are given:

1. Research is needed to define the relationship between oxygen uptake, cell population, predominating species, and substrate removal in low energy systems such as in the BOD bottle.

2. It may be advisable to accomplish work with biological systems having a lower energy level than normally used in the BOD test. This work may help define the relationship between the utilization of endogenous and exogenous carbon and may allow greater precision in making the seed subtraction.

3. The mechanistic and kinetic relationship between high and low energy biological systems needs to be investigated.

A SELECTED BIBLIOGRAPHY

1. O'Brien, W. J. and J. W. Clark, "The History Development of the Biochemical Oxygen Demand Test." New Mexico State Univ. Eng. Exp. Sta. Bull. No. 20 (Jan. 1962).

2. Theriault, E. J., "The Oxygen Demand of Polluted Waters. I. A Critical Review. II. The Rate of Deoxygenation." Pub. Health Bull., 173 (1927).

3. Phelps, E. B., "The Disinfection of Sewage and Sewage Filter Effluents." U.S. Geol. Survey Water Supply Paper, 229, 74 (1909).

4. _____, Stream Sanitation, John Wiley and Sons, New York, N. Y. (1944).

5. Butterfield, C. T., "Studies of Natural Purification in Polluted Water. II. Development of Suitable Dilute Medium." Pub. Health Rept., 44, 2647 (1929).

6. Heukelekian, H., and N. S. Chamberlain, "The Effect of the Dilution Water on the Biochemical Oxygen Demand Determination." Sewage Works Jour., 3, 187 (April 1931).

7. Holderby, J. M., and W. L. Lea, "The Relation of the pH of Dilution Water to the Biochemical Oxygen Demand of Various Wastes." Sewage Works Jour., 6, 734 (July 1934).

8. Lea, W. L., and M. S. Nichols, "Influence of Substrate on Biochemical Oxygen Demand." Sewage Works Jour., 8, 435 (May 1936).

9. Ruchhoft, C. C., "Report on the Cooperative Study of Dilution Waters Made for the Standard Methods Committee of the Federation of Sewage Works Associations." Sewage Works Jour., 13, 669 (July 1941).

10. Standard Methods for the Examination of Water and Wastewater, 11th Ed., A.P.H.A., Inc. N. Y. (1960).

11. Heukelekian, H., "The Influence of Nitrifying Flora, Oxygen and Ammonia Supply on the Nitrifying of Sewage." Sewage Works Jour., 14, 964 (Sept. 1942).

12. Ruchhoft, C. C., O. R. Placak, and M. B. Ettinger, "Variations in BOD Velocity Constants of Sewage Dilutions." Ind. and Eng. Chem., 40, 1290 (July 1948).

13. Lea, W. L., and M. S. Nichols, "Influence of Phosphorus and Nitrogen on Biochemical Oxygen Demand." Sewage Works Jour., 9, 34 (Jan. 1937).

14. Ruchhoft, C. C., O. R. Placak, and M. B. Ettinger, "Correction of BOD Velocity Constants for Nitrification." Sewage Works Jour., 20, 832 (Sept. 1948).

15. National Research Council Subcommittee on Sewage Treatment, "Sewage Treatment at Military Installations. II. Characteristics of Military Sewage." Sewage Works Jour., 18, 830 (May 1946).

16. Eldridge, E. F., "Studies of Biochemical Oxygen Demand of Trade Wastes." Eng. News-Rec., 105, 12 (July 1939).

17. Sawyer, C. N., and A. W. Williams, "The Solution of a Dilution Water for the Determination of the BOD of Industrial Wastes." Sewage Works Jour., 14, 1000 (Sept. 1942).

18. Mills, E. J., Jr., and V. T. Stack, Jr., "Acclimation of Microorganisms for the Oxidation of Pure Organic Compounds." Ninth Ind. Waste Conf., Purdue Univ., Proceedings, 87, 492 (1954).

19. Butterfield, C. T., "Studies of Natural Purfication in Polluted Waters. III. A Note on the Relation Between Food Concentration in Liquid Media and Bacteria Growth." Pub. Health Rept., 44, 2864 (1929).

20. _____, W. C. Purdy, and E. J. Theriault, "Studies of Natural Purification in Polluted Water. IV. The Influence of Plankton on the Biochemical Oxidation of Organic Matter." Pub. Health Rept., 46, 393 (1931).

21. _____, and E. Wattie, "Studies of Sewage Purfication. VIII. Observations on the Effect of Variation in the Initial Numbers of Bacteria and the Dispersion of Sludge Flocs on the Coarse of Oxidation of Organic Matter by Bacteria in Pure Culture." Pub. Health Rept., 53, 1912 (1938).

22. Heukelekian, H., "Effects of Dilution of Sewage Bacteria Numbers." Sewage Works Jour., 5, 774 (Sept. 1934).

23. Tidwell, W. L., and J. H. Sorrels, "The Effect of Bacterial Flora on Deoxygenation." Sew. and Ind. Wastes, 27, 167 (Feb. 1955).

24. Hoover, C. B., L. Jasewicz, and N. Porges, " An Interpretation of the BOD Test in Terms of Endogenous Respiration of Bacteria." Sew. and Ind. Wastes, 25, 1163 (Oct. 1953).

25. Buswell, A. M., H. F. Muller, and I. Van Meter, " Bacterialogical Explanation of Rates of oxygen Consumption in the BOD Test." Sew. and Ind. Wastes, 26, 276 (Mar. 1954).

26. Zehnpfennig, R., and M. S. Nichols, "Inoculation Studies in BOD Determination on Sewage and Pulp Mill Waste." Sew. and Ind. Wastes, 25, 61 (Jan 1953).

27. Lea, W. L., and M. S. Nichols, "Influence of Substrate on the Biochemical Oxygen Demand." Sewage Works Jour., 3, 434 (May 1936).

28. Busch, A. W., "BOD Progression in Soluble Substrate." Sew. and Ind. Wastes, 30, 1336 (Nov. 1953).

29. _____, and N. Myrick, "Aeorbic Bacteria Degradation of Glucose." Jour. Water Poll. Control Fed., 33, 897 (Sept. 1961).

30. Myrick, N., and A. W. Busch, "The Selective Stimulation of Respiration in Mixed Cultures of Bacteria and Protozoa." Jour. Water Poll. Control Fed., 32, 741 (July 1960).

31. Tsivoglou, E. C., "Discussion - Stream Data Applied to Waste Treatment Plant Design." Oxygen Relationships in Stream, Tech. Rept., W58-2, USPHS (1958).

32. Lee, E. W., and W. J. Oswald, "Effects of Seeding on BOD." Water and Waste Treatment Jour., 1, 400 (1959).

33. McWhorter, T. R., and H. Heukelekian, "Growth and Endogenous Phases in the Oxidation of Glucose." 1st Int. Conf. on Water Poll. Res., London (1962).

34. Wilson, I. S., and M. E. Harrison, "The Biochemical Treatment of Chemical Wastes." Jour. Inst. Sewage Purfi., 3, 261 (1960).

35. Gaudy, A. F., Jr., M. N. Bhatla, and F. Abu-Niaaj, "Studies on the Occurrence of the Plateau in BOD Exertion." Presented at the 18th Industrial Waste Conference, Purdue University(May 1963).

36. _____,_____, and _____, "Studies on the Occurrence of the Plateua in BOD Exertion. I. Heterogeneous and Pure Culture Seeds with Various Concentrations of Glucose as Carbon Source." Submitted to Jour. of the Water Poll. Control Federation (1963).

37. _____, and _____, "Studies on the Occurrence of the Plateau in BOD Exertion. II. Experiments Using Different Carbohydrates and Multicomponent Systems." Submitted to Jour. of the Water Poll. Control Federation (1963).

38. Abu-Niaaj, F. N., "Study of the Velocity of Expression of the BOD." Unpublished Masters Report, Oklahoma State University (1962).

39. Rao, T. S., and A. W. Busch, "BOD Progression in Soluble Substrates. V. TOD Acceleration with Log-phase Seeding. " Presented at the 17th Annual Industrial Waste Conference, Purdue University (May 1962).

40. Gaudy, A. F., Jr., F. Abu-Niaaj, and E. T. Gaudy, "A Statistical Study of the Spot Plate Technique for Cell Counts." Applied Microbiology (In Press).

41. McKinney, R. E., H. E. Langley and H. D. Tomlinson, "Survival of Solmonelia typhosa during Anaerobic Digestion. I. Experimental Methods and High Rate Digester Studies." Sew. and Ind. Wastes, 30, 1469 (Dec. 1958).

42. Eckenfielder, W. W., Jr., and D. J. O'Connor, Biological Waste Treatment. Peramon Press, New York (1961).

43. Rich, L. G., Unit Processes of Sanitary Engineering. John Wiley & Sons, New York (1963).

APPENDIX

TABLE I

DISSOLVED OXYGEN AND BOD DATA, EXPERIMENT 1

	SEED		2 mg/l			5 mg/l			8 mg/l		
TIME	DO	BOD	DO	BOD	NET	DO	BOD	NET	DO	BOD	NET
0	7.6	0	7.9	0	0	7.9	0	0	8.0	0	0
12	7.6	0	7.9	0	0	7.7	0.2	0.2	7.8	0.2	0.2
22	7.5	0.1	7.7	0.2	0.1	7.4	0.5	0.4	7.2	0.8	0.7
36	7.4	0.2	6.8	1.1	0.9	6.7	1.2	1.0	5.8	2.2	2.0
44	---	---	6.7	1.2	---	5.8	2.1	---	4.8	3.2	---
60	7.4	0.2	6.2	1.7	1.5	5.5	2.4	2.2	4.5	3.5	3.3
72	7.0	0.6	6.6	1.3	0.7	5.2	2.7	2.1	4.2	3.8	3.2
84	7.0	0.6	6.6	1.3	0.7	4.5	3.4	2.8	4.0	4.0	3.4
96	7.1	0.5	6.1	1.8	1.3	4.9	3.0	2.5	3.3	4.7	4.2
108	7.1	0.5	6.1	1.8	1.3	4.3	3.6	3.1	3.0	5.0	4.5
120	7.0	0.6	6.0	1.9	1.3	3.9	4.0	3.4	3.0	5.0	4.4
155	7.0	0.6	5.8	2.1	1.5	3.9	4.0	3.4	2.7	5.3	4.7
264	6.9	0.7	5.6	2.3	1.6	3.8	4.1	3.4	2.5	5.5	4.8
420	6.8	0.8	5.4	2.5	1.7	3.4	4.5	3.7	2.2	5.8	5.0
480	6.8	0.8	5.3	2.6	1.8	3.3	4.6	3.8	2.0	6.0	5.2

TABLE II

VIABLE BACTERIA COUNTS, EXPERIMENT 1*

	SEED			2 mg/l			5 mg/l			8 mg/l		
TIME	N	COLONIES PER PLATE	ORGANISM PER ML X 1000	N	COLONIES PER PLATE	ORGANISM PER ML X 1000	N	COLONIES PER PLATE	ORGANISM PER ML X 1000	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	3	1	12.5	3	4	43.8	3	1	12.5	3	1	12.5
12	3	1	18.7	3	1	6.25	3	0	----	3	9	106
22	3	4	56.4	3	4	50.0	3	6	75.0	3	9	106
36	2	42	52.5	2	65	81.3	2	103	129	3	45	56.3
44	-	TMC	----	2	51	63.8	-	TMC	----	3	125	1560
60	3	5	61.5	2	118	231	3	92	1150	3	68	850
72	2	220	276	3	80	1000	3	200	2500	3	186	2320
84	-	TMC	----	-	TMC	----	3	65	815	3	85	1060
96	1	280	35.0	3	67	850	3	125	1560	4	5	625
108	2	120	150	3	90	1120	3	61	764	4	8	940
120	2	66	82.5	3	40	500	3	35	438	3	45	563
155	2	280	350	3	25	313	3	30	375	3	32	400
264	2	100	125	3	15	188	3	20	250	3	55	688
420	2	115	144	3	20	250	3	35	438	3	70	877
480	2	95	119	3	18	225	3	27	338	3	85	1060

* N is equal to the dilution factor (10^{-N}). TMC- Too Many To Count.

TABLE III

DISSOLVED OXYGEN AND BOD DATA, EXPERIMENT 2

	SEED		2 mg/l			5 mg/l			8 mg/l		
TIME	DO	BOD	DO	BOD	NET	DO	BOD	NET	DO	BOD	NET
0	8.2	0	8.2	0	0	8.2	0	0	8.2	0	0
19	8.2	0	8.2	0	0	8.2	0	0	8.2	0	0
28	8.1	0.1	8.1	0.1	0	8.1	0.1	0	7.7	0.5	0.4
43	8.0	0.2	7.5	0.7	0.4	7.8	0.4	0.2	7.1	1.1	0.9
52	7.8	0.4	7.4	0.8	0.4	6.1	2.1	1.7	5.4	3.1	2.7
58	7.8	0.4	7.3	0.9	0.5	7.2	1.0	0.6	5.0	3.2	2.8
69	7.8	0.4	7.2	1.0	0.6	5.9	2.3	1.9	4.0	4.2	3.8
80	7.8	0.4	7.1	1.1	0.7	5.8	2.4	2.0	3.7	4.5	4.1
93	7.7	0.5	7.0	1.2	0.7	5.2	3.0	2.5	3.2	5.0	4.5
120	7.7	0.5	7.0	1.2	0.7	5.0	3.2	2.7	2.8	5.4	4.9
149	7.6	0.6	6.7	1.5	0.9	4.8	3.4	2.8	2.8	5.4	4.8
197	7.6	0.6	6.6	1.6	1.0	4.6	3.8	3.2	2.5	5.7	5.1
238	7.6	0.6	6.6	1.6	1.0	4.8	3.4	2.8	2.3	5.9	5.3
310	7.5	0.7	6.4	1.8	1.1	4.3	3.9	3.2	2.1	6.1	5.4
387	7.5	0.7	6.4	1.8	1.1	4.2	3.9	3.2	2.1	6.1	5.4
480	7.5	0.7	6.4	1.8	1.1	4.2	3.9	3.2	2.1	6.1	5.4

TABLE IV

VIABLE BACTERIA COUNT, EXPERIMENT 2

TIME	SEED N*	SEED COLONIES PER PLATE	SEED ORGANISM PER ML X 1000	2 mg/l N	2 mg/l COLONIES PER PLATE	2 mg/l ORGANISM PER ML X 1000	5 mg/l N	5 mg/l COLONIES PER PLATE	5 mg/l ORGANISM PER ML X 1000	8 mg/l N	8 mg/l COLONIES PER PLATE	8 mg/l ORGANISM PER ML X 1000
0	2	3	3.76	2	1	1.25	2	2	2.50	2	2	2.50
19	2	3	3.76	2	1	1.25	2	2	2.50	-	-	----
28	1	19	2.38	1	8	1.00	1	27	3.38	1	23	2.88
43	1	101	12.6	1	168	20.9	1	69	8.60	2	136	17.0
52	1	90	11.3	2	22	22.5	1	125	15.6	2	56	70.0
58	1	12	1.50	2	10	12.5	-	---	----	3	12	150
69	1	38	4.75	2	18	22.5	2	15	18.8	3	2	25.0
80	1	99	12.4	2	15	18.8	2	50	62.5	3	1	12.5
93	1	62	7.80	2	8	10.0	2	6	7.50	2	126	157
120	1	140	17.0	1	360	45.0	-	TMC	----	-	TMC	---
149	2	13	16.2	2	180	21.5	2	160	200	2	110	137
197	2	9	11.2	2	25	31.2	2	125	157	2	220	275

* N is equal to the dilution factor (10^{-N}). TMC - Too Many To Count.

TABLE IV CONTINUED

238	2	11	13.8	2	20	25.0	2	77	96.2	2	100	125
310	1	80	10.0	2	28	35.0	2	110	138	2	128	160
387	1	50	6.25	2	19	23.8	2	52	65.0	2	130	162
480	1	36	4.50	2	13	16.2	2	22	27.7	2	14	17.5

TABLE V

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 3

	SEED						12 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.6 8.6	0	1	39 37	4.75		8.6 8.5	0	0	1	18 18	2.25
19	8.6 8.6	0	1	12 9	1.44		8.4 8.4	0.2	0.2	1	14 12	1.62
31	8.4 8.4	0.2	1	56 100	9.65		8.2 8.2	0.4	0.2	1	47 45	5.75
43	8.3 8.3	0.3	1	115 95	13.1		7.4 7.4	1.2	0.9	1	246 240	30.4
55	8.3 8.3	0.3	1	228 230	27.8		5.7 5.8	2.8	2.6	2	95 70	103
67	8.2 3.1	0.5	-	TMC	----		3.4 3.3	5.2	4.8	2	200 240	275
79	7.9 7.9	0.7	2	60 23	52.0		2.7 2.7	5.9	5.2	2	240 320	350
95	7.8 7.8	0.8	2	39 53	57.5		2.0 2.0	6.6	5.8	2	101 125	142
120	7.8 7.6	0.8	2	58 40	56.5		1.3 1.7	7.1	6.3	2	250 160	236
146	7.7 7.6	0.9	2	38 43	47.2		0.8 1.1	7.8	6.8	3	14 40	310

*N is equal to the dilution factor (10^{-N}). TMC- Too Many To Count.

TABLE V CONTINUED

194	7.5 7.5	1.1	2	29 29	33.4	0.7 0.5	8.0	6.9	3	23 20	253
269	7.4 7.4	1.2	2	13 22	26.0	0.2 0.2	8.4	7.2	3	11 29	250
339	7.3 7.4	1.3	2	11 16	16.9	0.1 0.1	8.5	7.2	2	80 144	137

TABLE VI

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 4

	SEED						8 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.5	0	1	10 8	1.12	8.5	0	0	1	11 13	1.50
8	8.5	0	0	64 --	0.80	8.2	0.3	0.3	0	101 ---	1.26
18	8.4	0.1	-	TMC	---	8.1	0.4	0.3	-	TMC	----
28	8.2	0.3	0	200	2.50	5.0	3.5	3.2	-	TMC	----
40	8.0	0.5	2	110 120	187	4.7	3.8	3.3	3	144 124	1680
51	8.0	0.5	2	124 126	157	4.4	4.1	3.6	2	540 ---	675
60	7.9	0.6	2	121 130	156	4.4	4.1	3.5	3	90 86	1100
70	7.8	0.7	-	TMC ---	---	4.4	4.1	3.4	3	86 100	1160
82	7.8	0.7	2	72 70	88.9	4.3	4.2	3.5	3	30 37	418
107	7.6	0.8	2	128 130	161	4.1	4.4	3.6	3	63 82	813
131	7.6	0.8	-	---	---	4.0	4.5	3.7	-	TMC	---
210	7.4	1.1	2	148 150	186	3.1	5.4	4.3	3	27 23	313
384	7.4	1.1	2	120 142	164	1.9	6.6	5.5	2	35 40	47.0

* N is equal to the dilution factor (10^{-N}). TMC - Too Many To Count.

TABLE VII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 5 A

	SEED						12 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.0	0	2 3	15 1	18.8		7.9	0	0	-	---	----
12	8.0	0	2	9 10	11.9		7.4	0.5	0.5	2	52 52	65.0
23	7.9	0.1	1	320 148	29.3		4.0	3.9	3.8	2	256 144	250
35	7.8	0.2	-	TMC ---	----		3.5	4.4	4.2	2	120 136	160
46	7.8	0.2	-	TMC ---	----		3.3	4.6	4.4	3	44 40	525
60	7.8	0.2	2	95 106	126		2.7	5.2	5.0	3	69 73	883
74	7.8	0.2	3	20 26	288		2.6	5.3	5.1	3	47 53	625
95	7.8	0.2	2	68 65	83.1		2.1	5.9	5.7	2	230 214	278
118	7.8	0.2	2	39 51	56.4		1.8	6.1	5.9	2	180 201	238
154	7.8	0.2	2	68 63	82.0		---	---	---	2	200 197	248

* N is equal to the dilution factor (10^{-N}). TMC - Too Many To Count.

TABLE VIII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 5 B

	SEED						12 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.0	0	1 3	4 1	0.50		8.0	0	0	-	-	---
12	8.0	0	2	10 10	12.5		8.0	0	0	2	8 8	10.0
23	8.0	0	2	44 33	48.3		7.3	0.7	0.7	2	256 153	256
35	7.8	0.2	-	-- --	----		3.5	4.5	4.3	-	--- ---	---
46	7.7	0.3	2	202 ---	253		3.3	4.7	4.4	3	148 ---	1850
60	7.7	0.3	2	150 ---	188		2.8	5.2	4.9	3	250 300	3440
74	7.6	0.4	3	35 38	456		2.4	5.6	5.2	4	12 23	2190
95	7.5	0.5	2	113 86	124		1.8	6.2	5.7	3	171 188	2240
118	7.5	0.5	2	193 203	156		1.6	6.4	5.9	3	188 169	2230
154	7.5	0.5	2	--- ---	---		1.0	7.0	6.5	3	151 160	1920

* N is equal to the dilution factor (10^{-N}). TMC - Too Many To Count.

TABLE IX

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 6

	SEED						12.5 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	3 4 4	280 4 1	3500	8.1	0	0	3 4 4	280 26 37	3500
7	8.0	0.2	4	36 28	4000	3.1	5.0	4.8	4	67 55	7640
15	7.8	0.4	3	304 281	3660	2.3	5.8	5.4	3	320 360	4250
22	7.5	0.7	3	208 212	2630	1.8	6.3	5.6	3	230 308	3360
29	7.4	0.8	4	29 31	3760	1.5	6.6	5.8	4	26 41	4180
35	7.1	1.1	4	15 20	2190	0.8	7.3	6.2	4	16 27	2680
41	6.9	1.3	4	10 15	1570	0.7	7.4	6.1	4	22 25	2940
73	6.6	1.6	3	63 63	788	0.4	7.7	6.1	3	129 128	1610
53	6.3	1.9	3	42 47	556	0.1	8.0	6.2	3	74 82	975

* N is equal to the dilution factor (10^{-N}). TMC- Too Many To Count.

TABLE X

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 7 A

	SEED						9 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.3	0	2	161	201		8.3	0	0	2	151	310
12	8.1	0.2	3	39	494		5.2	3.1	2.9	3	236	2950
16	8.1	0.2	3	55	657		4.8	3.5	3.5	3	370	4640
23	8.0	0.3	3	36	456		4.4	3.9	3.6	4	44	5510
35	8.0	0.3	3	45	557		3.9	4.4	4.1	4	35	4190
47	8.0	0.3	3	50	632		3.8	4.5	4.2	4	25	3190
59	7.9	0.4	3	66	825		3.4	4.9	4.5	4	23	2880
71	7.8	0.5	3	50	625		3.1	5.2	4.7	3	67	845
84	7.8	0.5	3	51	637		2.7	5.6	5.1	3	48	606
95	7.7	0.6	3	20	256		2.5	5.8	5.2	3	28	356
107	7.6	0.7	2	117	212		---	---	---	-	--	---
120	7.5	0.8	-	---	---		2.3	6.0	5.2	3	10	138
161	7.5	0.8	2	138	172		1.9	6.4	5.6	2	172	149
209	7.5	0.8	2	167	208		1.7	6.6	5.8	2	154	134
257	7.5	0.8	2	80	99.5		1.2	7.1	6.3	2	---	---

* N is equal to the dilution factor (10^{-N}). + Average of Two Plates.

TABLE XI

DISSOLVED CXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 7 B

	SEED					9 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	3	107	1340	8.2	0	0	3	100	1240
12	7.6	0.6	3	169	2110	4.4	3.8	3.2	3	103	1280
16	7.5	0.7	4	19	2380	4.0	4.2	3.5	4	49	6140
23	7.3	0.9	3	158	1970	3.7	4.5	3.6	4	38	4760
35	7.1	1.1	3	150	1930	3.2	5.0	3.9	3	247	3080
47	7.0	1.2	4	16	1940	2.9	5.3	4.1	4	28	3560
59	6.6	1.6	3	78	948	2.2	6.0	4.4	4	14	1810
71	6.3	1.9	3	28	350	1.8	6.4	4.5	3	51	643
84	6.2	2.0	3	36	444	1.4	6.8	4.7	3	50	625
95	6.1	2.1	3	27	344	1.2	7.0	4.9	3	15	181
107	5.8	2.4	2	83	103	0.7	7.5	5.1	2	96	122
120	5.7	2.5	2	167	210	0.7	7.5	5.0	2	102	128
161	5.5	2.7	2	128	160	0.1	8.1	5.4	2	193	168

* N is equal to the dilution factor (10^{-N}). + Average of Two Plates.

TABLE XII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 8

	SEED						8 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE +	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.3	0	2	126	157		8.2	0	0	2	118	147
7	8.3	0	2	39	49.4		8.0	0.2	0.2	2	96	120
15	8.3	0	2	31	38.2		7.3	0.9	0.9	2	304	386
22	8.2	0.1	2	206	258		5.2	3.0	2.9	3	168	2110
29	7.9	0.4	2	342	543		4.8	3.4	3.0	4	29	3690
35	8.2	0.1	2	207	232		4.6	3.6	3.5	4	25	3060
41	8.2	0.1	2	151	190		4.5	3.7	3.6	4	21	2630
47	8.2	0.1	2	132	165		4.4	3.8	3.7	3	115	2000
53	8.2	0.1	3	21	263		4.2	4.0	4.9	3	112	1570
60	8.2	0.2	2	78	97.5		3.9	4.3	4.1	3	193	2410
72	8.1	0.2	2	137	165		3.7	4.5	4.3	3	72	906
84	8.1	0.2	2	126	159		2.9	5.3	5.1	3	53	663
102	8.1	0.2	3	19	231		---	---	---	3	47	707
120	8.1	0.2	2	81	101		2.7	5.5	5.3	2	137	170
145	8.0	0.3	2	61	76.4		1.8	6.4	6.1	2	181	229
210	7.9	0.4	2	110	135		2.4	5.8	5.4	2	179	223

* N is equal to the dilution factor (10^{-N}). + Average of Two Plates.

TABLE XIII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 9

	SEED						8 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE +	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	7.8	0	4	42	5500		7.7	0	0	4	50	6500
4	7.5	0.3	4	59	7500		4.7	3.0	2.7	4	87	10,200
11	6.8	1.0	4	56	7100		4.0	3.7	2.7	4	85	10,200
14	6.6	1.2	4	39	4750		3.6	4.1	2.9	4	60	7430
23	5.9	1.9	3	218	2830		2.7	5.0	3.1	3	264	3250
33	4.7	3.1	3	84	1050		1.2	6.5	3.4	3	125	1500
45	3.0	4.8	3	77	950		0.1	7.6	2.8	3	121	1500

TABLE XIV

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 10

	SEED						8 mg/l					
TIME	DO	BOD	N*	COLONIES PER PLATE +	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	7.6	0	4	121	15,800		7.4	0	0	4	76	9500
4	5.6	2.0	4	68	8500		3.4	4.0	2.0	4	61	7400
9	3.3	4.3	4	67	8100		1.1	6.3	2.0	4	56	7000

* N is equal to the dilution factor (10^{-N}). + Average of two plates.

TABLE XV

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 11

	SEED					NUTRIENT BROTH					
TIME	DO	BOD	N*	COLONIES PER PLATE +	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	1	15	1.94	8.2	0	0	1	14	1.87
5	8.2	0	1	2	0.19	8.2	0	0	1	6	0.69
12	8.2	0	1	6	0.75	8.2	0	0	1	22	2.69
23	8.2	0	1	112	14.0	8.2	0	0	1	107	13.4
31	8.1	0.1	2	127	159	7.8	0.4	0.3	-	TMC	----
37	8.0	0.2	-	TMC	---	7.1	1.1	0.9	-	TMC	----
47	7.9	0.3	2	42	52.5	6.4	1.8	1.5	-	TMC	----
55	7.8	0.4	3	20	250	6.2	2.0	1.6	3	110	1380
60	7.5	0.7	2	159	199	6.1	2.1	1.5	3	123	1530
72	7.2	1.0	2	166	208	5.9	2.3	1.3	3	99	1240
80	7.2	1.0	-	TMC	---	5.9	2.3	1.3	3	130	1630
86	7.1	1.1	3	55	688	5.9	2.3	1.2	4	32	4000
95	7.1	1.1	3	51	638	5.7	2.5	1.4	3	98	1230
103	7.0	1.2	-	TMC	---	5.7	2.5	1.3	3	113	1410
119	6.9	1.3	2	269	336	5.7	2.5	1.2	3	149	1870

* N is equal to the dilution factor (10^{-N}). + Average of two plates. TMC-Too Many To Count.

TABLE XVI

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 12

	SEED						NUTRIENT BROTH					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	1	296	37.0		8.3	0	0	1	25	3.19
6	8.3	0	1	300	37.5		8.3	0	0	1	53	6.56
12	8.3	0	1	TMC	----		8.1	0.2	0.2	1	400	50.0
18	8.2	0	2	252	314		7.0	1.3	1.3	2	TMC	----
24	8.2	0	2	280	350		6.5	1.8	1.8	3	212	2650
30	8.2	0	3	27	338		5.8	2.5	1.5	4	25	3119
36	8.1	0.1	2	284	355		4.7	3.6	3.5	5	2	2500
42	8.1	0.1	2	232	290		2.1	5.2	5.1	4	32	4010
48	8.0	0.2	1	TMC	---		1.9	6.4	6.2	3	TMC	----
57	8.1	0.1	1	TMC	---		1.7	6.6	6.5	3	TMC	----
72	8.0	0.2	2	246	3080		1.3	7.0	6.8	4	59	3780
100	8.1	0.1	3	290	2620		0.1	8.2	8.1	4	16	2000

* N is equal to the dilution factor (10^{-N}). + Average of two plates. TMC-Too Many To Count.

TABLE XVII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 13

	SEED					STERILE SEWAGE					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	1	3	0.38	8.1	0	0	1	13	1.62
10	8.2	0	-	-	----	7.9	0.2	0.2	1	92	11.5
24	8.1	0.1	1	3	0.38	5.4	2.7	2.6	-	TMC	----
28	8.1	0.1	1	43	5.38	5.1	3.0	2.9	-	TMC	----
33	8.1	0.1	1	148	18.5	4.8	3.3	3.2	3	95	1210
38	8.1	0.1	1	200	25.0	4.7	3.4	3.3	3	107	1340
47	8.0	0.2	1	122	27.8	4.4	3.7	3.5	3	101	1260
52	8.0	0.2	2	60	74.5	4.1	4.0	3.8	3	78	963
57	8.0	0.2	2	34	41.8	4.0	4.1	3.9	2	327	408
62	8.0	0.2	2	38	50.6	3.7	4.4	4.2	2	240	300
70	8.0	0.2	2	31	38.2	3.3	4.8	4.6	2	52	65.6
120	8.0	0.2	2	69	86.2	2.9	5.2	5.0	2	20	25.6
168	7.9	0.3	2	45	57.0	2.2	5.9	5.6	2	91	113
288	7.9	0.3	2	79	99.0	1.6	6.5	6.2	2	82	102
360	7.6	0.6	2	36	45.0	1.4	6.7	6.1	2	67	83.2
480	7.6	0.6	2	25	31.3	0.7	7.4	6.8	2	37	46.4

* N is equal to the dilution factor (10^{-N}). + Average of two plates. TMC - Too Many To Count.

TABLE XVIII

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 14

	SEED						NEUTRAL SULFITE SEMICHEMICAL					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000	DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.0	0	2	140	17.5	7.5	0	0	2	160	20.0
6	7.8	0.2	-	---	----	5.5	2.0	1.8	2	204	25.5
14	7.7	0.3	3	23	19.3	4.6	2.9	2.6	3	50	62.5
18	7.6	0.4	2	127	15.9	4.4	3.1	2.7	3	41	502
22	7.6	0.4	3	20	244	4.3	3.3	2.9	3	51	630
30	7.6	0.4	3	27	331	3.7	3.8	3.5	3	131	1640
38	7.5	0.5	3	38	475	3.3	4.2	3.7	3	66	825
50	7.4	0.6	2	66	82.5	2.8	4.7	4.1	3	29	363
62	7.2	0.8	2	47	59.4	2.6	4.9	4.1	3	13	168
74	7.2	0.8	2	77	97.0	2.1	5.4	4.6	2	212	266
98	7.1	0.9	2	56	68.0	1.8	5.7	4.8	2	266	331
120	7.1	0.9	2	3	3.75	1.5	6.0	5.1	-	---	----

* N is equal to the dilution factor (10^{-N}). +Average of two plates.

TABLE XIX

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 15

	SEED						NEUTRAL SULFITE SEMICHEMICAL					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.3	0	-	TMC	----		7.5	0	0	1	480	60.2
6	8.3	0	-	TMC	----		7.3	0.2	0.2	1	TMC	----
12	8.3	0	2	66	84.5		5.7	1.8	1.8	2	209	621
18	8.3	0	2	64	83.2		5.1	2.4	2.4	2	TMC	---
24	8.2	0.1	3	11	144		4.6	2.9	2.8	3	73	914
30	8.2	0.1	4	1	125		4.1	3.4	3.3	4	14	1820
36	8.1	0.2	2	54	67.5		3.8	3.7	3.5	4	15	1880
42	8.2	0.1	2	56	70.0		3.7	3.8	3.7	4	4	437
48	8.1	0.2	-	TMC	----		3.6	3.9	3.7	3	35	463
57	8.1	0.2	-	TMC	----		3.2	4.3	4.1	3	60	752
72	8.1	0.2	2	172	222		3.0	4.5	4.3	3	62	776
100	8.0	0.3	3	8	106		2.1	5.4	5.1	3	16	218
120	7.9	0.4	2	158	204		1.2	6.3	5.9	2	59	74.5
192	7.8	0.5	2	134	167		1.1	6.4	5.9	2	57	71.4

* N is equal to the dilution factor (10^{-N}). + Average of two plates. TMC - Too Many To Count.

TABLE XX

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 16 A

	SEED						KRAFT BLACK LIQUOR					
TIME	DO	BOD	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000		DO	BOD	NET	N	COLONIES PER PLATE	ORGANISM PER ML X 1000
0	8.2	0	1	113	13.5		7.5	0	0	1	65	8.13
6	8.2	0	1	47	5.87		7.2	0.3	0.3	1	53	6.13
12	8.2	0	-	---	----		7.2	0.3	0.3	2	6	7.50
18	8.2	0	-	---	----		7.2	0.3	0.3	2	10	12.5
24	8.2	0	2	21	26.2		7.1	0.4	0.4	2	5	6.25
31	8.2	0	2	320	400		6.9	0.6	0.6	2	104	129
38	8.2	0	1	30	3.81		5.3	2.2	2.2	-	TMC	----
45	8.2	0	-	TMC	----		5.6	2.9	2.9	-	TMC	----
51	8.2	0	1	110	13.7		4.2	3.3	3.3	-	TMC	----
57	8.2	0	1	88	11.0		3.6	3.9	3.9	-	TMC	----
63	8.2	0	1	107	13.4		3.5	4.0	4.0	4	87	10,900
71	8.1	0.1	-	TMC	----		2.8	4.7	4.6	4	92	11,500
83	8.1	0.1	2	22	26.9		1.8	5.7	5.6	4	68	8500
96	8.1	0.1	2	65	81.2		1.6	5.9	5.8	4	87	10,900
120	8.0	0.2	2	65	82.0		1.2	6.3	6.1	4	78	9820
192	7.9	0.3	2	53	64.5		0.6	6.9	6.6	4	36	5660
264	7.9	0.3	2	26	31.8		0.2	7.3	7.0	4	8	1000

* N is equal to the dilution factor (10^{-N}). + Average of two Plates. TMC - Too Many To Count.

TABLE XXI

DISSOLVED OXYGEN, BOD AND VIABLE BACTERIA COUNT DATA, EXPERIMENT 16 B

KRAFT MILL EFFLUENT					
TIME	DO	BOD	NET	N*	COLONIES PER PLATE+	ORGANISM PER ML X 1000
0	8.0	0	0	1	127	15.9
6	7.8	0.2	0.2	1	186	23.2
12	7.7	0.3	0.3	2	83	104
18	7.1	0.9	0.9	2	278	348
24	6.1	1.9	1.9	-	TMC	----
31	5.6	2.4	2.4	-	TMC	----
38	5.2	2.8	2.8	3	256	3200
45	5.1	2.9	2.9	3	214	2680
51	5.1	2.9	2.9	3	260	3250
57	4.7	3.3	3.3	3	97	1220
63	4.3	3.7	2.7	4	30	3810
71	3.1	4.9	4.8	4	44	5500
83	2.8	5.2	5.1	4	38	4680
96	2.5	5.5	5.4	4	26	3180
120	1.6	6.4	6.2	4	18	2250
192	1.2	6.8	6.5	3	105	1310
264	0.8	7.2	6.9	3	9	106

* N is equal to the dilution factor (10^{-N}).

\+ Average of two plates.

TMC - Too Many To Count.

VITA

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