THE EVALUATION OF BACTERIAL GROWTH RATE CONSTANTS FOR MUNICIPAL WASTES

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Jacob Nicholas Dick
1964

This is to certify that the

thesis entitled

THE EVALUATION OF BACTERIAL GROWTH
RATE CONSTANTS FOR
MUNICIPAL WASTES

presented by

Jacob Nicholas Dick

has been accepted towards fulfillment
of the requirements for

M. S. degree in Sanitary Engineering

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Major professor

Date May 22, 1964

O-169

[redacted]

MAY 25 '66

Circulate Item 6

[redacted]

ABSTRACT

THE EVALUATION OF BACTERIAL GROWTH RATE CONSTANTS FOR MUNICIPAL WASTES

by Jacob Nicholas Dick

This thesis reports the results of a study made on several municipal wastes to determine the growth rate constants of bacteria and their relationship to B.O.D. The growth rate constants were determined by measuring the respiration rates per unit sample volume in the Warburg apparatus. A semi-logarithmic plot was then made of the respiration rates and the slope of that portion of the curve which displayed a straight line was taken to be the growth rate constant.

It was found that an approximately linear relationship existed between the growth rate constant and the B O.D. at low B.O.D. concentrations and that the growth rate constant became independent of the B.O.D. at high concentrations.

The maximum hourly respiration rates per unit sample volume were related to the B O.D. It was found that a linear relationship existed between the maximum hourly respiration rate per unit sample volume and the initial B.O.D. concentration.

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By

Jacob Nicholas Dick

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Civil Engineering

1964

ACKNOWLEDGMENT

The author wishes to express his indebtedness and appreciation to Dr. K. L. Schulze, Department of Civil Engineering, Michigan State University, whose generous help and encouragement made this thesis possible.

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K	a constant defining the rate of deoxygenation
k_{10}	growth rate constant to the base 10
k	growth rate constant to the base e
mg/l	milligrams per litre
mg/l/hr	milligrams per litre per hour
B.O.D.	Biochemical Oxygen Demand
$B.O.D_5$	5-day Biochemical Oxygen Demand
ml.	millilitres
mg.	milligrams
ul	microlitres
mm.	millimetres
O_2	oxygen
ppm	parts per million
hr^{-1}	per hour
S.S.	suspended solids
hrs	hours
D.O.	dissolved oxygen
pH	inverse logarithm of the hydrogen ion concentration
C.O.D.	Chemical Oxygen Demand

1.0 LITERATURE REVIEW

Phelps, as found in Clark (12, p. 9), was the first to establish a mathematical relationship describing the observed rate of deoxygenation of sewage. The law may be expressed as:

$$d \frac{(L - L_t)}{dt} = Kt$$

where,

K = a constant defining the rate of deoxygenation.

L_t = the organic matter at time (t), or the oxygen requirement of the sample at time (t).

L = the organic load present at the beginning.

Pleissner, as found in Clark (12, p. 12), also sought to derive an expression of the rate of deoxygenation of polluted waters on the assumption that the velocity of the reaction depended not only upon the concentration of organic matter but also upon the concentration of dissolved oxygen. Realizing the importance of referring to a standard point on the deoxygenation curve when comparing sewages, he proposed a forty-eight hour incubation period. Using this incubation period he derived a mathematical expression as follows:

$$m_t = 17.0 \log^2 t$$

where,

m_t = a number that when divided into the oxygen demand value for the time t gives the standard hourly oxygen loss.

t = incubation time in days.

Mueller as found in Clark (12, p. 14), compared the relationship between bacterial count and hourly loss of oxygen in water using Bacillus fluorescens liquefaciens and Bacterium coli. He found that a direct proportionality did not exist between the two parameters but that the maximum loss of oxygen per hour did correspond to the maximum bacterial count.

"The Oxygen Demand of Polluted Waters" written by Theriault (32) and published by United States Public Health Service as Public Health Bulletin Number 173 in 1927 ranks as one of the great classics of this field. The report consists of two parts: first a critical review of the pertinent literature to that date, and second an experimental verification of the rate of sewage deoxygenation. The first, or carbonaceous, stage of deoxygenation was found to conform to the monomolecular formula proposed by Phelps previously.

Penfold (25) in 1912 stated that little attention had been paid to the influence of the concentration of the culture medium employed when considering the generation time of bacteria. Working with a culture of Bacillus typhosus, Penfold showed that the rate of growth was greatly influenced by a peptone concentration when it is below 0.4 per cent.

The generation-time was inversely proportional to the concentration of peptone at values below 0.2 per cent.

Callow (10) washed bacteria free from culture medium and investigated the oxygen uptake rate. Various cultures of bacteria were used and the oxygen uptake rates varied from 5 to 25 c.c. of oxygen per gram of dry weight under these conditions.

Butterfield (6) explored the relationship between food concentration and bacterial growth in 1929. He found that in pure cultures of Bacterium aerogenes the relationship between the limiting bacterial numbers and the concentration of food supply was logarithmic. In the case of grossly mixed cultures including plankton, the increase in the limiting bacterial numbers produced by an increase in food concentration was always less than in the case of pure cultures.

Martin (21) measured the oxygen consumption of Escherichia coli during the lag and logarithmic phases of growth. He observed that the rate of oxygen consumption per cell increased rapidly from the time of inoculation to a point of maximum respiration near the end of the lag phase of the growth curve. The maximum surface area per cell coincided with the point of maximum oxygen consumption per cell.

Lea and Nichols (19) showed the importance of certain essential elements in a bacterial medium. They stated that maximum bacterial growth can only be obtained when sufficient

cell-building elements, such as nitrogen, phosphorus, and potash are present in the substrate.

Using pure cultures of bacteria isolated from activated sludge, Butterfield, Ruchhoft, and McNamee (7) found that the addition of fresh nutrient substrate to these pure culture activated sludges under aeration very greatly increased the quantities of oxygen utilized. Butterfield and Wattie (8) explained this fact by stating that the rate of oxidation of bacterial food during the first hours of incubation was dependent upon the number of living bacteria. The greater the number of bacteria, the more extensive was the initial oxidation. The rate of oxidation was also found to be influenced by the degree of dispersion of bacteria, or bacterial floc. Adequate dispersion was necessary to produce extensive oxidation.

In 1939 Sawyer and Nichols (28) studied the effect of various factors on the oxygen utilization rate of activated sludge. They found that the oxygen uptake rate was directly proportional to the sludge concentration. At higher temperatures, the rate of oxygen utilization greatly exceeded that at the lower temperatures. The change in activity with temperature, however, was not linear.

Grieg and Hoogerheide (17) determined in 1941 that the oxygen uptake of growing cultures of bacteria was directly proportional to the bacterial content and that rate of oxygen uptake constituted a convenient method for the measurement of

the rate of growth. They measured the oxygen consumption in Warburg manometers and found it to be directly proportional to the bacterial content when the latter was determined nephelometrically.

Monod (23) reported extensive studies on the growth of bacteria under aerobic conditions in simple media containing a single carbohydrate. The increase in cell mass of Escherichia coli and Bacillus subtilis grown on many different carbohydrates was found to be directly proportional to the initial concentration of the nutrient, indicating that exhaustion of the food supply was the factor limiting growth.

The rate of growth was studied in relation to the concentration of nutrient and was found to follow the relationship,

$$k = k_m \frac{c}{c' + c}$$

where,

k_m = maximum rate of growth

c = concentration of nutrient remaining

c' = a constant

In 1946 a comprehensive study was released by the Subcommittee on Sewage Treatment at Military Installations (24) on all phases of sewage treatment and on oxygen demand characteristics. The average value of K was found to be 0.18, but extreme values ranging from 0.10 to 0.30 were reported.

Hinshelwood (18) in 1947 stated that the logarithmic law was found to be a very good approximation of bacterial growth over quite a wide range of conditions, but lacked absolute character. He also stated that over quite wide ranges of media concentrations the growth rate was almost independent of concentration and not until very dilute concentrations was there a decrease in the growth rate. He pointed out that a quantitative effect of the media on growth would be difficult to establish since the growth rate also depended upon the degree of adaptation.

Ruchhoft, Placak, Kackmar, and Calbert (26) found the value of K and L in a series of B.O.D. determinations to be dependent upon the sewage concentration to a large extent. They observed that in their specific case at a dilution of one per cent or greater consistent results were obtained, whereas below this dilution the data became inconsistent and were somewhat effected by seeding.

Caldwell and Langelier (9), working at the University of California, used the direct oxygen utilization method for sewage analyses. They reported a proportionately higher rate of oxygen demand in undiluted sewage samples. They reported the concentration of the sewage to be a factor in the rate of oxidation but that a limit existed above which the reaction velocity was not proportionally increased.

Garret and Sawyer (16) in studies pertaining to the removal of soluble B.O.D. and oxygen utilization of mixed

cultures developed from a sewage seed showed that the growth of the organisms in mixed cultures was in agreement with the laws of growth found by investigators to be applicable to pure cultures of bacteria. At high concentrations of B.O.D. the rate of growth was constant, and at low concentrations the rate of growth was directly proportional to the remaining soluble B.O.D.

Smith (31) studied the respiratory activity of activated sludge. Within a range of about 0.2 to 6 ppm of dissolved oxygen the unit rate of oxygen utilization was not significantly affected. The oxygen uptake rate decreased with increasing sludge age and increased with increasing B.O.D. loading.

Buswell, Mueller, and Van Meter (5) discussed the rate of oxygen consumption in the B.O.D. test. They found a direct relationship between maximum bacterial population and total pollutional load. They suggested that the oxygen consumption curve consists of two rates (1) a higher rate associated with cell multiplication and (2) a lower rate associated with resting or dying cultures.

Longmuir (20) used a polarograph to determine the relation between respiration rate of bacteria and the concentration of dissolved oxygen. The relationship could be expressed in the form of the Michaelis-Menten equation.

Balmat (2) discussed the rate at which settleable and supra-colloidal sewage solids underwent decomposition and

found that the larger particles were characterized by a relatively low rate of biochemical oxidation due to the slow action of the hydrolyzing exoenzymes of the bacteria.

Fisichelli and Palombo (14) in 1960 analyzed a total of 170 24-hour composite samples of raw and primary effluent to determine the velocity reaction constant K. For raw sewage samples they found K to range from 0.05 to 0.29 with a mean of 0.16 and for primary sewage samples they measured K values from 0.055 to 0.29 with a mean of 0.15.

Sawyer (27) stated that for a great many years the B.O.D. reaction was considered to have a rate constant K equal to 0.10 per day at 20°C. As the application of the B.O.D. test spread to the analyses of industrial wastes and the use of synthetic dilution water became established, it was noted that the K values of different waste materials varied considerably from the accepted value of 0.10 per day. It was also noted that the K value varied from day to day as shown by Schroepfer, Robins, and Susag (29) who reported values of experimentally determined rate constants from the Minneapolis-St. Paul Sanitary District and Mississippi River.

Schuller (30) using the Warburg technique in 1961 concluded that it was possible, by multiplication with a constant, to calculate the total oxygen demand after the highest hourly respiratory value had been determined.

2.0 PROCEDURE

2.1 Sampling

Two sampling procedures were used in this study. The first procedure consisted of collecting a series of grab samples at one sewage treatment plant throughout the day. When this method was used the samples were collected in screw cap bottles and stored in a refrigerator until used.

The second procedure consisted of obtaining grab samples from different sewage treatment plants for comparison. The samples in this case were obtained in gallon jugs and stored in a pail of ice until used. All samples were collected from the primary effluent of the respective sewage treatment plants.

2.2 Suspended Solids Determination

The total suspended matter was determined in this study as described in Standard Methods (1).

2.3 Biochemical Oxygen Demand Determination

2.31 Standard B.O.D.$_5$.--The standard 5-day Biochemical Oxygen Demand was determined as described in Standard Methods (1). An equivalent amount of potassium nitrate, however, was utilized as a nitrogen source instead of the ammonium chloride as stated in Standard Methods to prevent errors due to nitrification. Gaffney and Heukelekian (15)

have recommended a nitrate source of nitrogen rather than an ammonium source to reduce nitrification.

The dilution water control was performed by filling four B O.D. bottles with the dilution water and determining the dissolved oxygen content of two of these bottles immediately. The other two bottles were set into the B.O.D. incubator for 5 days and on the fifth day the dissolved oxygen content was determined for these bottles. In all cases the Alsterberg azide modification of the Winkler method as found in Standard Methods (1) was employed. The two initial D.O. determinations were repeated until two consecutive determinations within 0.1 mg/l of D.O. were observed.

If either of the incubated dilution water control bottles showed an oxygen uptake of 0.2 mg/l, or above, the B.O.D. determinations were considered unsatisfactory.

All B.O.D. bottles were washed with chromic acid solution and then rinsed several times with distilled water before being used.

The incubated B.O.D bottles were examined from time to time to insure a good water seal.

2.32 Dissolved B.O.D.--The dissolved B.O.D. was determined by filtering the sample through a Gooch crucible. The filter was made up as described in Section 2.2.

The B.O.D. was determined on the filtrate as described in Section 2.31.

2.4 Warburg Apparatus Procedure

Before the Warburg flasks were used the grease from the glass joints was removed with a piece of cheese cloth dipped in unleaded gasoline. The flasks were next placed in a Hemosol soap solution for 12 hours, rinsed with water, washed in chromic acid solution, and rinsed 5 times with distilled water.

A 20 per cent potassium hydroxide solution was placed in the centre well of the flask to absorb the carbon dioxide that was given off. To increase the surface area of the potassium hydroxide a small folded piece of filter paper was placed into the centre well.

As previously mentioned two sampling procedures were used. When the first procedure was used duplicate flasks were set up each containing 5 ml of the sample. The duplication was made to determine the reproduction of results.

When the second procedure was used a part of the sample was filtered through a Gooch crucible as described in Section 2.2. This was done to eliminate any particulate matter from contributing to the B.O.D. so that only soluble B.O.D. would contribute to the growth rate. Then ten 100 ml graduates were set in a row, five were filled with the filtered sample and the remaining 5 filled with the unfiltered sample. To four graduates, both of the filtered and unfiltered sample, the following amounts of glucose were added: 6.7 mg, 13.4 mg, 26.8 mg, and 53.6 mg. These

quantities of glucose were added to increase the original B.O.D_5 by 50, 100, 200, and 400 mg per litre respectively. The five filtered samples were next seeded with 1 ml of the original primary effluent per 100 ml of sample. Five ml of sample was carefully transferred from each of the 10 graduates to 10 Warburg flasks. The pipette was cleaned with distilled water after each 5 ml sample had been transferred.

Stop-cock grease was next applied to the side-arm stopper of the flask and the manometer arm to insure that no air leaks would develop. The flasks were attached to the manometer, with the valves on the manometer open, placed in the water bath, and allowed to shake for approximately 15 minutes. This was done to allow the temperature and pressure to equalize. The shaker was stopped, all valves closed, the manometer fluid adjusted to read 150 mm on each leg and the apparatus again started. The shaking rate employed in this study was 85 oscillations per minute. The manometers were read at approximately 1-hour intervals and the results recorded.

When the 150 microlitre supply was almost consumed, the manometer cocks were opened, the manometer again filled with air, and the fluid carefully adjusted to read 150 on each leg. Then the manometer cocks were closed and the vibrator again started.

During the night hours, 12:00 P.M. to 7:00 A.M., it happened on occasion that the oxygen consumed was greater

than the 150 microlitre capacity of the manometer. Cheng (11) developed a formulation whereby two settings of the manometer under these conditions would still give results with errors of only one per cent. To achieve this the manometer was adjusted so that the liquid level in the open leg was just on the scale and the readings on both of the legs recorded. The reading on the closed leg above the 150 mark was observed. The manometer was next adjusted so that the closed leg reading would be twice the value of the previous reading of the closed leg. The readings on both legs were again recorded and by the following formulation the oxygen consumed evaluated.

$$y = \frac{We}{f - e}$$

where,

y = the distance below the scale of the liquid level in the open leg.

e = the distance above zero that the fluid has moved in the closed leg when the open leg is just on zero.

f = the distance above zero that the fluid has moved in the closed leg when the fluid in the open arm is some distance on the open leg.

W = the difference of the two readings on the open leg.

If f were chosen to equal 2e, then y = W.

Each test was conducted at a temperature of 20°C. The temperature was maintained at this level by circulating water from the University water system through the water bath. An overflow mechanism was installed into the water tank to maintain a constant water level. Since the University water supply had a nearly constant temperature of 17°C., the heater in the water bath maintained the temperature at a constant 20°C. $\pm$ 0.05°C.

A thermobarometer was employed to correct for atmospheric pressure changes.

The logs of the observed oxygen uptake rates were plotted versus time for each sample. The growth rate constant k_{10} was then evaluated from the slope of the straight line portion of this plot.

3.0 THEORY

The commonly accepted equation for the growth of organisms can be expressed as:

$$y = y_o e^{kt}$$

y = the organism concentration at time t

y_o = the original organism concentration

k = the growth rate constant

t = the interval of time

$$\frac{y}{y_o} = e^{kt}$$

$$\ln \left(\frac{y}{y_o}\right) = kt$$

$$\log_{10} \left(\frac{y}{y_o}\right) = 2.31\ kt = k_{10}t$$

Burk and Milner (4) working at the Fixed Nitrogen Research Laboratory in Washington, D. C., studied the nitrogen fixation of micro-organisms with the Warburg technique. They stated that nitrogen fixation was indicated by an increase in the rate of oxygen consumption, which was caused by the growth of organisms, and this in turn was dependent on nitrogen fixation. These quantities are, under ordinary conditions, proportional to growth increases as measured by dry weight, cell number, or turbidity.

Burk (3) in 1934 stated that the growth of Azotobacter may be measured by either cell nitrogen, cell number, dry matter, turbidity, respiration rate, or amount of azotase. Under any set of reasonably favorable physiological conditions each of these quantities increases logarithmically with time. Owing to this simple relationship the initial velocity of growth or nitrogen fixation at unit cell concentration can easily be obtained as a function of any particular variable, such as temperature, pH, concentration of sugar, nitrogen pressure or source of fixed nitrogen. It is measured by the first order velocity constant g:

$$g = 2.30\ d\ \frac{\log (a + y)}{dt}$$

where,

a = the initial Azotobacter concentration

y = the increase in t hours

Burk used g as a notation for the growth rate constant and stated that it was conveniently evaluated experimentally from the slope of a plot of the logarithm of the respiration rate against time.

Grieg and Hoogerheide (17) studied the growth of microorganisms in the Warburg apparatus and found the oxygen uptake of growing cultures of bacteria to be proportional to the bacterial content. They further stated that since the oxygen uptake is directly proportional to bacterial content, the measurement of the rate of oxygen uptake constitutes a convenient method for the measurement of the rate of growth.

4.0 RESULTS

4.1 Respiration Rates

In experiments No. 1 and 2 a series of grab samples collected from the primary effluent of the East Lansing sewage treatment plant on September 25 and on October 3, 1962 were analyzed. The results are shown in Tables 1 to 13 and in Figures 1 to 8. The curves show that the logarithmic and declining growth phases were of relatively short duration and approximately of equal length. This can be observed from the symmetry displayed in most of the respiration rate curves. The curves in Figures 1 to 8 inclusive indicate a logarithmic phase of approximately 4 hours duration. As shown in Figures 1 to 8 inclusive all East Lansing samples demonstrated a great similarity. They all approached a maximum hourly rate of 10 mg O_2/l/hr. The minimum respiration rate at the beginning of each sample appeared to be 2 or 3 mg O_2/l/hr. These curves did not indicate the presence of a lag phase.

In experiments No. 3, 4, and 5 grab samples from the primary effluent of the East Lansing, Lansing, and Williamston sewage treatment plants were prepared according to procedure 2 described in Section 2.

The data from the East Lansing sample are listed in Tables 14 and 15 and shown graphically in Figures 9 and 10.

The curve in Figure 9 represents a typical sample of East Lansing unfiltered primary effluent dated November 5, 1962, where 6.7 mg of glucose was added. The curve is quite similar to those in Figures 1 to 8 inclusive. The curve in Figure 10 resulted from a typical filtered sample with 6.7 mg glucose added; however, it shows some distinct differences. A considerable lag phase is observed as compared to the curve in Figure 10 and the respiration rates are quite small at the beginning. The log growth phase appears to be somewhat longer than in the previously mentioned curves.

The data for the samples from the Lansing plant are listed in Tables 16 and 17 and shown graphically in Figures 11 and 12. Figure 11 represents a typical unfiltered Lansing sample with 6.7 mg of glucose added. The initial respiration rate was approximately 1 mg O_2/l/hr which increased to a maximum of approximately 3.5 mg O_2/l/hr. Figure 12 shows a curve typical for a filtered Lansing sample with 6.7 mg of glucose added. The very low initial oxygen uptake rate continued for approximately 60 hours before it increased to approximately 5 mg O_2/l/hr.

The curves of Figures 11 and 12 showed a very distinct lag phase. The unfiltered sample showed a lag phase of approximately 20 hours duration whereas in the filtered sample logarithmic growth started only after approximately 60 hours. The curves further showed a different form possibly due to an inhibitory effect. In Figure 11 the

logarithmic growth phase appeared to be 20 hours long and the declining growth phase had a duration of approximately 10 hours. Figure 12 showed a good symmetrical curve with shorter logarithmic and declining growth phases. This difference may be due to the inhibitory agents adhering to the particulate matter which were being removed by filtration.

The data for the samples from the Williamston plant are listed in Tables 18 and 19 and shown graphically in Figures 13 and 14. Figure 13 represents a typical unfiltered sample with no glucose addition. The respiration rate began at approximately 1 mg O_2/l/hr and increased to a maximum of 8 mg O_2/l/hr. The curve was very similar to those obtained for the unfiltered samples from the East Lansing plant. In Figure 14 the curve for a typical filtered sample is shown also with no glucose addition. The respiration rate began at a rather low value and increased to approximately 6.5 mg O_2/l/hr. The curves of Figures 13 and 14 are very similar except for the lower initial respiration rate of the filtered sample.

The respiration rate curves obtained from those samples where 13.4, 26.8, and 53.6 mg glucose were added are not shown; however, the curves were very similar to the ones depicted except that higher maximum respiration rates were obtained. The higher respiration rates were caused by the fact that a larger microbial population developed from the higher concentration of substrate.

The respiration rates per unit volume of sample varied considerably. As shown in Table 22 the maximum respiration rate of 45 mg O_2/l/hr was observed when the initial B.O.D. of the sample was 477 ppm. This value was obtained from a filtered sample of the Williamston plant with 536 mg glucose added. The lowest respiration rate of 3.0 mg O_2/l/hr. was obtained from an unfiltered sample of the Lansing plant.

Some lag phase was observed with all the filtered samples. In this case the lag phase was possibly caused by a new environment or due to the very low bacterial cell concentration at the start.

Table 20 is a listing of the data on pH, suspended solids and B.O.D_5 which were obtained on the original samples from the East Lansing, Lansing, and Williamston plants. In addition this Table contains the B.O.D. values measured for the filtered samples where no glucose was added. The B.O.D_5 values for those samples where glucose was added were assumed to be higher by 50, 100, 200, or 400 mg/l according to the amount of glucose added to the filtered or unfiltered samples.

In Figures 15, 16, and 17 the maximum respiration rates observed for the East Lansing, Lansing, and Williamston samples with glucose addition were plotted against the respective B.O.D_5 data for these samples. The data used for these graphs are listed in Table 22. The curves indicate a definite relationship between the initial B.O.D. concentration

and the maximum hourly respiration rate per unit volume of sample. It should also be noted that the slope of the line differed with each sample. This was probably caused by the characteristics of the particular type of waste.

Schuller (30) working in Germany in 1961 compared the maximum hourly respiration rate with the 24 hour B.O.D. as obtained from Warburg measurements and the chemical oxygen demand (C.O.D.) whereas in this study the 5-day B.O.D. was used. Schuller (30) obtained maximum hourly respiration rates from primary effluents ranging from 7 mg O_2/l/hr to 30 mg O_2/l/hr. These values are quite similar to those reported in this study.

4.2 Growth Rate Values and B.O.D.

Figure 18 is a plot of the k_{10} values obtained for the unfiltered samples from the East Lansing plant as listed in Table 21 versus the B.O.D_5 concentration of these same samples. The curve shows the specific growth rate to be linear with respect to the B.O.D. for most of the data. It is possible however that at B.O.D. values above 200 ppm the specific growth rate constant k_{10} approached a maximum value.

In Figures 19, 20, and 21 the k_{10} values for the East Lansing, Lansing, and Williamston samples with glucose addition were plotted against the respective B.O.D_5 values as listed in Table 22.

Figure 19 demonstrates an approximately linear relationship between the specific growth rate constant k_{10} and

the B.O.D. up to a growth rate value of 0.13 per hour. The concentration of soluble B.O.D. at which the growth rate became B.O.D. independent appeared to be approximately 200 ppm. In the unfiltered sample k_{10} appeared to reach its maximum value at $B.O.D_5$ of 400 mg/l. It should also be noted that the unfiltered curve is identical to that of the filtered curve except that the unfiltered curve is shifted to the right on the graph.

For the Lansing sample as shown in Figure 20 the curves representing the filtered and unfiltered portion were quite different. The curve for the filtered portion began very much like the curves shown in Figures 18 and 19; however, the specific growth rate did not exceed a value of 0.11 and decreased with increasing glucose concentrations. The unfiltered curve had the general appearance of the already mentioned curves but the specific growth rate did not exceed a value of 0.06 per hour.

In Figure 21, representing the Williamston sample, the unfiltered sample portion produced extremely scattered data, probably due to a malfunction of the Warburg apparatus. The filtered portion of the sample was similar to those shown in Figures 18 and 19. The maximum growth rate value appeared to be 0.13 per hour at approximately 200 mg/l B.O.D.

Figure 22 is a graph prepared by plotting the k_{10} values obtained for all filtered samples versus their

respective soluble B.O.D_5 concentrations. This plot demonstrates clearly that the specific growth rate approaches a maximum value of 0.13 at a B.O.D. concentration of 200 mg/l.

Under the most ideal conditions as shown in Figure 19, there was no appreciable difference between the filtered and unfiltered sample portions except that the curve for the filtered portion was moved more to the right on the graph.

Figure 20, however, showed a clear difference in the two curves. The curve for the unfiltered portion reached a maximum growth rate k_{10} of 0.06 per hour whereas the filtered curve obtained a maximum growth rate k_{10} of 0.105 per hour. It appeared that certain inhibiting effects were removed by the filtering process. Another interesting phenomenon was the inhibiting effect of higher glucose concentrations demonstrated for the filtered portion of the sample.

5.0 DISCUSSION

In a study such as this it is extremely important to be able to duplicate the results obtained. In Figures 2, 3, 5, 6, and 7 data from duplicate samples are plotted together. It should be noted that the same slope has been obtained for either of the two samples.

It was originally the intent of this study to measure the mass of sludge growth by weight and to obtain a growth rate value by plotting the logarithm of the sludge increase against time. Due to the low concentrations of suspended solids in the samples and the difficulty experienced in determining the suspended solids precisely, this method proved unsatisfactory. It was then decided to determine the sludge growth by utilizing respiration rate as a measure of the sludge growth rate.

All biological systems need certain chemical substances which are required for their growth and normal function. In this study a reagent grade glucose was added to a number of sewage samples as an additional carbon source. The nitrogen source and other nutritional elements necessary for growth had to be supplied by the sample. Garret and Sawyer (16) who also evaluated growth rates from mixed bacterial cultures utilized a synthetic nutrient solution where all essential elements were present to obtain a maximum growth rate. In

this study the maximum growth rate was produced by increasing only the concentration of the carbon source.

Garret and Sawyer (16) determined the growth rate constant by plotting the accumulated oxygen consumption versus time on a semi-logarithmic scale and by measuring the slope of the straight line portion of this curve. The writer does not believe this to be a true determination of the growth rate constant. It is generally assumed that the oxygen consumed in a biological oxidation reaction is used partly for cell synthesis and partly for direct oxidation of the organic matter. Therefore a measure of the total oxygen consumed is not a measure of the cell increase but a value which is larger than the specific growth rate constant.

It should be noted that the respiration rate unit employed was a unit of volume rather than a unit of weight. Respiration rate units commonly employed are mg O_2 per gram of sludge per unit of time. The unit employed in this study was mg O_2 per unit volume of original sample per unit of time.

Figures 15, 16, and 17 were graphs of maximum respiration rates as related to the standard 5-day B.O.D. It should be observed that each plot has a different slope. The slope of the line was characteristic of the particular waste and would be different for each municipality. The greater the slope of the line the more readily the waste was broken down and conversely the smaller the slope the more difficult it was to break down the organic matter.

As shown in Table 22, certain samples did not obtain a logarithmic growth phase. This occurred in samples of relatively low B.O.D where the margin of error inherent in the equipment was exceeded.

All the filtered samples, as demonstrated in Figures 10, 12, and 14, showed small initial respiration rates. This may be due at least in part to insufficient seeding of the sample; however, caution had to be observed to avoid too much seeding and thereby eliminating the log growth phase.

Soluble organic matter can be assimilated directly by biologically active bacterial cell material whereas particulate matter must first be hydrolyzed to be available to the cells. Consequently the soluble B.O.D. fraction will be depleted directly and the particulate matter removed by coagulation, entrainment, adsorption, and only later on by oxidation of components made soluble by enzymes. The logarithmic growth rate constant k_{10} as determined in this study was related to the soluble B.O.D. as demonstrated in Figures 19, 20, 21, and 22. The data indicate that a maximum growth rate would be reached if the soluble B O.D. fraction should exceed 200 ppm B.O.D. At lower soluble B.O.D. concentrations the growth rate of the bacteria would be dependent on the substrate concentration.

Normally activated sludge plants are designed to operate at an aeration period of 4 to 8 hours, an air supply of

0.5 to 2.0 cubic feet per gallon of sewage, and a return sludge capacity of 25 per cent of the sewage flow. Haseltine (22) in 1955 criticized this design procedure because no mention of the amount of activated sludge to be carried in the aeration tanks was stipulated. He proposed to design the plant around the Sludge Volume Index, B.O.D. to solids loading, and the return sludge capacity. In this procedure the total volume of the aeration tanks is set by the above mentioned criteria. This study would indicate a further criterion to be used, namely the growth rate k. It should be mentioned here, however, that at high growth rates the activated sludge becomes dispersed and the coagulant growth disappears. This would be an important factor in the utilization of large growth rates.

6.0 CONCLUSIONS

1. Some municipal wastes have an extended lag phase before the logarithmic growth phase begins.
2. There is a linear relationship between the maximum hourly respiration rate per unit liquid volume and the initial B.O.D concentration.
3. The duration of the logarithmic growth phase under normal conditions is approximately 5 hours; however, it is dependent on the initial substrate concentration.
4. The curve relating B.O.D and growth rate constant is characteristic of each waste. This relationship could change; however, throughout the day depending on the various components that make up the waste at different times during the day.
5. The growth rate of bacteria in sewage is B.O.D. dependent below approximately 200 ppm dissolved B.O.D. Above 200 ppm dissolved B.O.D the growth rate is independent of the B.O.D. concentration and at its maximum rate.
6. The maximum growth rate value k at $20^{\circ}C$. was found to be approximately 0.30 per hour in this study. The mean generation time corresponding to this k value is 2.31 hours.

7.0 APPENDICES

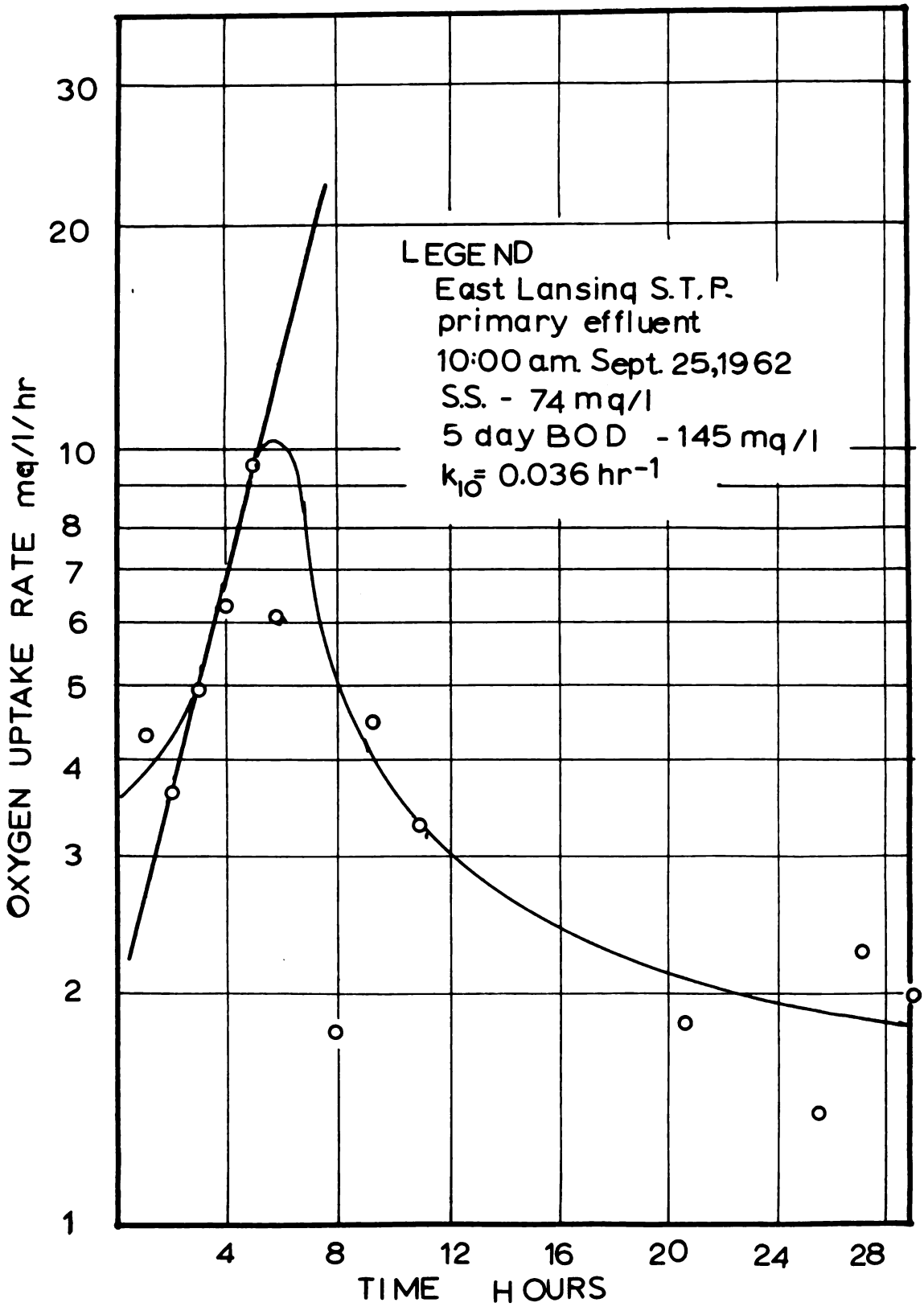


Figure 1.- Relationship of Respiration Rate and Time

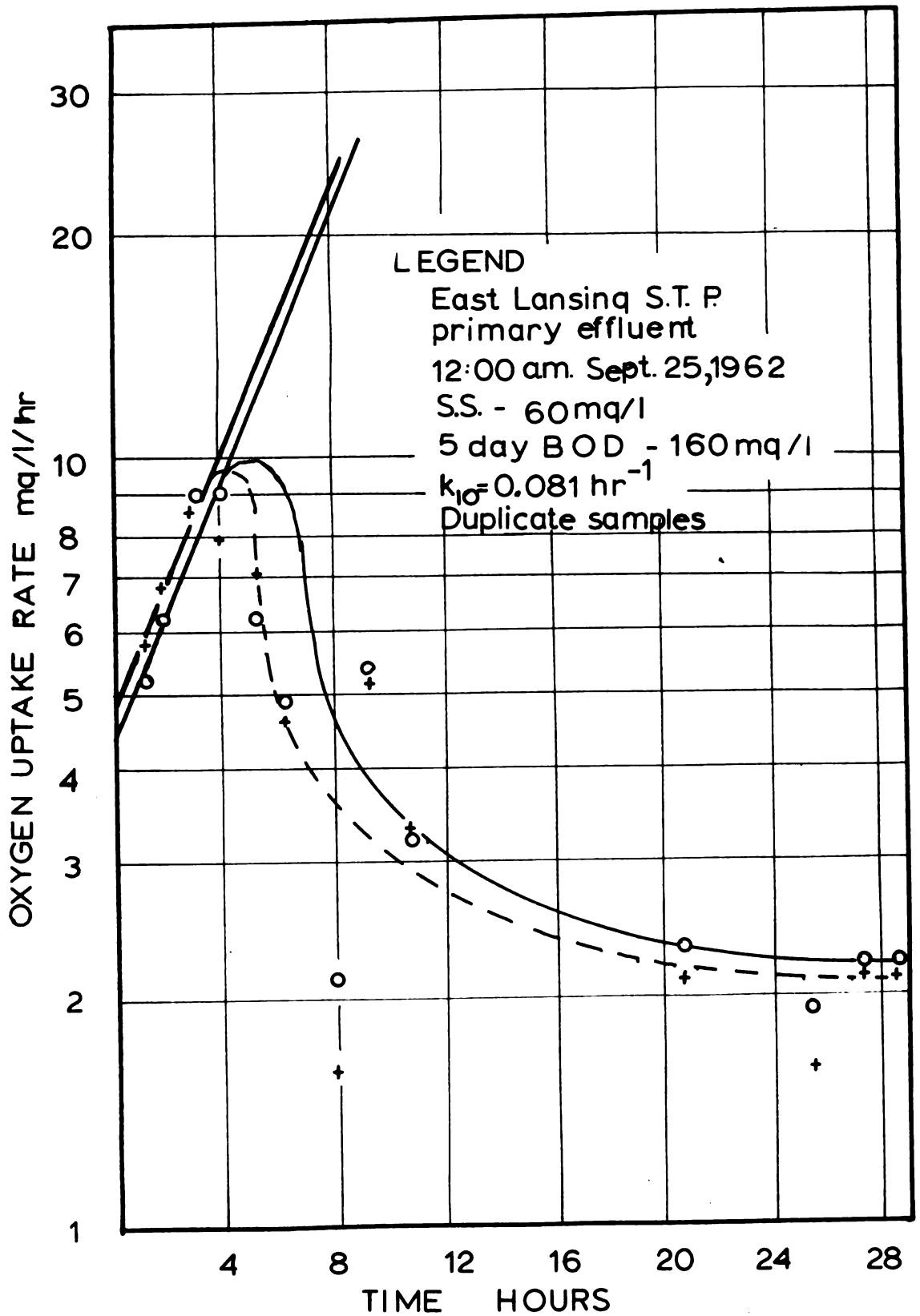


Figure 2- Relationship of Respiration Rate and Time

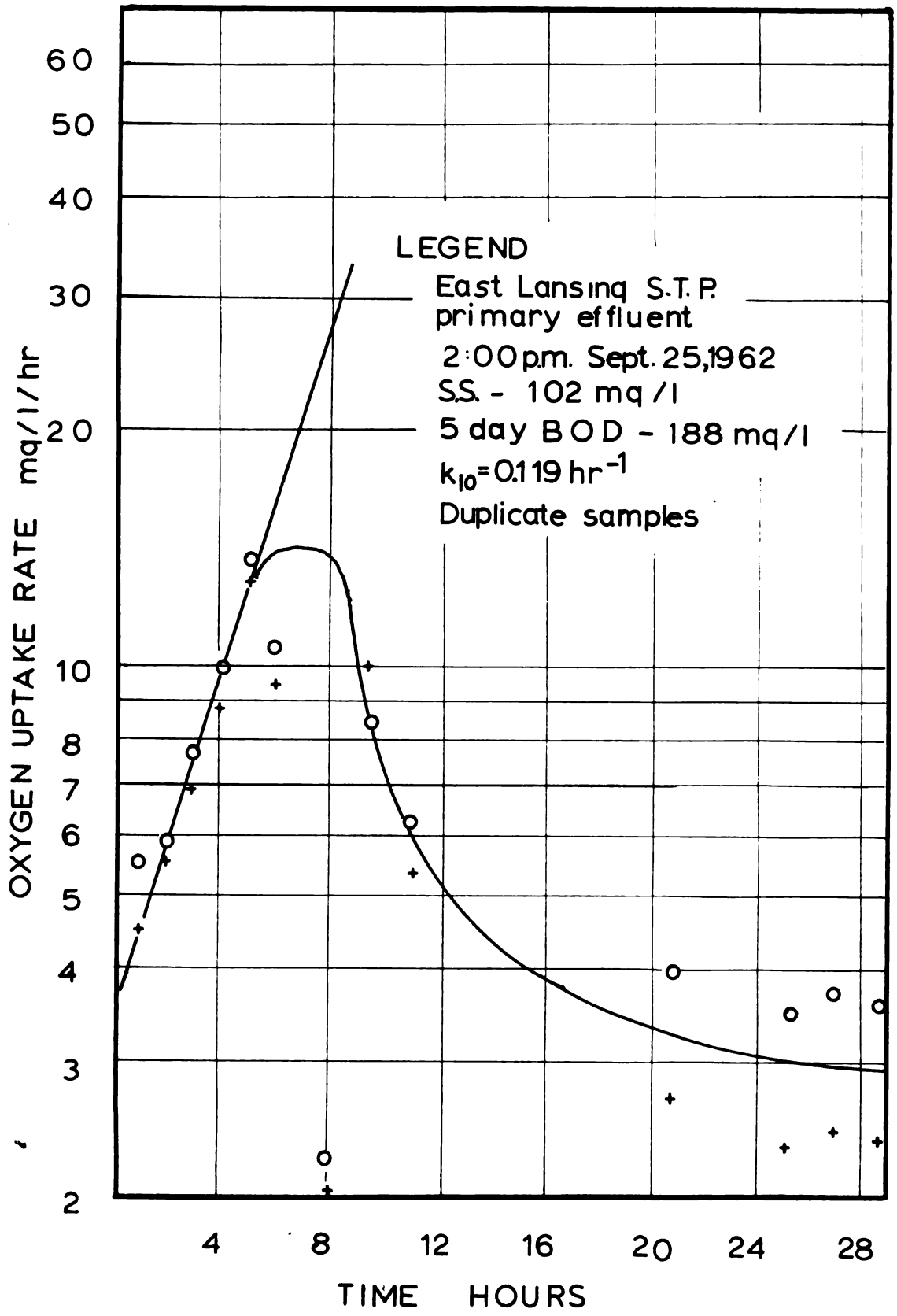


Figure 3.-Relationship of Respiration Rate and Time

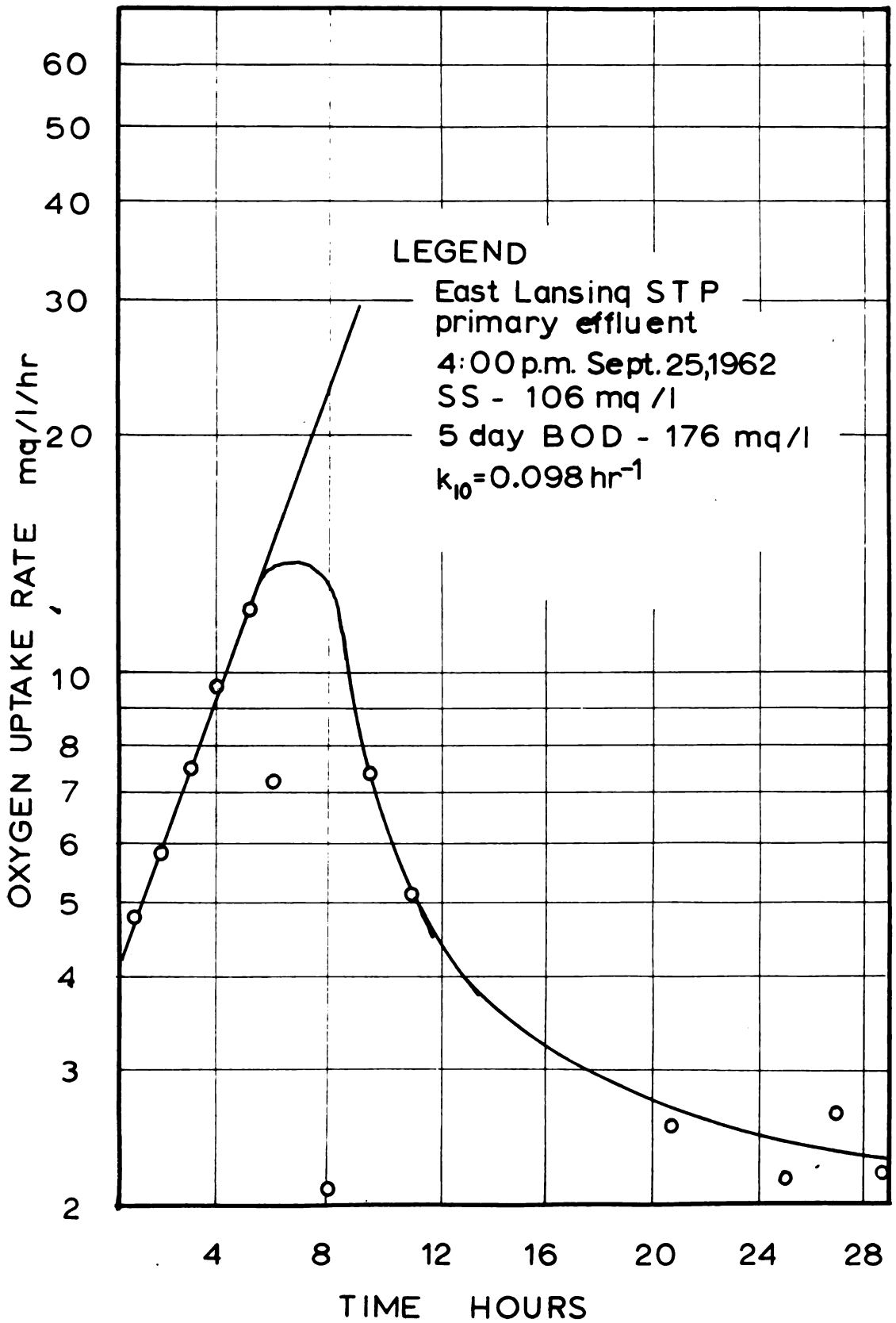


Figure 4 Relationship of Respiration Rate and Time

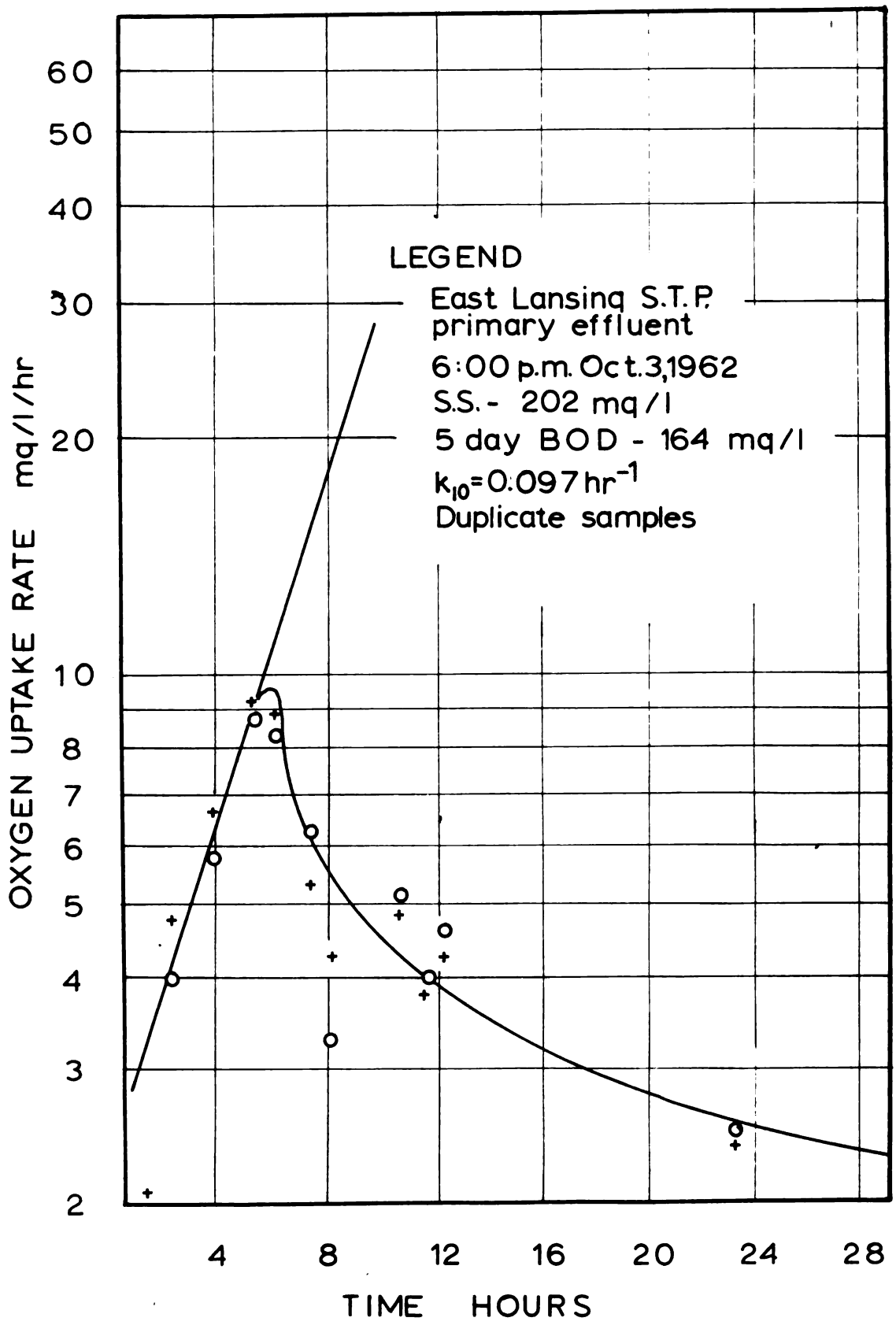


Figure 5 Relationship of Respiration Rate and Time

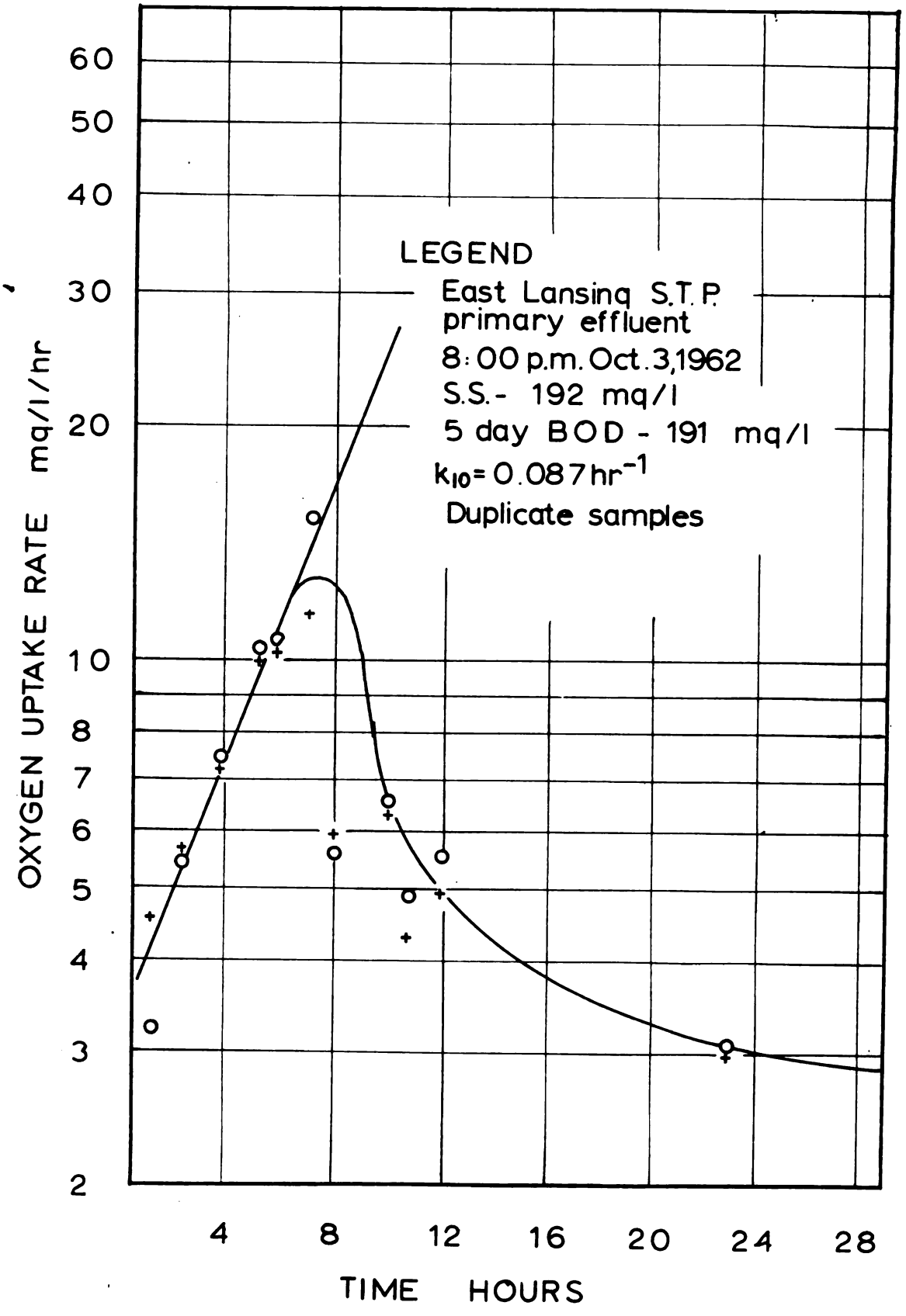


Figure 6.-Relationship of Respiration Rate and Time

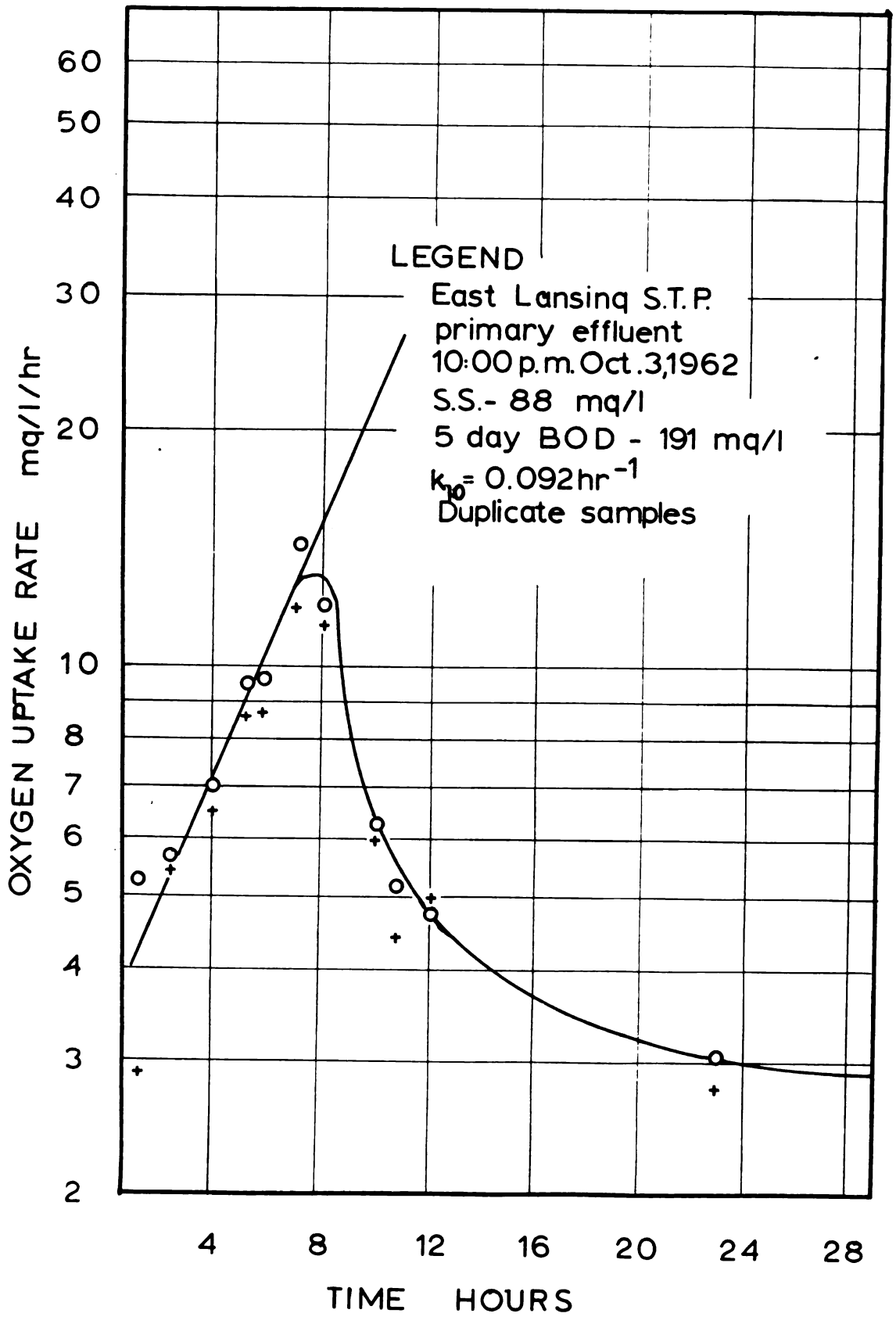


Figure 7.-Relationship of Respiration Rate and Time

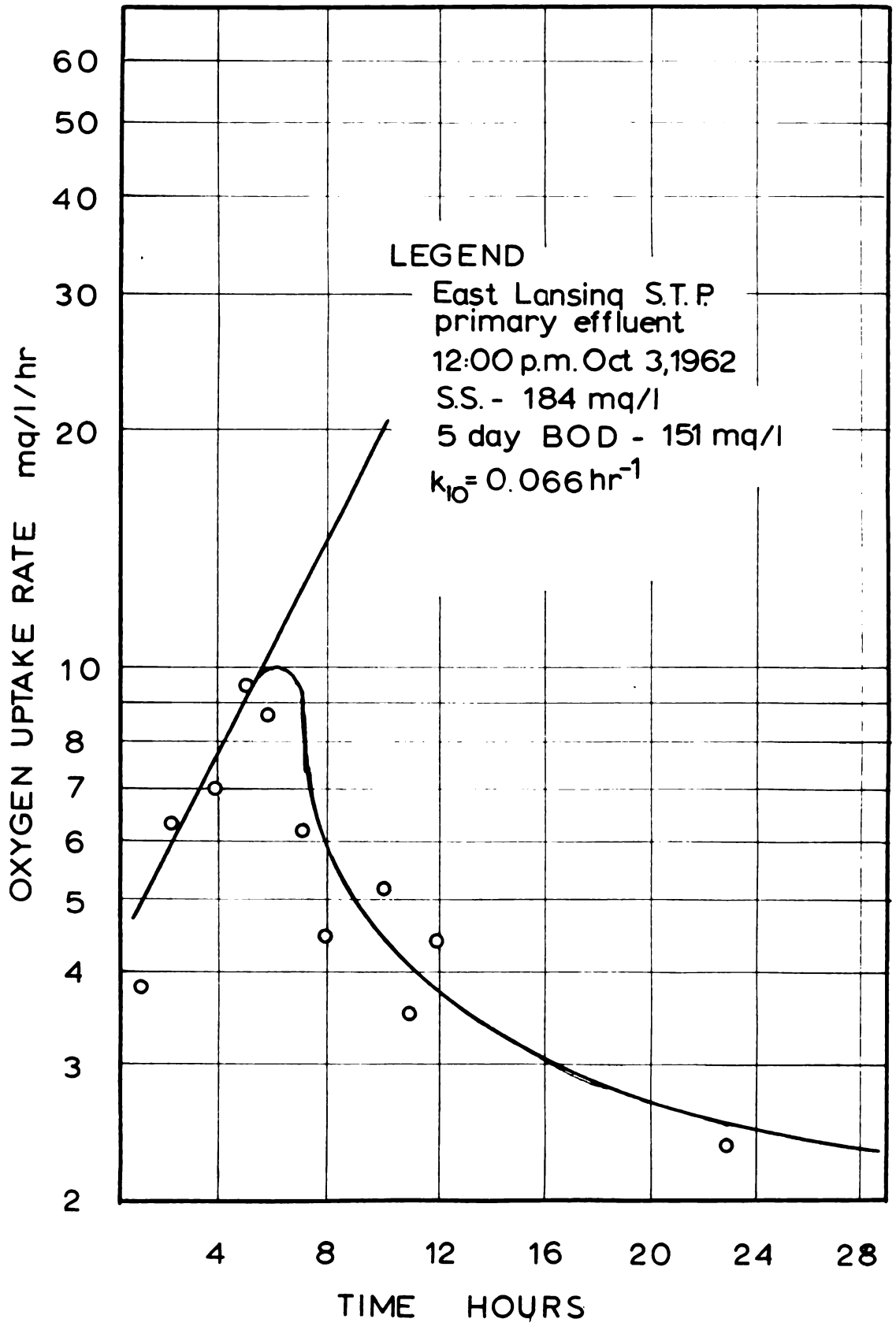


Figure 8.- Relationship of Respiration Rate and Time

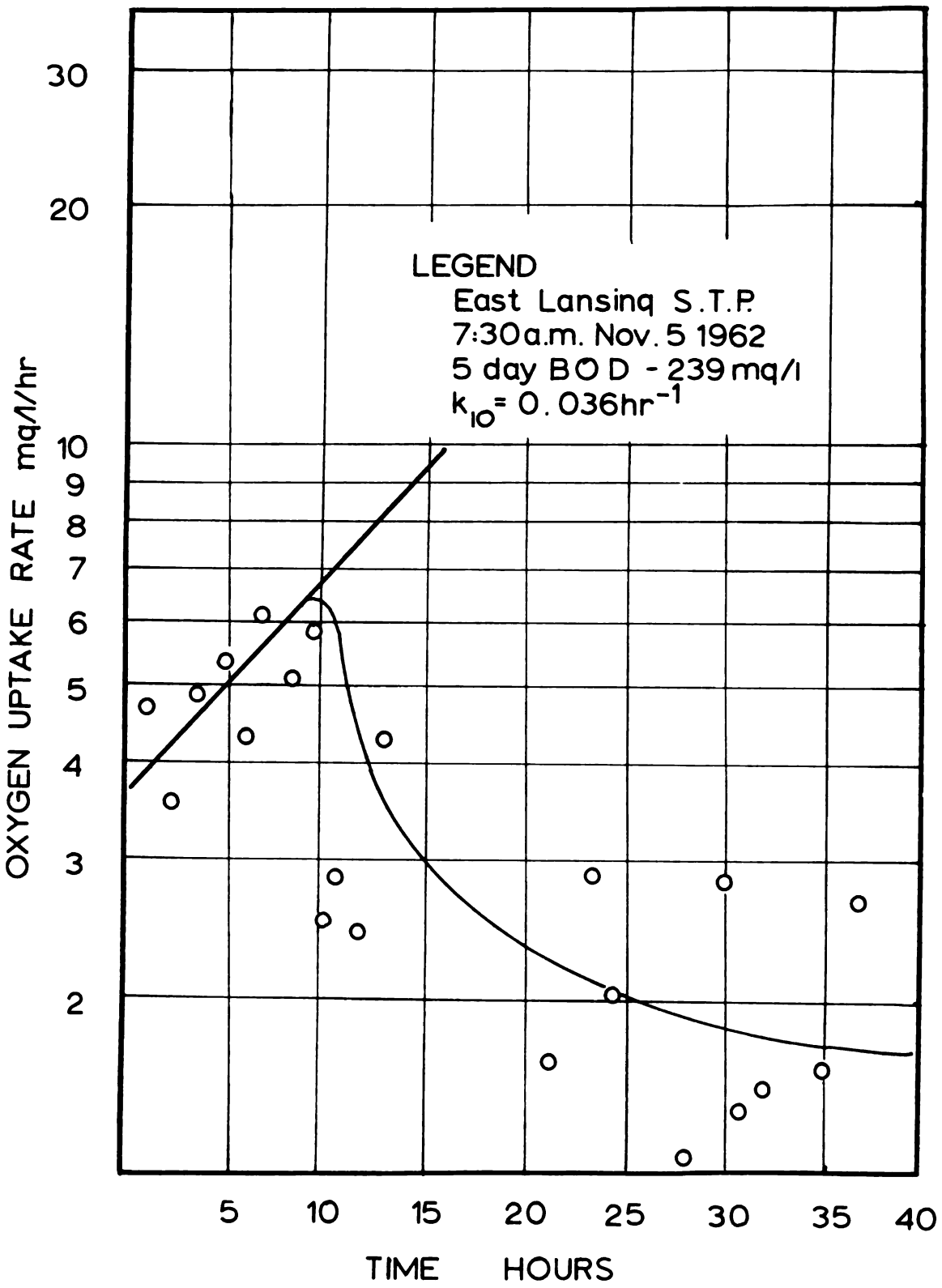


Figure 9 - Typical Respiration Rate plot of unfiltered sample with glucose added

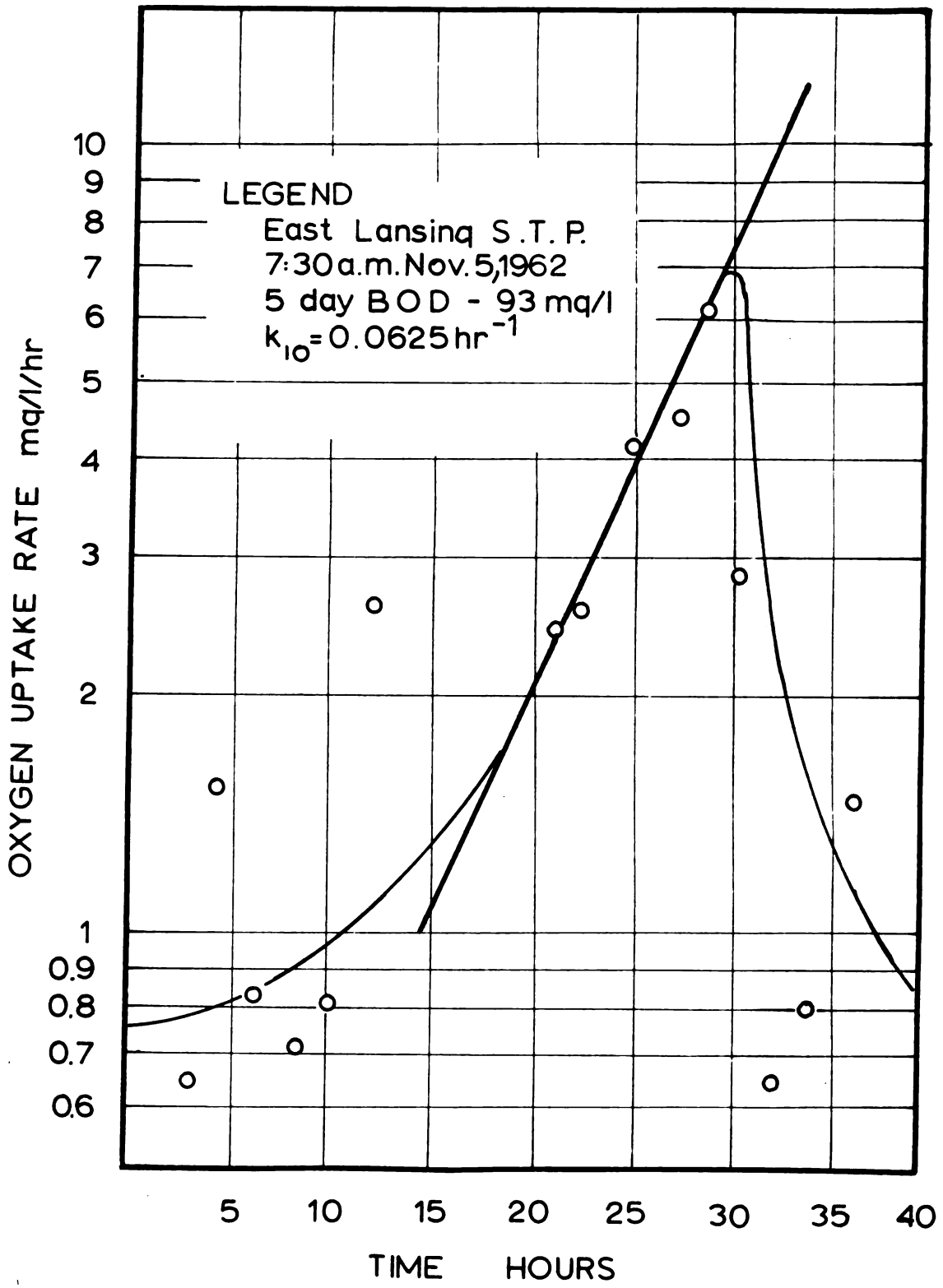


Figure 10 - Typical Respiration Rate plot of filtered sample with glucose added

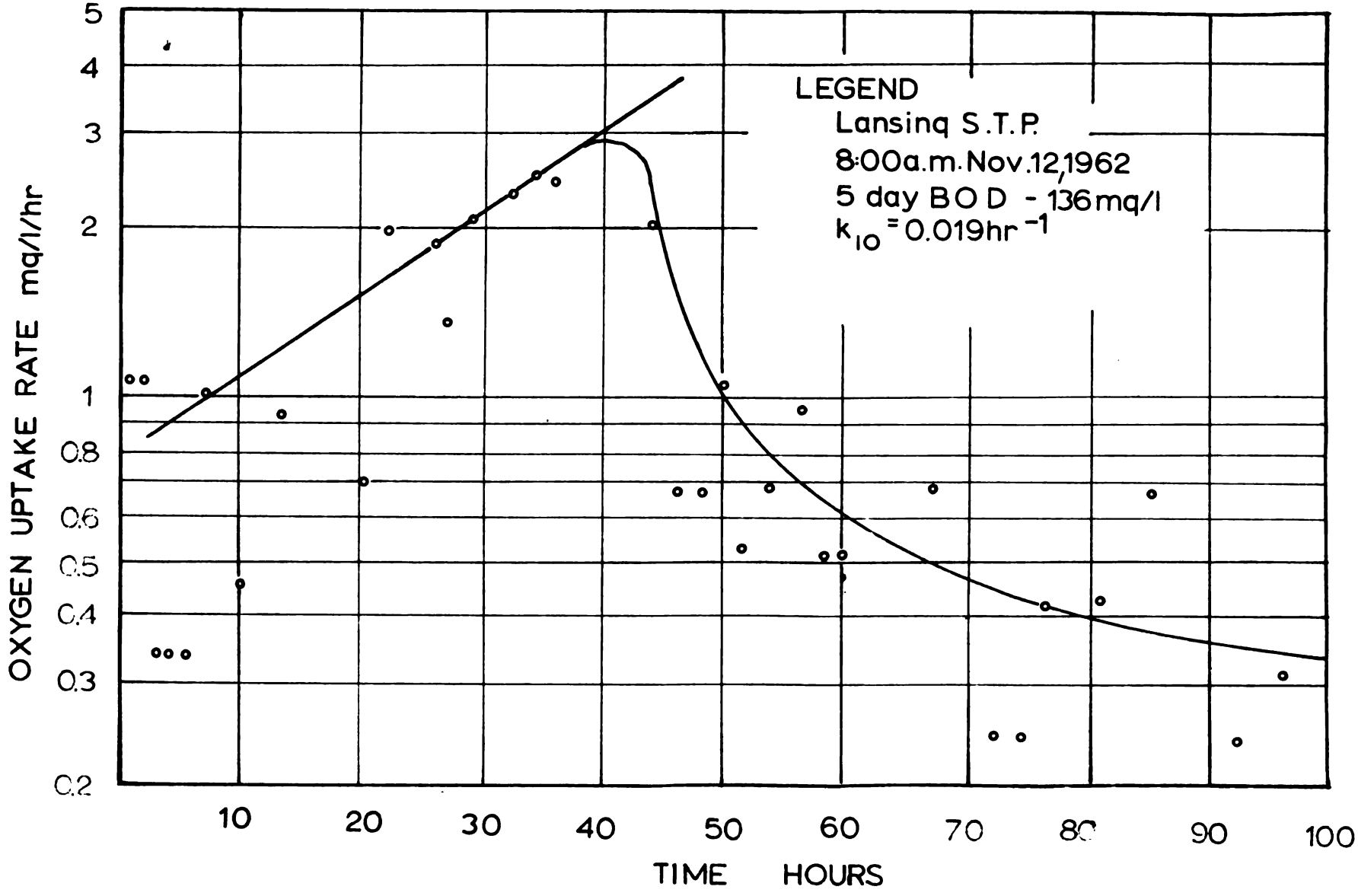


Figure11- Typical Respiration Rate plot of unfiltered sample with glucose added

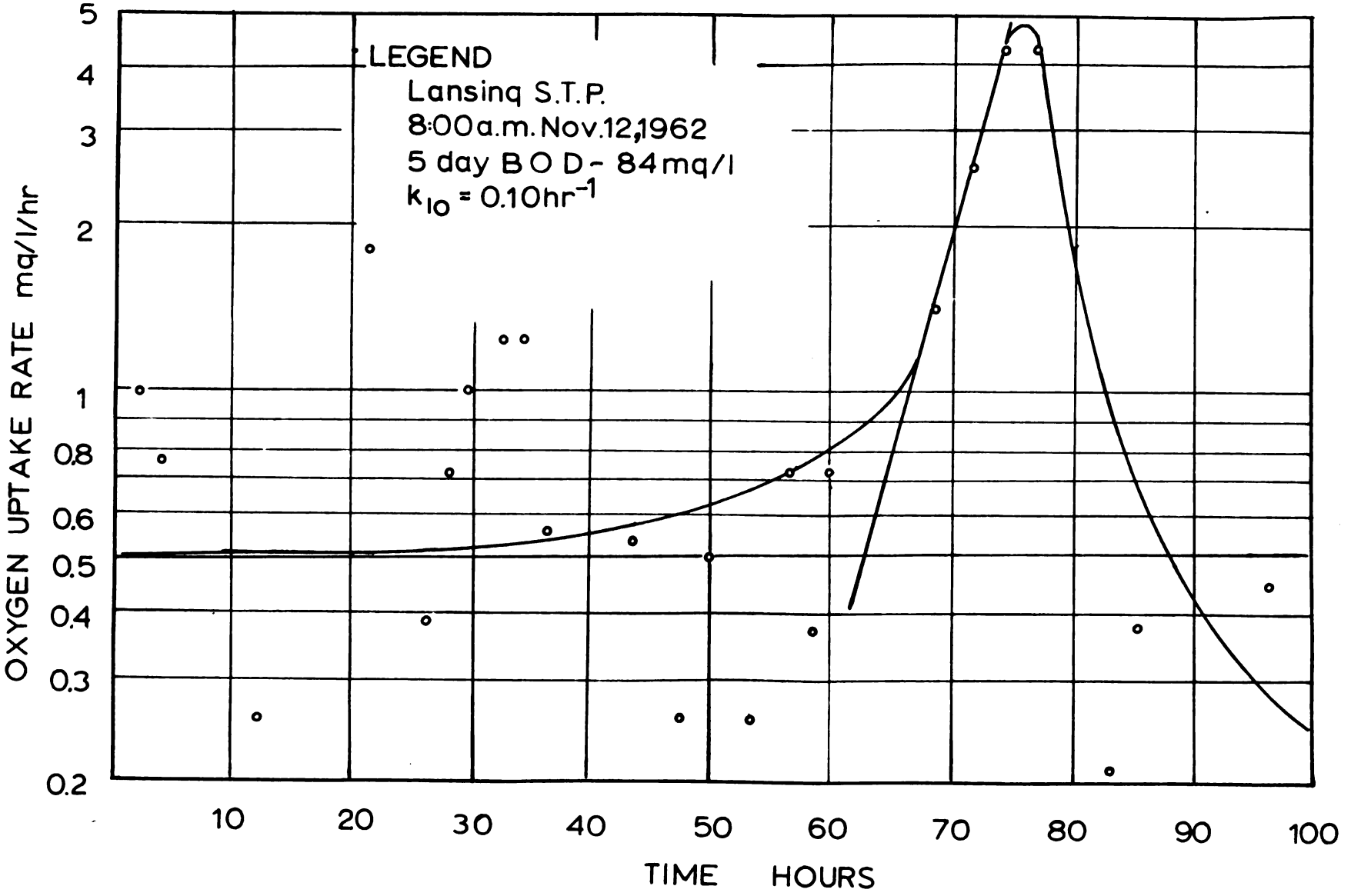


Figure12 - Typical Respiration Rate plot of filtered sample with glucose added

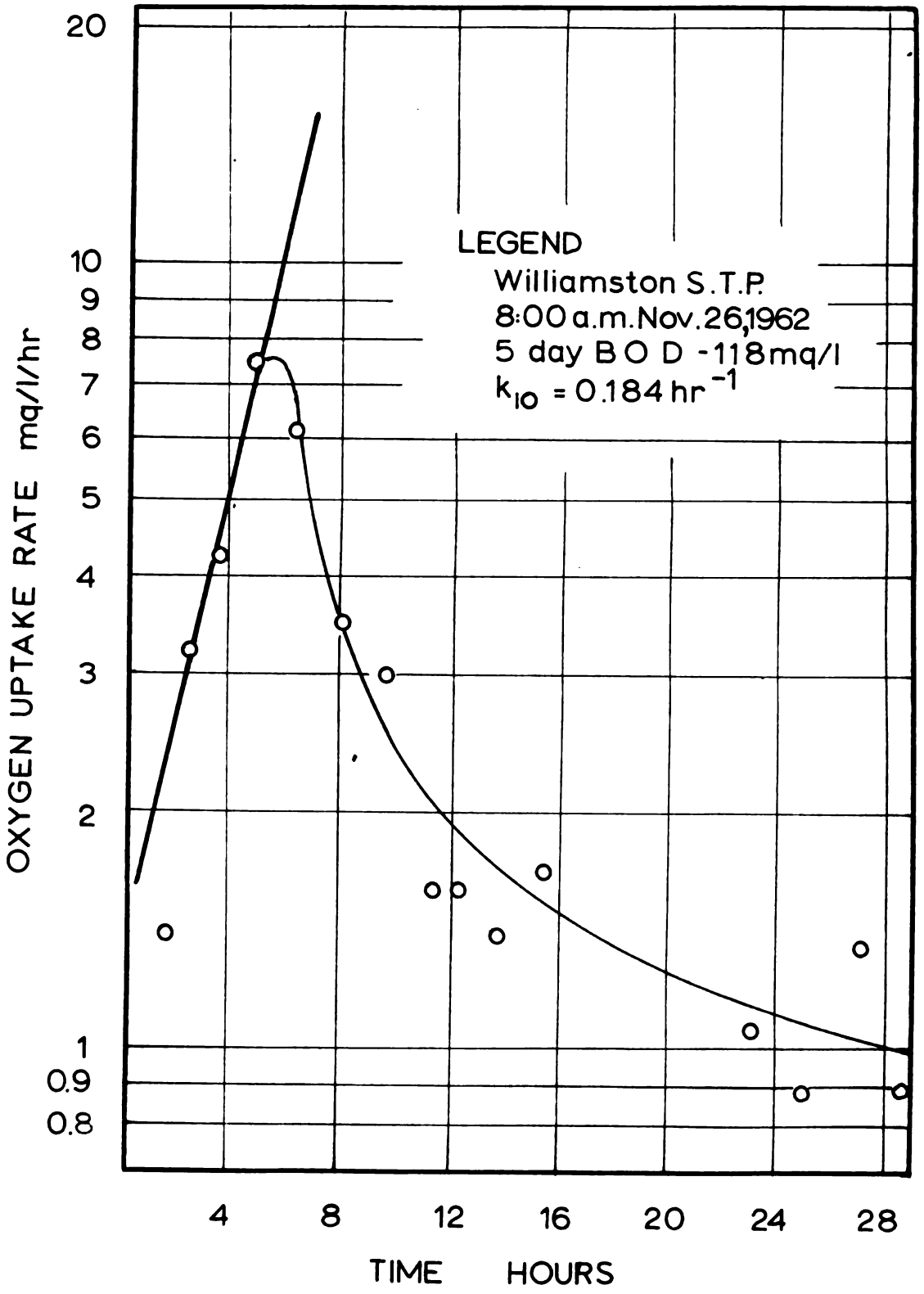


Figure 13 - Typical Respiration Rate plot of unfiltered sample with glucose added

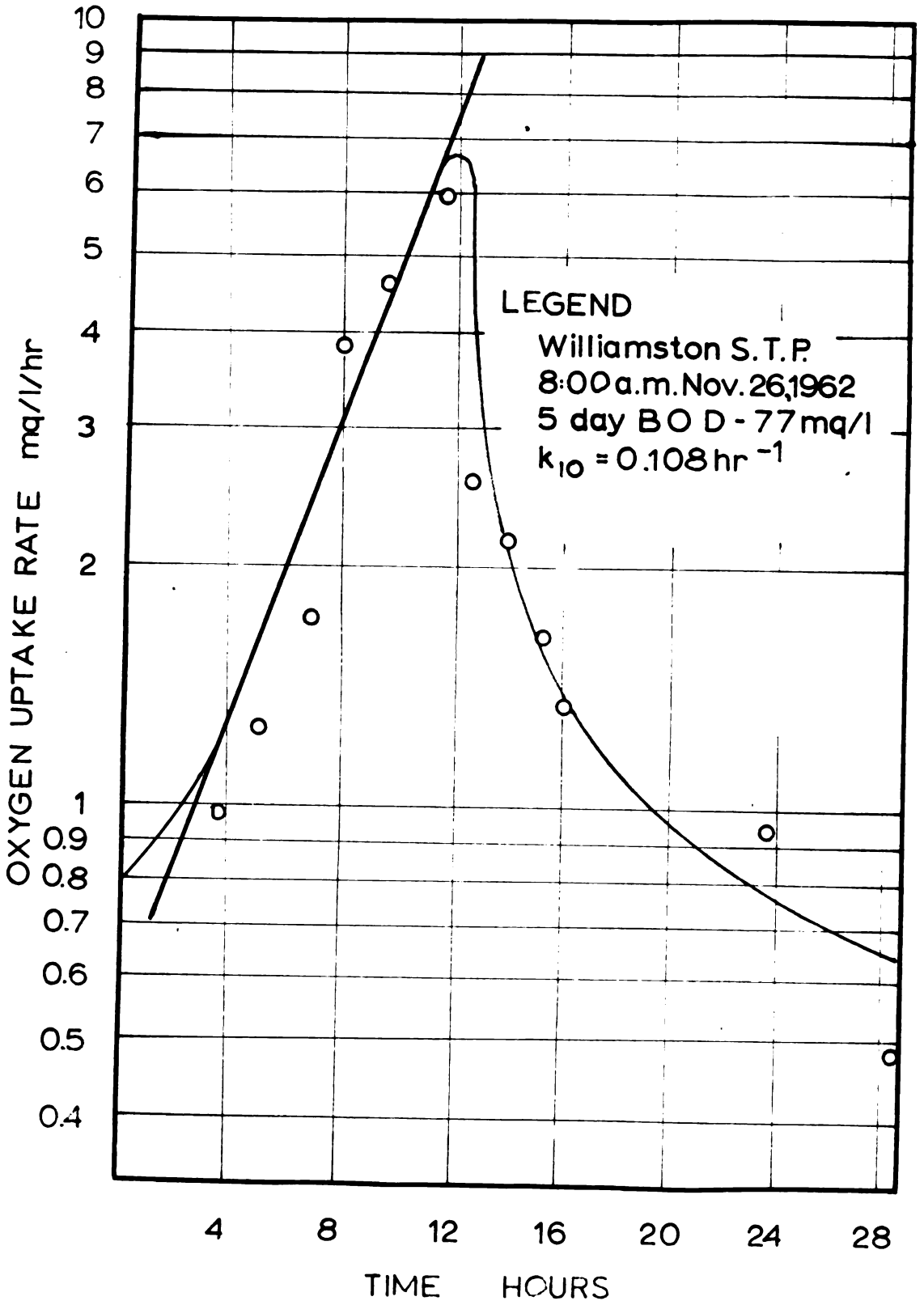


Figure 14 - Typical Respiration Rate plot of filtered sample with glucose added

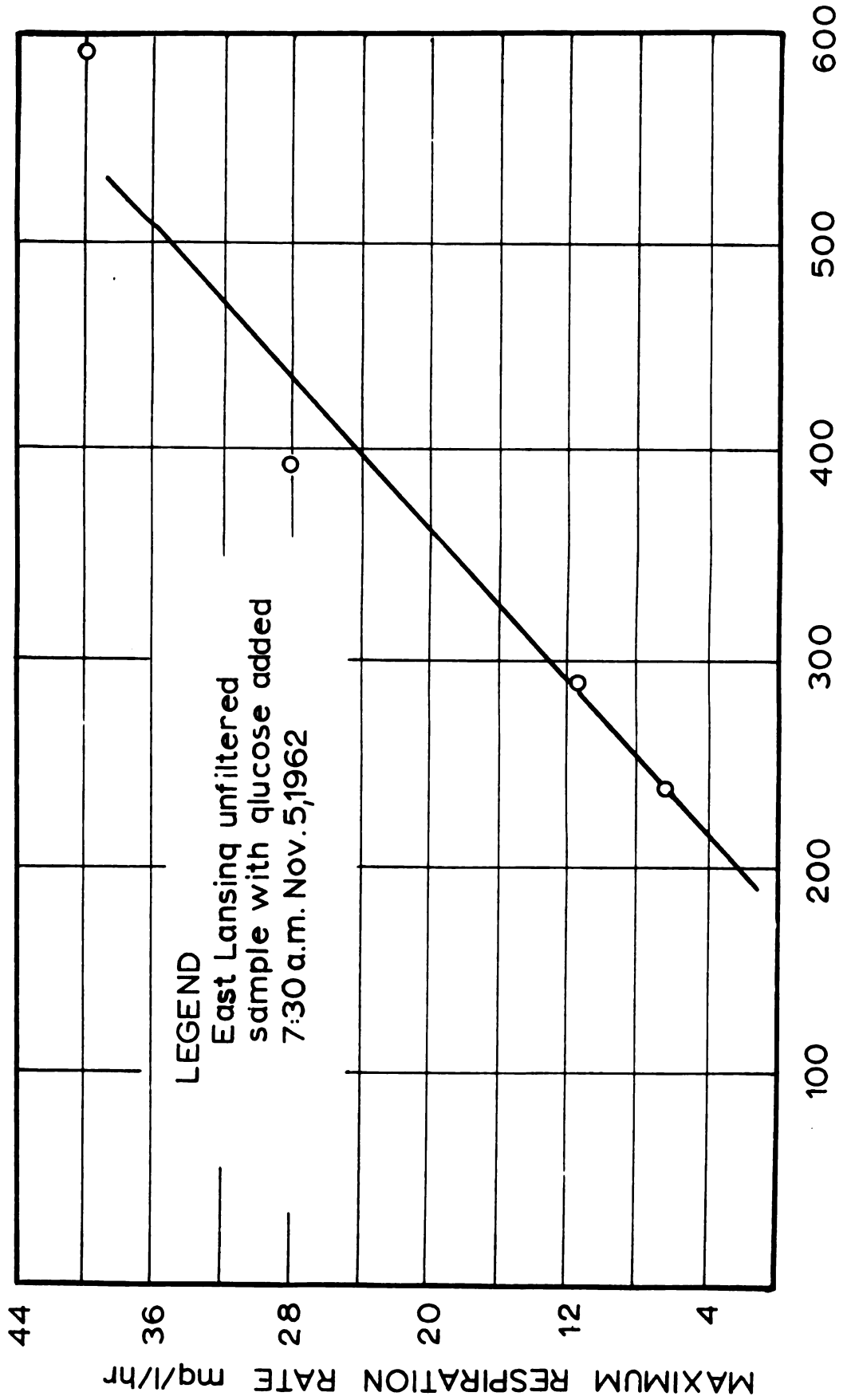
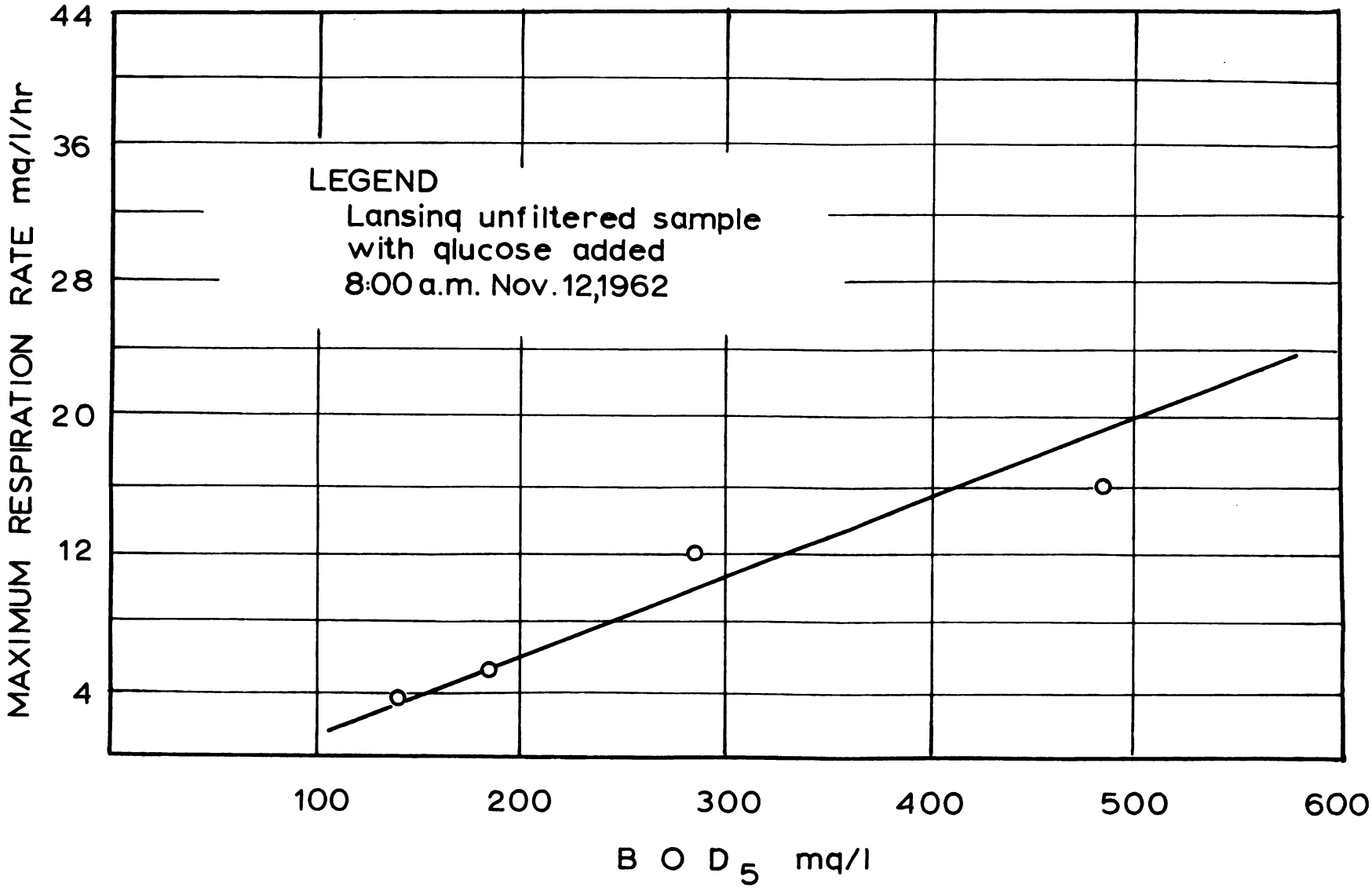


Figure 15 -Plot of Maximum Respiration Rate versus BOD_5



Fiqure 16 - Plot of Maximum Respiration Rate versus BOD_5

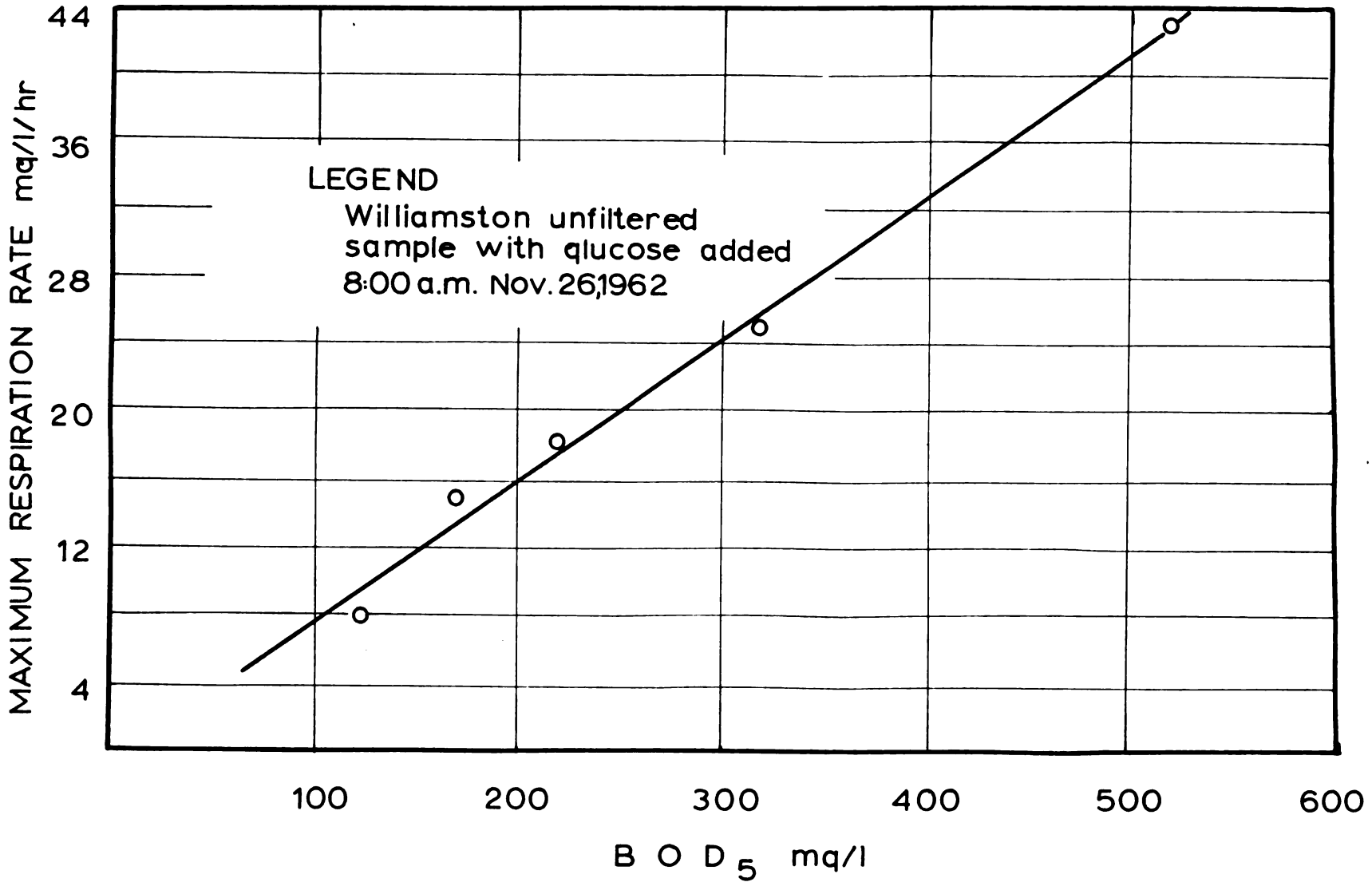


Figure 17 - Plot of Maximum Respiration Rate versus BOD_5

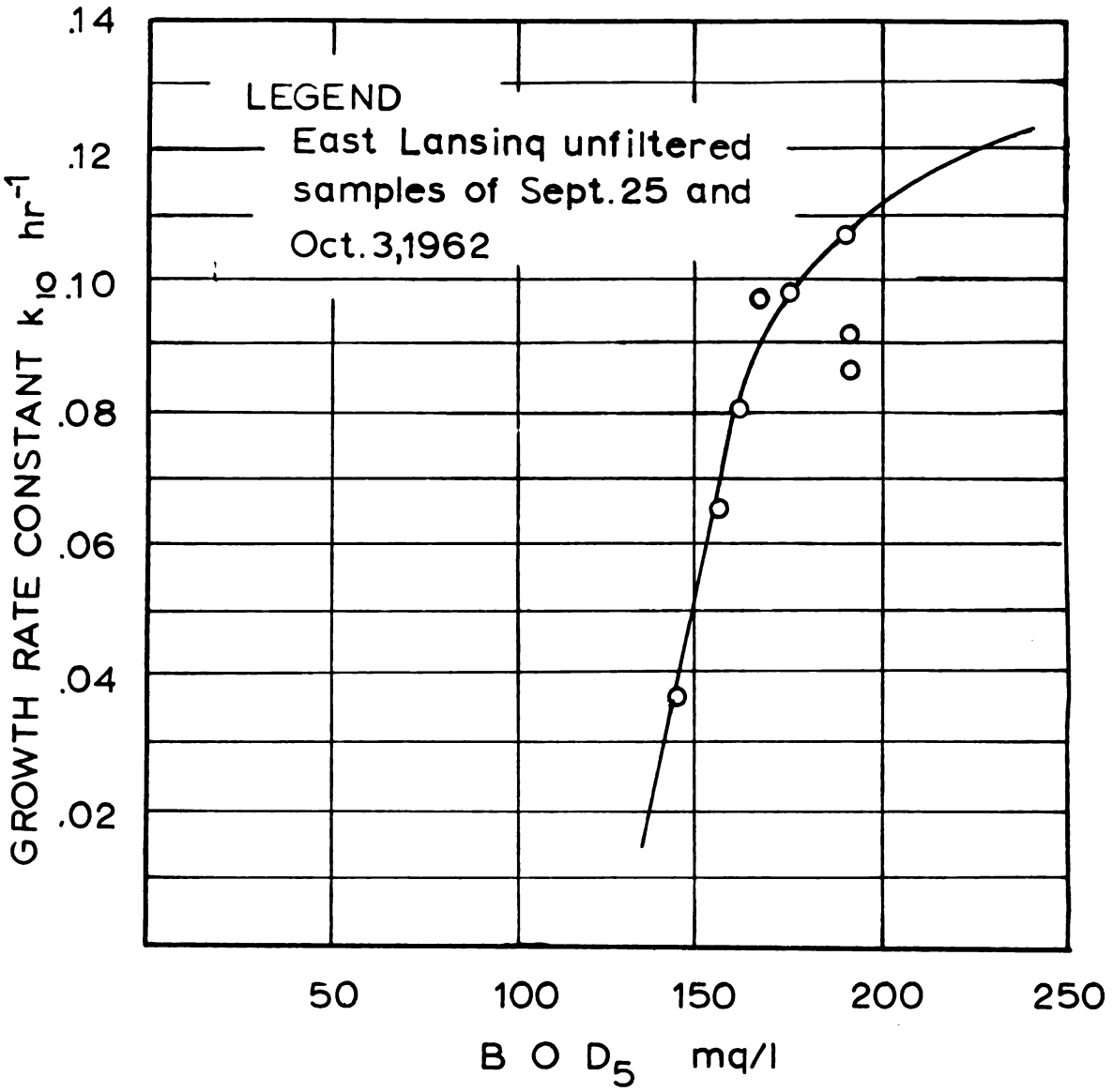


Figure 18 - Plot of Growth Rate Constant k_{10} and BOD_5

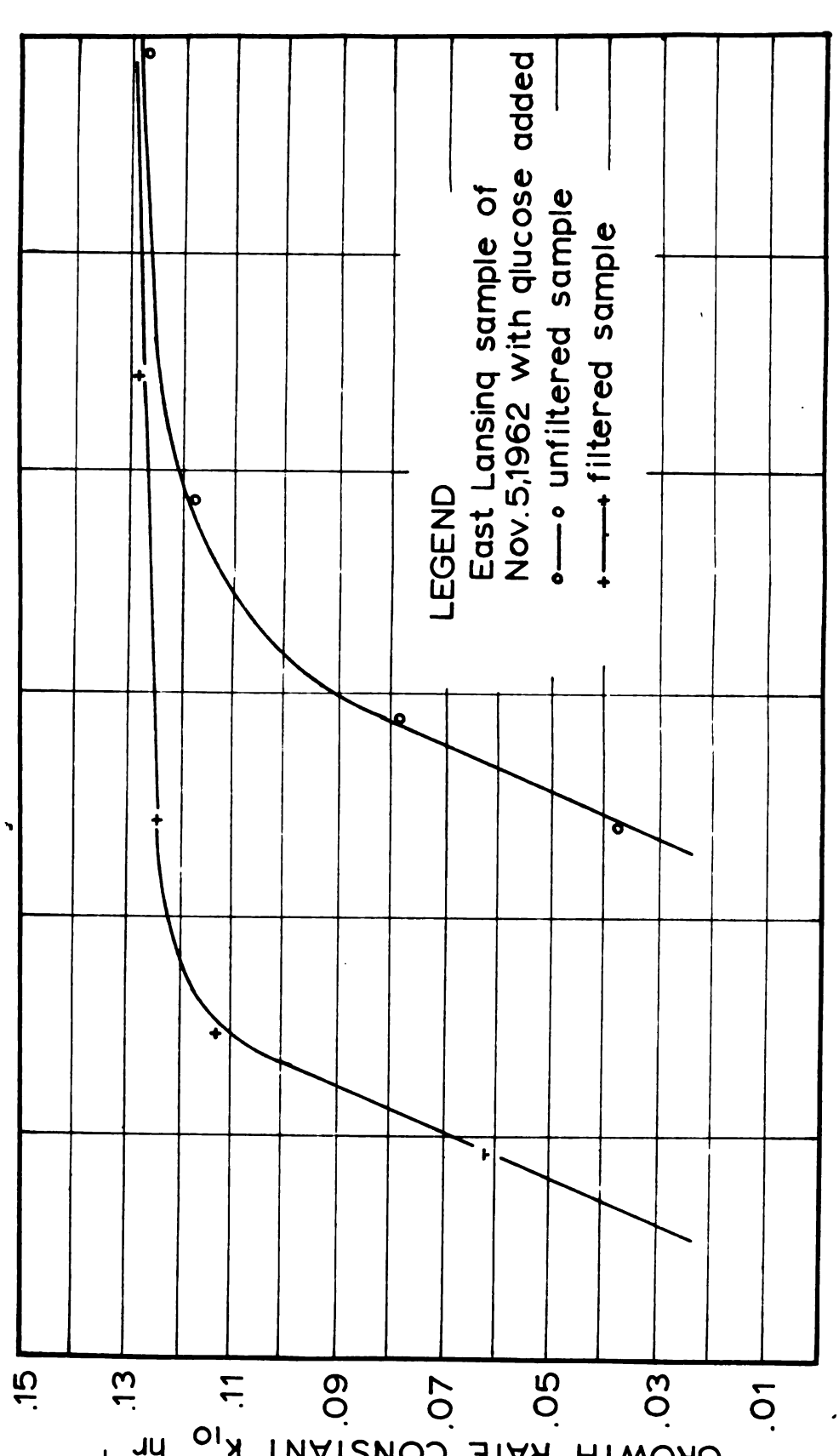


Figure 19 - Plot of Growth Rate Constant k_{10} and BOD_5

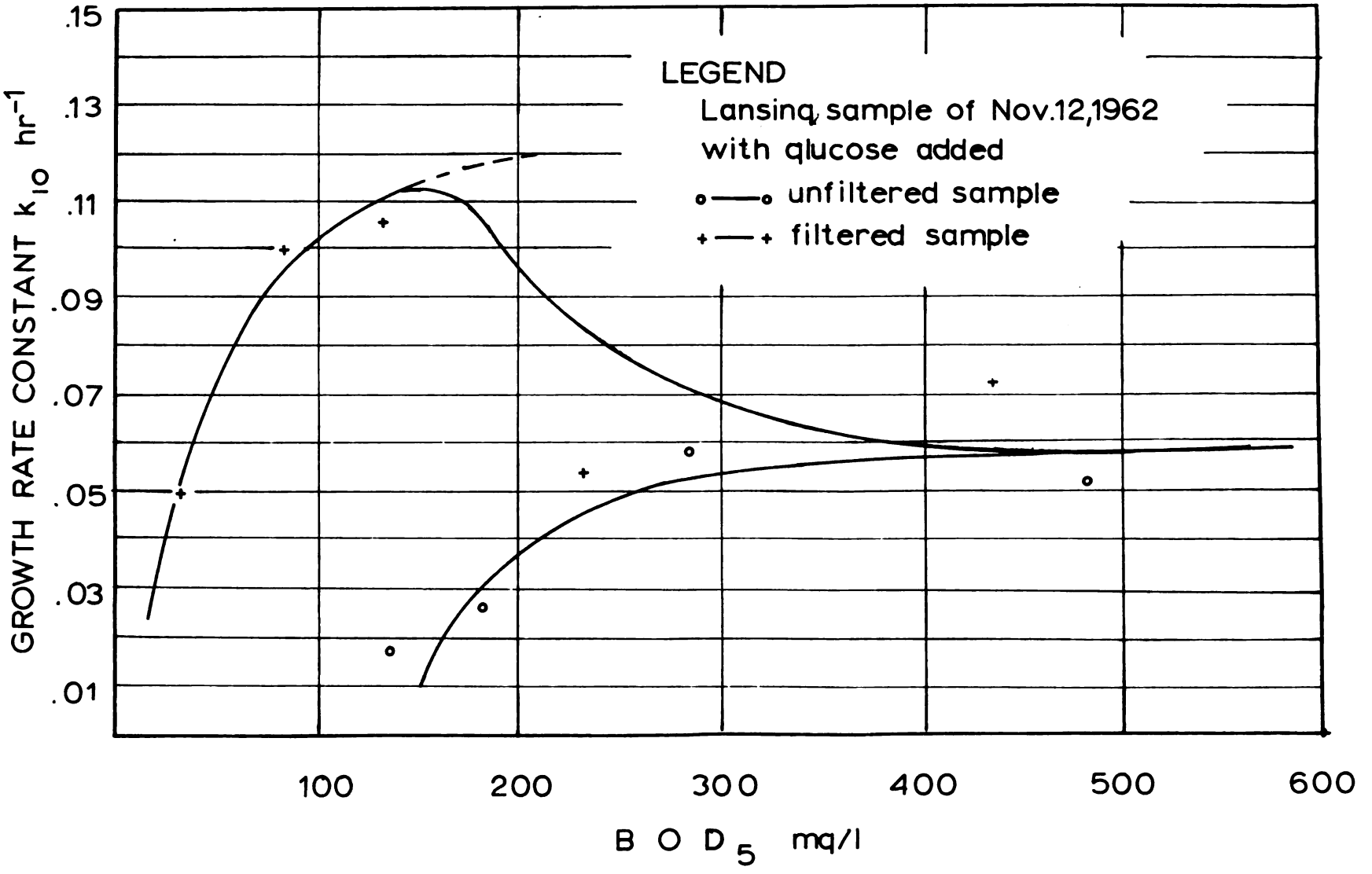


Figure 20- Plot of Growth Rate Constant k_{10} and BOD_5

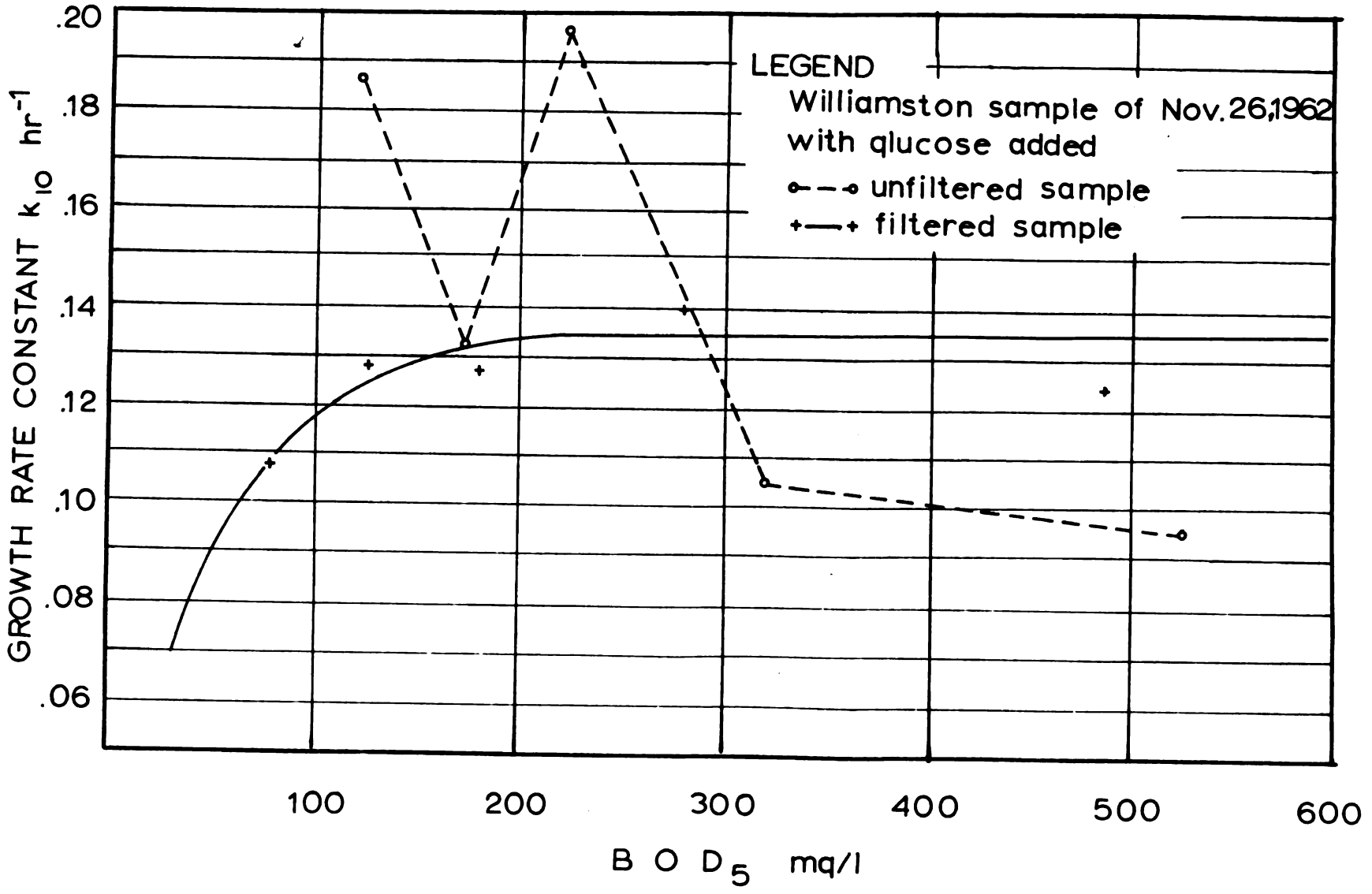


Figure 21 - Plot of Growth Rate Constant k_{10} and BOD_5

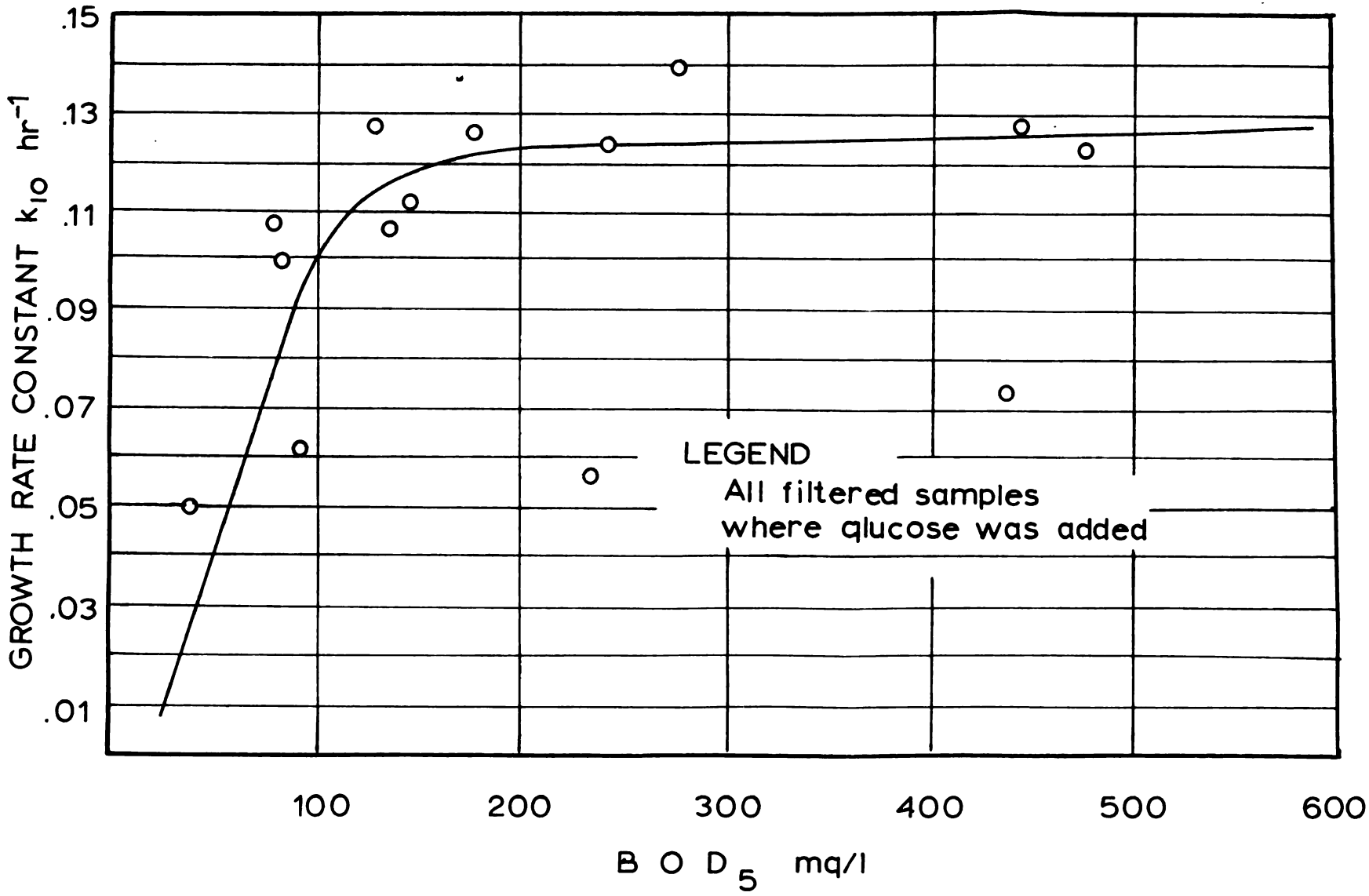


Figure 22 - Plot of Growth Rate Constant k_{10} and BOD_5

TABLE 1

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+13	13	4.3	4.3
2.0	- 4	+15	11	3.6	3.6
3.0	0	+15	15	4.9	4.9
4.0	- 2	+21	19	6.3	6.3
5.1	- 2	+31	29	9.5	9.5
6.0	- 4	+22	18	6.0	6.0
8.0	-16	+27	11	3.6	1.8
9.25	0	+17	17	5.6	4.5
10.75	- 5	+20	15	4.9	3.3
20.75	- 4	+60	56	18.5	1.8
25.5	+12	+ 8	20	6.6	1.4
27.25	+15	- 3	12	4.0	2.3
29.25	+ 5	+ 7	12	4.0	2.0

Type of sample - primary effluent
Date - 10:00 a.m. Sept. 25, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 2

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+16	16	5.1	5.1
2.0	- 4	+25	19	6.1	6.1
3.0	0	+28	28	8.9	8.9
4.0	- 2	+30	28	8.9	8.9
5.1	- 2	+21	19	6.1	6.1
6.0	- 4	+19	15	4.8	4.8
8.0	-16	+29	13	4.2	2.1
9.25	- 1	+22	21	6.7	5.3
10.75	- 5	+20	15	4.8	3.2
20.75	- 4	+75	71	22.7	2.3
25.5	+12	+16	28	9.0	1.9
27.25	+15	- 3	12	3.8	2.2
29.25	+ 5	+ 9	14	4.5	2.2

Type of Sample - primary effluent
Date - 12:00 a.m. Sept. 25, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 3

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+16	16	5.7	5.7
2.0	- 4	+23	19	6.7	6.8
3.0	0	+24	24	8.5	8.5
4.0	- 2	+24	22	7.8	7.8
5.1	- 2	+18	20	7.1	7.1
6.0	- 4	+17	13	4.6	4.6
8.0	-16	+25	9	3.2	1.6
9.25	- 1	+19	18	6.4	5.1
10.75	- 5	+19	14	5.0	3.3
20.75	- 4	+64	60	21.0	2.1
25.5	+12	+10	22	7.8	1.6
27.25	+15	- 4	11	3.9	2.2
29.25	+ 5	+ 7	12	4.3	2.1

Type of Sample - primary effluent
Date - 12:00 a.m. Sept. 25, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 4

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+16	16	5.5	5.5
2.0	- 4	+21	17	5.8	5.9
3.0	0	+22	22	7.8	7.8
4.0	- 2	+31	29	10.0	10.0
5.1	- 2	+42	40	13.7	13.7
6.0	- 4	+35	31	10.6	10.6
8.0	-16	+28	12	4.1	2.1
9.25	- 1	+32	31	10.6	8.5
10.75	- 5	+32	27	9.3	6.2
20.75	- 4	+91	87	30.0	3.0
25.5	+12	+23	35	12.0	2.5
27.25	+15	- 1	14	4.8	2.7
29.25	+ 5	+10	15	5.2	2.6

Type of sample - primary effluent
Date - 2:00 p.m. Sept. 25, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 5

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+13	13	4.6	4.6
2.0	- 4	+19	15	5.5	5.5
3.0	0	+19	19	7.0	7.0
4.0	- 2	+26	24	8.9	8.9
5.1	- 2	+37	35	12.8	12.8
6.0	- 4	+30	26	9.6	9.6
8.0	-16	+27	11	4.0	2.0
9.25	- 1	+27	26	12.8	10.2
10.75	- 5	+27	22	8.1	5.4
20.75	- 4	+81	77	28.5	2.8
25.5	+12	+19	31	11.4	2.4
27.25	+15	- 3	12	4.4	2.5
29.25	+ 5	+ 9	14	5.2	2.6

Type of Sample - primary effluent
Date - 2:00 p.m. Sept. 5, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 6

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+14	14	4.8	4.8
2.0	- 4	+21	17	5.8	5.8
3.0	0	+22	22	7.5	7.5
4.0	- 2	+30	28	9.6	9.6
5.1	- 2	+37	35	12.0	12.0
6.0	- 4	+25	21	7.2	7.2
8.0	-16	+28	12	4.1	2.1
9.25	- 1	+28	27	9.3	7.4
10.75	- 5	+27	22	7.6	5.1
20.75	- 4	+76	72	24.7	2.5
25.5	+12	+19	31	10.6	2.2
27.25	+15	- 1	14	4.7	2.7
29.25	+ 5	+ 8	13	4.4	2.2

Type of sample - primary effluent
Date - 4:00 p.m. Sept. 25, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 7

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+ 2	5	1.6	1.6
2.2	+ 7	+ 7	14	4.6	4.0
4.0	+ 7	+25	32	10.6	5.8
5.25	+ 2	+31	33	10.9	8.7
6.0	+ 4	+15	19	6.3	8.4
7.0	+ 2	+17	19	6.3	6.3
8.0	- 2	+12	10	3.3	3.3
10.0	- 3	+34	31	10.2	5.1
11.0	- 3	+15	12	4.0	4.0
12.0	0	+14	14	4.6	4.6
23.0	+ 8	+76	84	28.0	2.5
25.0	+ 1	+ 5	6	2.0	1.0

Type of Sample - primary effluent
Date - 6:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Plant

TABLE 8

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+ 3	6	2.1	2.1
2.2	+ 7	+ 9	16	5.6	4.8
4.0	+ 7	+27	34	12.0	6.6
5.25	+ 2	+31	33	11.7	9.3
6.0	+ 4	+15	19	6.7	8.9
7.0	+ 2	+13	15	5.3	5.3
8.0	- 2	+14	12	4.3	4.3
10.0	- 3	+31	28	9.8	4.9
11.0	- 3	+14	11	3.9	3.9
12.0	0	+12	12	4.3	4.3
23.0	+ 8	+71	79	27.7	2.5
25.0	+ 1	+ 3	4	1.4	.7

Type of Sample - primary effluent
Date - 6:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 9

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+ 7	10	3.2	3.2
2.2	+ 7	+13	20	6.4	5.5
4.0	+ 7	+36	43	13.8	7.5
5.25	+ 2	+39	41	13.0	10.4
6.0	+ 4	+21	25	8.0	10.7
7.0	+ 2	+46	48	15.4	15.4
8.0	- 2	+20	18	5.7	5.7
10.0	- 3	+45	42	13.3	6.7
11.0	- 3	+18	15	4.8	4.8
12.0	0	+18	18	5.7	5.7
23.0	+ 8	+98	106	34.0	3.1
25.0	+ 1	+ 5	6	1.9	.9

Type of Sample - primary effluent
Date - 8:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 10

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+10	13	4.6	4.6
2.2	+ 7	+12	19	6.7	5.8
4.0	+ 7	+31	38	13.5	7.4
5.25	+ 2	+34	36	12.7	10.2
6.0	+ 4	+18	22	7.9	10.5
7.0	+ 2	+34	36	12.7	12.7
8.0	- 2	+19	17	6.0	6.0
10.0	- 3	+41	38	13.5	6.7
11.0	- 3	+15	12	4.3	4.3
12.0	0	+14	14	5.0	5.0
23.0	+ 8	+87	95	34.0	3.1
25.0	+ 1	+ 4	5	1.8	.9

Type of Sample - primary effluent
Date - 8:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 11

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+12	15	5.2	5.2
2.2	+ 7	+12	19	6.5	5.6
4.0	+ 7	+30	37	12.8	7.0
5.25	+ 2	+32	34	11.7	9.4
6.0	+ 4	+17	21	7.2	9.6
7.0	+ 2	+39	41	14.0	14.0
8.0	- 2	+38	36	12.4	12.4
10.0	- 3	+39	36	12.4	6.2
11.0	- 3	+18	15	5.2	5.2
12.0	0	+14	14	4.8	4.8
23.0	+ 8	+93	101	34.6	3.1
25.0	+ 1	+ 6	7	2.4	1.2

Type of Sample - primary effluent
Date - 10:00 p.m. Oct.3,1962
Place - East Lansing Sewage Treatment Plant

TABLE 12

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+ 5	8	2.9	2.9
2.26	+ 7	+10	17	6.4	5.5
4.0	+ 7	+26	33	11.8	6.5
5.25	+ 2	+27	29	10.6	8.5
6.0	+ 4	+14	18	6.6	8.8
7.0	+ 2	+33	35	12.5	12.5
8.0	- 2	+35	33	11.8	11.8
10.0	- 3	+37	34	12.0	6.0
11.0	- 3	+15	12	4.3	4.3
12.0	0	+14	14	5.0	5.0
23.0	+ 8	+80	88	31.5	2.8
25.0	+ 1	+ 5	6	2.2	1.1

Type of Sample - primary effluent
Date - 10:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 13

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	+ 3	+ 8	11	3.8	3.8
2.1	+ 7	+14	21	7.3	6.3
4.0	+ 7	+30	37	12.8	7.0
5.25	+ 2	+33	35	12.0	9.6
6.0	+ 4	+15	19	6.5	8.7
7.0	+ 2	+16	18	6.2	6.2
8.0	- 2	+15	13	4.5	4.5
10.0	- 3	+33	30	10.4	5.2
11.0	- 3	+13	10	3.5	3.5
12.0	0	+13	13	4.5	4.5
23.0	+ 8	+71	79	27.0	2.4
25.0	+ 1	+ 5	6	2.1	1.1

Type of Sample - primary effluent
Date - 12:00 p.m. Oct. 3, 1962
Place - East Lansing Sewage Treatment Plant

TABLE 14

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	+13	13	4.8	4.8
2.0	0	+10	10	3.7	3.7
3.25	+ 2	+15	17	6.3	5.0
4.25	+ 1	+14	15	5.5	5.5
5.25	- 4	+16	12	4.4	4.4
6.25	- 4	+21	17	6.3	6.3
7.25	- 4	+18	14	5.2	5.2
8.41	- 4	+23	19	7.0	6.0
9.25	- 4	+10	6	2.2	2.6
10.25	- 6	+14	8	2.9	2.9
11.5	-10	+15	5	1.8	1.4
12.25	+ 1	+ 8	9	3.3	4.4
21.5	- 3	+46	43	16.0	1.7
22.75	- 3	+13	10	3.7	2.9
24.5	+ 9	+ 1	10	3.7	2.1
27.0	+20	-11	9	3.3	1.3
28.25	+ 4	- 2	2	.7	.6
29.25	+ 5	+ 3	8	2.9	2.9
30.25	+ 3	+ 1	4	1.5	1.5
32.75	+10	+ 1	11	4.0	1.6
34.25	+ 4	+ 3	7	2.6	1.7
36.25	+ 9	+ 6	15	5.5	2.8
45.75	+42	+13	54	20.0	2.1
47.25	-13	+12	0	-	-
49.25	+ 9	+14	23	8.5	4.2

Type of Sample - primary effluent (unfiltered)
Date - 7:30 a.m. Nov. 5, 1962
Place - East Lansing Sewage Treatment Plant
Glucose added - 67 mg/l

TABLE 15

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.0	0	- 1	0	--	--
2.0	0	+ 1	1	.4	.4
3.25	+ 2	0	2	.8	.6
4.25	+ 1	+ 3	4	1.6	1.6
5.25	- 4	+ 4	0	--	--
6.25	- 4	+ 6	2	.8	.8
7.25	- 4	+ 5	1	.4	.4
8.41	- 4	+ 6	2	.8	.7
9.25	- 4	+ 4	0	--	--
10.25	- 6	+ 8	2	.8	.8
11.5	-10	+ 8	0	--	--
12.25	+ 1	+ 4	5	2.0	2.6
21.5	- 3	+57	54	22.0	2.4
22.75	- 3	+11	8	3.2	2.5
24.5	+ 9	+ 9	18	7.3	4.2
27.0	+20	+ 7	27	11.0	4.4
28.25	+ 4	+15	19	7.7	6.2
29.25	+ 5	+ 2	7	2.8	2.8
30.25	+ 3	- 2	1	.4	.4
32.75	+10	- 6	4	1.6	.6
34.25	+ 4	- 1	3	1.2	.8
36.25	+ 9	- 2	7	2.8	1.4
45.75	+42	-29	13	5.3	.5
47.25	-13	+ 5	0	--	--
49.25	+ 9	+ 5	4	1.6	.8

Type of Sample - primary effluent (filtered)
Date - 7:30 a.m. Nov. 5, 1962
Place - East Lansing Sewage Treatment Plant
Glucose added - 67 mg/l

TABLE 16

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.5	+ 7	- 2	5	1.7	1.2
3.0	+ 8	- 3	5	1.7	1.2
4.0	- 1	+ 2	1	.3	.3
5.0	- 4	+ 5	1	.3	.3
6.0	- 3	+ 4	1	.3	.3
7.0	- 3	+ 6	3	1.0	1.0
8.5	- 5	+ 6	1	.3	.2
10.0	- 5	+ 7	2	.7	.5
11.5	- 5	+ 6	1	.3	.2
13.0	+ 2	+ 2	4	1.3	.9
20.0	- 4	+19	15	5.2	.7
22.33	0	+15	15	5.2	2.0
24.5	-11	+12	1	.3	.2
26.5	+ 8	+ 3	11	3.8	1.9
28.0	- 1	+ 7	6	2.1	1.4
29.5	- 2	+11	9	3.1	2.1
32.0	-13	+30	17	5.8	2.3
34.0	- 5	+20	15	5.2	2.6
36.0	-12	+26	14	4.8	2.4
44.5	-12	+63	51	17.5	2.1
47.0	- 1	+ 6	5	1.7	.7
48.5	+ 3	0	3	1.1	.7
50.0	+19	-14	5	1.7	1.1
52.5	+27	-23	4	1.4	.6
54.0	+ 1	+ 2	3	1.1	.7
56.5	+19	-12	7	2.4	1.0
58.5	+ 6	- 3	3	1.1	.5
60.0	+ 7	- 4	3	1.1	.5
68.0	+50	-36	14	4.8	.7
70.5	- 5	+ 6	1	.3	.1
72.5	+ 6	- 4	2	.7	.4
74.5	+11	- 9	2	.7	.4
77.0	+ 3	0	3	1.1	.4
81.0	-11	+16	5	1.7	.4
82.5	+ 1	0	1	.3	.2
84.5	- 1	+ 2	1	.3	.2
85.5	0	+ 2	2	.7	.7
93.5	+25	-17	8	2.8	.3
96.0	- 1	+ 4	3	1.1	.4

Type of Sample - primary effluent (unfiltered)
Date - 8:00 a.m. Nov. 12, 1962
Place - Lansing Sewage Treatment Plant
Glucose added - 67 mg/l

TABLE 17

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.5	+ 7	- 3	4	1.5	1.0
3.0	+ 8	- 8	0	--	--
4.0	- 1	+ 3	2	.7	.7
5.0	- 4	+ 4	0	--	--
6.0	- 3	+ 3	0	--	--
7.0	- 3	+ 3	0	--	--
8.5	- 5	+ 5	0	--	--
10.0	- 5	+ 5	0	--	--
11.5	- 5	+ 4	0	--	--
13.0	+ 2	- 1	1	.4	.3
20.0	- 4	+ 5	1	.4	.1
22.5	0	+13	13	4.9	2.0
24.5	-11	+ 5	0	--	--
26.5	+ 8	- 6	2	.8	.4
28.0	- 1	+ 4	3	1.1	.7
29.5	- 2	+ 6	4	1.5	1.0
32.0	-13	+22	9	3.4	1.4
34.0	- 5	+12	7	2.6	1.3
36.0	-12	+15	3	1.1	.6
44.5	-12	+23	12	4.5	.5
47.0	- 1	+ 2	1	.4	.2
48.5	+ 3	- 2	1	.4	.3
50.0	+19	-17	2	.8	.5
52.5	+27	-27	0	--	--
54.0	+ 1	0	1	.4	.2
56.5	+19	-14	5	1.8	.8
58.5	+ 6	- 4	2	.8	.4
60.0	+ 7	- 4	3	1.1	.7
68.0	+ 50	-18	32	12.0	1.5
70.5	- 5	+23	18	6.7	2.7
72.5	+ 6	+16	22	8.4	4.2
74.5	+11	+10	22	8.4	4.2
77.0	+ 3	+ 1	4	1.5	.6
81.0	-11	+15	4	1.5	.4
82.5	+ 1	- 1	0	--	--
84.5	- 1	+ 2	1	.4	.2
85.5	0	+ 1	1	.4	.4
93.5	+25	-22	3	1.1	.1
96.0	- 1	+ 4	3	1.1	.4

Type of Sample - primary effluent (filtered)
Date - 8:00 a.m. Nov. 12, 1962
Place - Lansing Sewage Treatment Plant
Glucose added - 67 mg/l

TABLE 18

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.5	+ 7	- 1	6	2.1	1.4
2.5	+ 9	0	9	3.2	3.2
3.5	+ 8	+ 4	12	4.3	4.3
5.0	+17	+15	32	11.3	7.5
6.5	+ 1	+25	26	9.2	6.2
8.0	-10	+25	15	5.3	3.5
9.5	- 6	+19	13	4.6	3.1
11.25	- 1	+ 9	8	2.8	1.6
12.5	+ 2	+ 4	6	2.1	1.7
14.0	+ 1	+ 5	6	2.1	1.4
15.0	+ 5	0	5	1.7	1.7
16.0	0	+ 1	1	.4	.4
23.25	-11	+35	24	8.5	1.2
25.25	- 2	+ 7	5	1.7	.9
27.0	+15	- 8	7	2.5	1.4
29.0	+ 3	+ 2	5	1.8	.9

Type of Sample - primary effluent (unfiltered)
Date - 8:00 a.m. Nov. 26, 1962
Place - Williamston Sewage Treatment Plant
No glucose added

TABLE 19

RESPIRATION RATES

Time Hrs.	Thermobarometer Reading	Respirometer Reading	Corrected Reading µl	Oxygen Consumed mg/l	Respiration Rate mg/l/hr
1.5	+ 7	-15	0	--	--
2.5	+ 9	-11	0	--	--
3.5	+ 8	- 5	3	1.0	1.0
5.0	+17	-11	6	1.9	1.3
6.5	+ 1	+ 7	8	2.6	1.7
8.0	-10	+28	18	5.9	3.9
9.5	- 6	+27	21	6.9	4.6
11.25	- 1	+33	32	10.5	6.0
12.5	+ 2	+ 8	10	3.3	2.6
14.0	+ 1	+ 9	10	3.3	2.2
15.0	+ 5	0	5	1.6	1.6
16.0	0	+ 4	4	1.3	1.3
23.25	-11	+32	21	6.9	1.0
25.25	- 2	+ 4	2	.6	.3
27.0	+15	-13	2	.6	.3
29.0	+ 3	0	3	1.0	.5

Type of Sample - primary effluent (filtered)
Date - 8:00 a.m. Nov. 26, 1962
Place - Williamston Sewage Treatment Plant
No glucose added

TABLE 20

TYPE OF SAMPLE, S.S., B.O.D., AND pH

Date	Time	Type of Sample	S.S.	B.O.D.	pH
		East Lansing			
Sept.25,1962	10:00 a.m.	primary effluent	74	145	7.6
Sept.25,1962	12:00 a.m.	"	60	160	7.3
Sept.25,1962	2:00 p.m.	"	102	188	7.1
Sept.25,1962	4:00 p.m.	"	106	176	7.0
Oct. 3,1962	6:00 p.m.	"	202	164	7.8
Oct. 3,1962	8:00 p.m.	"	192	191	7.3
Oct. 3,1962	10:00 p.m.	"	88	191	7.3
Oct. 3,1962	12:00 p.m.	"	184	156	7.8
Nov. 5,1962	7:30 a.m.	unfiltered primary effluent	252	189	--
Nov. 5,1962	7:30 a.m.	filtered primary effluent	--	43	
		Lansing			
Nov. 12,1962	8:00 a.m.	unfiltered primary effluent	153	86	7.7
Nov. 12,1962	8:00 a.m.	filtered primary effluent	--	34	
		Williamston			
Nov. 26,1962	8:00 a.m.	unfiltered primary effluent	74	118	--
Nov. 26,1962	8:00 a.m.	filtered primary effluent	--	77	

TABLE 21

GROWTH RATES, MAXIMUM RESPIRATION RATE, AND BOD_5

Sample	Growth Rate k_{10}, hr^{-1}	Max. Resp. Rate mg/l/hr	BOD_5 mg/l
East Lansing Sept. 25, 1962			
10:00 a.m.	0.036	10.0	145
12:00 a.m.	0.081	10.0	160
2:00 p.m.	0.119	14.0	188
4:00 p.m.	0.098	14.0	176
East Lansing Oct. 3, 1962			
6:00 p.m.	0.097	9.5	168
8:00 p.m.	0.087	12.5	191
10:00 p.m.	0.092	13.0	191
12:00 p.m.	0.066	10.0	156

TABLE 22

GROWTH RATES, MAXIMUM RESPIRATION RATE, AND BOD_5

Sample	Growth Rate k_{10}, hr^{-1}	Max. Resp. Rate mg/l/hr	BOD_5 mg/l
East Lansing Nov. 5, 1962			
unfiltered no glucose added	*	*	189
unfiltered +67 mg/l glucose	0.036	6.5	239
unfiltered +134 mg/l glucose	0.078	11.5	289
unfiltered +268 mg/l glucose	0.118	28.0	389
unfiltered +536 mg/l gulcose	0.126	38.0	589
filtered no glucose added	*	*	43
filtered +67 mg/l glucose	0.062	6.0	93
filtered +134 mg/l glucose	0.112	15.0	143
filtered +268 mg/l glucose	0.125	25.0	243
filtered +536 mg/l glucose	0.128	32.0	443
Lansing Nov. 12, 1962			
unfiltered no glucose added	*	*	86
unfiltered +67 mg/l glucose	0.019	3.3	136
unfiltered +134 mg/l glucose	0.026	5.9	186
unfiltered +268 mg/l glucose	0.059	12.0	286
unfiltered +536 mg/l glucose	0.053	17.0	486
filtered no glucose added	*	*	34
filtered +67 mg/l glucose	0.100	4.9	84

TABLE 22--Continued

Sample	Growth Rate k_{10}, hr^{-1}	Max. Resp. Rate mg/l/hr	BOD_5 mg/l
filtered +134 mg/l glucose	0.106	11.5	134
filtered +268 mg/l glucose	0.055	18.0	234
filtered +536 mg/l glucose	0.072	35.0	434
Williamston Nov. 26, 1962			
unfiltered no glucose added	0.184	8.0	118
unfiltered +67 mg/l glucose	0.132	15.0	168
unfiltered +134 mg/l glucose	0.196	18.5	218
unfiltered +268 mg/l glucose	0.104	25.0	318
unfiltered +536 mg/l glucose	0.093	42.0	518
filtered no glucose added	0.108	6.5	77
filtered +67 mg/l glucose	0.128	13.0	127
filtered +134 mg/l glucose	0.127	20.0	177
filtered +268 mg/l glucose	0.140	44.0	277
filtered +536 mg/l glucose	0.123	44.0	477

*Samples for which k_{10} could not be obtained.

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