A DIFFERENTIAL MANOMETER FOR THE MEASUREMENT OF GROWTH RATES IN SEWAGE

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Thomas Charles Hoogerhyde

1965

ABSTRACT

A DIFFERENTIAL MANOMETER FOR THE MEASUREMENT

OF GROWTH RATES IN SEWAGE

by Thomas Charles Hoogerhyde

A differential manometer is presented which enables the investigator to measure oxygen consumption of sewage directly. The volume of gas absorbed is measured to the nearest 0.002 ml by a plastic micrometer syringe attached to the apparatus.

A comparison was made between the above differential manometer and the Warburg respirometer. The results indicate that the differential manometer provides much more accurate data.

Outside of the introduction of a short lag phase, it was found that storage at 0-5°C for 24 hours had no apparent effect on the oxygen uptake of samples.

The reproducibility of data was investigated by making parallel runs with primary effluent from the Lansing and East Lansing Sewage Treatment Plants. The results indicated good reproducibility.

Oxygen uptake rates were measured in samples taken from the primary effluent of the Lansing, East Lansing, Mason, and Williamston Sewage Treatment Plants. The oxygen uptake rates vs. time were plotted on semi-log paper and the bacterial growth rate determined from the slope of the linear portion of the curve.

A comparison was made between growth rates measured by the differential manometer, by photometric, and by gravimetric methods on replicate samples. It was found that the growth rates measured by the three methods agreed closely. The differential manometer produced a growth rate of 0.430/hr, while the gravimetric and photometric methods resulted in 0.420/hr, and 0.390/hr respectively.

It was found that the initial cell concentration had an effect on the initial oxygen consumption rate, the maximum oxygen uptake rate, and the time of occurrence for the maximum rate. Generally, the higher the initial cell concentration; the higher the initial oxygen consumption, the higher the maximum oxygen uptake rate, and the sooner the time of occurrence for the maximum rate. The initial cell concentration could be increased to a point where the maximum oxygen uptake rate would be reached instantaneously.

The relationship between the growth rate of bacteria and BOD was studied for the East Lansing Primary Effluent. It was found that the growth rate increased with increasing BOD and appeared to level off at higher BOD values. The results indicated that the growth rate was also a function of the composition of the substrate (BOD).

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by

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A THESIS

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

MASTER OF SCIENCE

Department of Civil and Sanitary Engineering

1965

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. Karl L. Schulze of the Department of Civil and Sanitary Engineering whose valuable guidance and assistance made this thesis possible.

This project was supported in part by a Public Health Traineeship from the Public Health Service, U.S. Department of Health, Education, and Welfare.

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LIST OF SYMBOLS

B.O.D. - Biochemical Oxygen Demand

$B.O.D._5$ - 5-day Biochemcial Oxygen Demand

k_1 - specific growth rate to the base e

r_r - oxygen uptake rate, mg/l/hr

O_2 - oxygen

μ - micron

O.D. - optical density

ml. - milliliters

mg. - milligrams

mm. - millimeters

ppm. - parts per million

mg/l - milligrams per liter

mg/l/hr - milligrams per liter per hour

hr^{-1} - per hour

min. - minutes

hrs. - hours

pH - inverse logarithm of the hydrogen ion concentration

mgd. - million gallons per day

cfs - cubic feet per second

I. INTRODUCTION

Over the last 75 years, various manometric devices have been used by several investigators for the measurement of oxygen consumption in sewage (Adeney, as found in Jenkins (1960); Lovett and Garner, 1936; Sierp, 1928; Falk and Rudolf, 1947; Bloodgood, 1938; Sawyer and Nichols, 1930; Caldwell and Langelier, 1948; Barcroft, 1908; Coaker and Murrary, as found in Jenkins (1960); Wheatland and Loyd, 1955; Snaddon and Harkness, 1959; and Jenkins, 1960). During that time the manometer and its technique has been refined and modified.

One of the persistant drawbacks that has limited the use of manometric techniques in the waste treatment field is the amount of time involved in manipulating cumbersome and inconvenient units. Manometer flasks and apparatus had to be carefully calibrated, and flask constants computed through the use of involved equations.

The differential manometer presented in this thesis measures directly the volume of oxygen consumed during a given time interval. Calibration of the flasks and apparatus is not necessary and cumbersome constants are eliminated.

The primary purpose of this thesis is to demonstrate the range and applicability of the differential manometer for the measurement of microbial growth rates in sewage. Preliminary investigations concerning a comparison with Warburg data, reproducibility of data, effect of storage and initial cell concentration, growth rate measurements, and the relationship between growth rates and BOD in sewage were made with this manometer.

II. LITERATURE REVIEW

Investigators have used manometric techniques for determining the rate of oxygen utilized by microorganisms since the late 1800's. Basically, these manometers measured the volume of oxygen consumed after removal of carbon dioxide produced. Jenkins (1960) has prepared a summary of the development of manometers and techniques used by past investigators.

In 1908 Adeney, as found in Jenkins (1960), made one of the first published attempts to measure oxygen consumption in sewage. Two vessels, one containing sewage and the other an equal amount of water, were connected to a graduated U-tube. At constant temperature and pressure, the oxygen consumption was determined by measuring the displacement of water in the U-tube. Dissolved oxygen was maintained in the sample by periodically shaking the apparatus.

Lovett and Garner (1936) developed a modified version of a manometer devised by Sierp (1928), which did not require corrections for variations in atmospheric pressure. Falk and Rudolf (1947) further modified the Sierp apparatus by making all connections with ground glass joints. They found that carbon dioxide was not immediately absorbed and that a correction factor should be applied.

Manometric devices employing air circulation through the sample were developed by Bloodgood (1938) and Sawyer and Nichols (1930) in the late 1930's. Oxygen consumption was determined by measuring the decrease in volume of the circulating air, at constant pressure.

The Warburg and Barcroft respirometers were not used for sewage analysis until about the 1930's.

The Warburg apparatus and its techniques have been described by several authors (Dixon, 1943; Caldwell and Langelier, 1948; Umbreit, Burris and Staffer, 1945). It is a constant volume device in which oxygen consumption is determined from changes in pressure between a closed reaction flask and the atmosphere. The Warburg is very sensitive to changes in temperature and atmospheric pressure. Because of this, a thermobarometer is used to compensate for the variations in temperature and atmospheric pressure. A constant temperature water bath reduces the effect of temperature.

The differential manometer developed by Barcroft (1908) operates under a closed system. One end of the manometer U-tube is connected to a reaction flask while the other end is connected to a temperature and pressure compensation flask. The system is not open to the atmosphere. There is no manometer fluid reservoir, so oxygen consumption is determined by measuring the difference between the readings of both sides of the U-tube.

The reaction flasks for the Warburg and Barcroft manometers are constructed with a center well for the addition of a strong alkali to remove the carbon dioxide produced in respiration. Dixon and Elliot (1930) pointed out that a high grade filter paper should be placed in the well to increase the absorptive surface. They also noticed that oxygen uptake tended to increase due to oxidation of the filter paper when alkali concentration exceeded 10%.

Caldwell and Langelier (1948) used 125 ml reaction flasks in order to overcome the disadvantage of small sample volumes in the 15 ml flasks used previously. The authors felt that a more representative sample could be analyzed with these flasks. They used volumes of up to 75 ml with close agreement on oxygen consumption in replicate samples. Wilson (1954) indicated that conditions in this unit are very similiar to those in an activated sludge plant employing mechanical aeration.

As the interest in the measurement of oxygen consumption in sewage increased, it became imperative that large-volume respirometers be developed. The smaller respirometers were not suited for work in the waste treatment field.

Coaker and Murray, as found in Jenkins (1960), constructed a respirometer based on the Barcroft principles, but using a one liter reaction flask. They were able to study sample volumes of up to 600 ml. The range of the apparatus was about 3.5 times that of the standard Barcroft. Wheatland and Loyd (1955) developed a large constant pressure respirometer, capable of holding up to 750 ml of sample. They devised a means of stirring in a closed system by rotating a polythene-covered magnet in the field of an electromagnet located below the reaction flask and under the water bath. Snaddon and Harkness (1959) modified the Wheatland and Loyd apparatus and simplified calculations necessary for its use. Jenkins (1960) designed a multi-purpose apparatus which was similiar to that of Snaddon and Harkness. He was able to add or remove samples during a test run, and isolate the reaction flask from the manometer and oxygen supply without interrupting the test.

Several authors have investigated the effect of the rate of shaking on oxygen-uptake. (Dixon and Elliot, 1930; Caldwell and Langelier, 1948; Dawson and Jenkins, 1949). They found that the rate of oxygen uptake must be independent of the rate of shaking. If this relationship is not maintained oxygen absorption from gas to liquid may limit the rate of respiration.

Several workers have established that, under aerobic conditions, carbon dioxide is the only gas liberated in the biological oxidation of sewage (Woolridge and Standfast, 1936; Caldwell and Langelier, 1943; and Dawson and Jenkins, 1949). Such gases as hydrogen, methane, hydrogen sulfide, and nitrogen were not formed.

Butterfield (1938) found the oxygen consumption per bacterial cell to be very small. In order to produce a measurable oxygen consumption of 0.1 mg/l., 100,000 - 500,000 cells per milliliter were required. Carpenter (1961) reports that domestic sewage contains between 0.5×10^6 and 20×10^6 microorganisms per milliliter. Martin (1932) relates the maximum oxygen consumption per cell to the maximum cell surface area attained near the end of the lag phase of the growth curve.

Longmuir (1954) found the respiration rate of bacteria to be a function of oxygen concentration. He expressed this relationship in the form of the Michaelis-Menten equation. Smith (1953) found that the respiration rate of activated sludge was not significantly affected by dissolved oxygen concentrations in the range of 0.2 to 6 ppm. Heukelekian (1936) studied the relationship between oxygen tension and bacterial numbers in sewage. He found that optimum rates of growth occurred between 2.0 and 3.5 ppm and between 12.0 and 18.0 ppm dissolved oxygen. Eckenfelder and O'Connor (1961) reported that when the oxygen concentration is larger than 0.2 - 0.5 ppm, the rate of bacterial respiration is independent of oxygen concentration.

Muller, as found in Clark (1962, p. 14), stated in 1911 that the maximum oxygen loss per hour coincided with the maximum bacterial count. He did not find a direct relationship between these two parameters, however. Clifton (1937)found that the rate of oxygen consumption increased in growing cultures of *Aerobacter aerogenes*, *Eberthella typhi*, and *Escherichia coli*.

Sawyer and Nichols (1930) studied the effect of sludge concentration on the rate of oxidation. They found that the rate of oxygen consumption was directly proportional to the sludge concentration. Crieg and Hoogerheide (1941) used Warburg manometers to study growing cultures of different species of bacteria. They found that the oxygen uptake of growing cultures was directly proportional to bacterial content.

The relationship between growth rate and respiration rate was investigated by Schulze and Lipe (1964). In their studies with a continuous flow culture of *E. coli*, they found that the respiration rate was directly proportional to the specific growth rate. Herbert, as found in Schulze (1964), obtained similar results for A. aerogenes.

Penfold and Norris (1912), in their studies concerning the nature of the relationship between the rate of growth and the concentration of food, found that the generation time varied inversely with food concentration. In 1944, while discussing the theory of the BOD equation, Phelps (1944) implied that the metabolic activities of bacteria proportion themselves exactly to the concentration of available organic matter remaining at any time. Garrett and Sawyer (1952) reported that the rate of bacterial growth in sewage is constant at high concentrations of soluble BOD, and at low concentrations the rate of growth is directly proportional to the remaining soluble BOD.

Several other authors have studied the relationship between substrate concentration and growth rate (Monod, 1949; Hinshelwood, 1946; Novick, 1955; Herbert, Elsworth, and Telling, 1956; Schulze and Lipe, 1964). The above authors reported that the growth rate asymptotically approaches a maximum value as the substrate concentration increases.

Dick (1964) measured growth rates at the East Lansing, Michigan Sewage Treatment Plant using a Warburg apparatus. He found the growth rate independent of BOD when dissolved BOD concentrations were above 200 ppm. The maximum growth rate obtained in his studies was 0.30 per hour.

Garrett and Sawyer (1952) established maximum reaction rates of 0.08 per hour at 10°C., 0.20 per hour at 20°C., and 0.30 per hour at 30°C. from studies with synthetic media containing glucose and peptone.

III. APPARATUS, MATERIALS AND METHODS

A. Sampling

1. Type of Sample

In order to obtain as much variety in sample composition as possible, samples were taken from several sewage treatment plants around the Lansing area. These plants were, in order of decreasing size, the Lansing, East Lansing, Mason, and Williamston Sewage Treatment Plants. A brief description of the essential characteristics concerning the primary effluent of each plant follows:

Lansing - The Lansing Sewage Treatment Plant is an activated sludge plant treating an average flow of 22 mgd., serving a population of 120,000. A substantial portion of the waste comes from industries located in the Lansing area. Supernatant from the digestors and filtrate from the sludge filters are returned through the primary tanks at the rate of 75 - 100,000 gallons/day. Excess activated sludge at the rate of 0.8 - 1.0 million gallons/day is also continually wasted through the primary tanks.

East Lansing - The East Lansing Sewage Treatment Plant is an activated sludge plant treating an average flow of 4.5 mgd., serving a population of 20-30,000 in the summer and 50-60,000 during the months of September through June. The fluctuations in population (and hence, the volume of the sewage) are caused by Michigan State University. Practically no industrial wastes are treated. Excess activated sludge at the rate of 10% of the total flow is continually wasted through the primary tank.

Mason - The Mason Sewage Treatment Plant is an activated sludge plant treating an average flow of 450,000 gallons/day, serving a population of 5,000. Industrial wastes from the Gerber plant is treated. Excess activated sludge at the rate of 15,000 gallons/day is fed into and settled out of the primary settling tank.

Williamston - The Williamston Sewage Treatment Plant uses only primary settling for its waste treatment. The plant treats an average flow of 180,000 gallons/day, serving a population of 2,100. The raw sewage is pre-chlorinated before it enters the primary settling tank. The sludge from the primary settling tank is filtered with a Komline-Sanderson Filter without the aid of chemical coagulants. When the filter is in operation, the filtrate is pumped into the primary settling tank.

2. Sampling Techniques

All samples were taken from the primary effluent of the various treatment plants. Grab samples were taken from all four plants and 24 hour composite samples were taken from the East Lansing Plant.

Grab samples were collected in 300 ml BOD bottles and stored in ice water (0-5^{o}C.) until ready for use. Since some of the samples were collected during the afternoon and evening, it was necessary to store them overnight. The storage time, however, never exceeded a period of 24 hours. In order to determine the changes that occurred in the sewage throughout the day, grab samples were taken at various times during a 24 hour period. When this was not possible, samples were collected at different hours in the day, on different days. Thus, a sample might be collected at 9:00 A.M. one day, 11:00 A.M. another, 3:00 P.M. another, and so on. This provided a wider range of BOD values since the waste usually has a lower BOD in the morning than it has in the afternoon.

Composite samples, proportional to the flow were obtained from the East Lansing Plant. The samples were collected with a modification of the automatic sampler described by Gard and Snavely (1952). The sample was kept at 0-5°C. during the 24 hour collection period by means of refrigeration.

3. Design of the Composite Sampler

As mentioned above, the basic design of the sampler scoop was taken from Gard and Snavely (1952). The sampler, as described by the above authors, was designed to set in place in a break made in a plant sewer line. The sampler operated in a wooden box ahead of a 90° V-notch steel weir. A float operated depth recorder made a continuous 24 hour record of the waste flow, while the sampler "scooped" a sample every two minutes. The sampler was designed to yield 10 gallons of sample based on an average 24 hour flow of 200,000 gallons.

A schematic drawing of the modified sampler installation and details of the scoop assembly are shown in Figure 1. The sampler was designed to operate in the effluent channel of the East Lansing primary tank.

Since the shape of the scoop determines the accuracy of the sampler in providing a proportionate composite sample, the scoop was designed according to the formulas employed by Gard and Snavely. The formulas given assume that the volume of each individual sample withdrawn by the scoop shall vary as d^n which is indicative of flow through a weir or Parshall flume. In our case the flow is open channel flow and Q is not proportional to d^n but is proportional to

$$\left[\frac{d^{5/2}}{2d + 1}\right]^{2/3}$$

The above relationship is derived from the Manning formula for open channel flow

$$V = \frac{1.486}{n} R^{2/3} \; S^{1/2}$$

For a one foot wide channel,

$$R = \frac{d}{2d + 1} \text{ and } A = (1)d = d$$

where, R = hydraulic radius, feet

A = Cross-sectional area, square feet

d = depth of water, feet

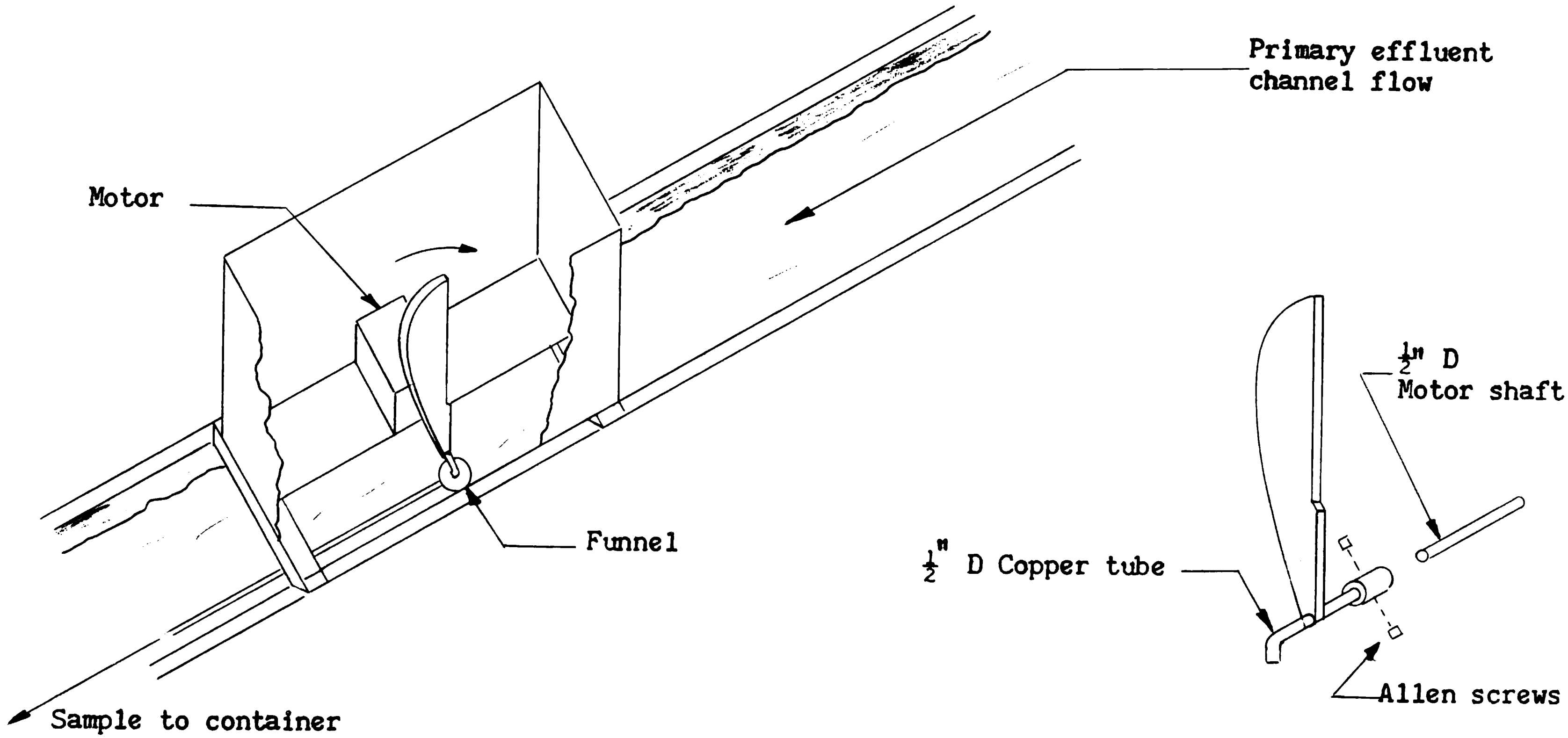
Also, for the East Lansing channels:

S = 0.014

n = 0.013 (assumption)

From the above conditions:

$$AV = dV = Q = 13.5 \left[\frac{d^{5/2}}{2d + 1}\right]^{2/3}$$



Schematic view of sampler installation

Scoop assembly

Figure 1. Sampler installation and details of scoop assembly

The average depth in the channel was computed to be 6.68 inches with the above formula based on an average flow of 2 mgd (3.09 cfs). Using this computed depth in the formula for flow over a rectangular weir, $Q = 3.33\ Ld^{1.5}$, L was found to be 2.14 feet for the same flow. The flow through a rectangular weir 2.14 feet wide compares quite favorably with the flow through a one foot wide rectangular channel as illustrated in Figure 2. From this we concluded that the scoop could be designed for a 2.14 foot wide rectangular weir and be used quite accurately in the one foot wide primary effluent channel.

The sampler was designed to yield a one gallon sample per 24 hours at a sampling rate of once every one-half hour based on an average flow of 2 mgd. through the primary tank. The thickness of the scoop was set at $\frac{1}{2}$ inch. The shape of the scoop obtained by computation is illustrated in Figure 3. The scoop was constructed of 26 gauge monel sheeting. All joints were soldered.

The scoop was mounted on a $\frac{1}{2}$ inch diameter shaft of a small motor. A $\frac{1}{2}$ inch diameter copper tube was inserted into the bottom of the scoop in such a manner that when the scoop was in an upright vertical position, the sample ran out. The sample poured out of the scoop into a 3 inch 60° funnel from which it was sucked into a container by means of an applied vacuum. A General Electric 30 minute cycle timer, model No. 3TSA14, was used to activate the motor at 30 minute intervals. During the period of activation, the scoop made exactly one complete revolution. A Cenco Hyvac 7 Mechanical Vacuum Pump was connected to the same timing system so that it was in operation during the same time interval as the scoop. The vacuum

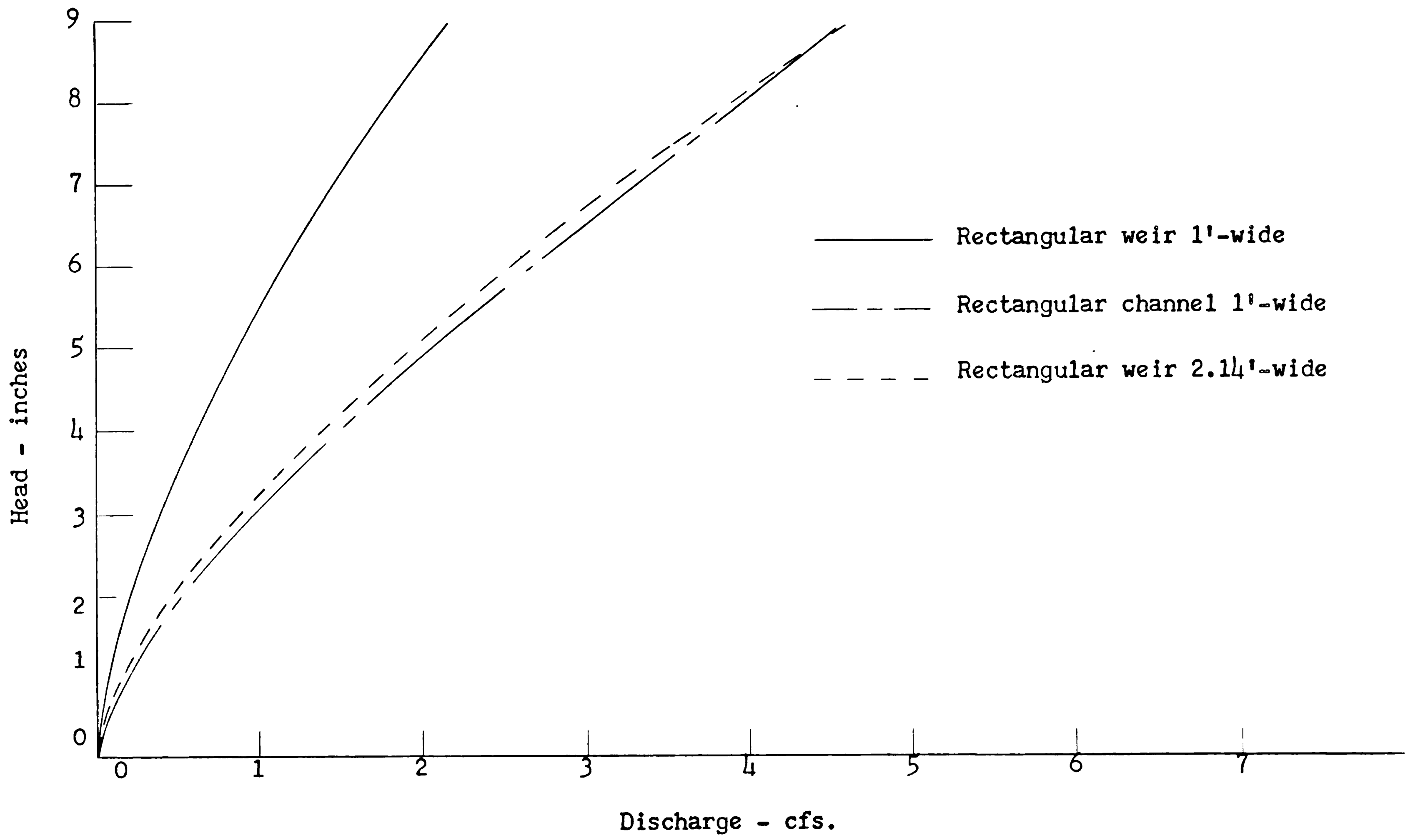


Figure 2. **Flow in a rectangular open channel compared to flow over a rectangular weir**

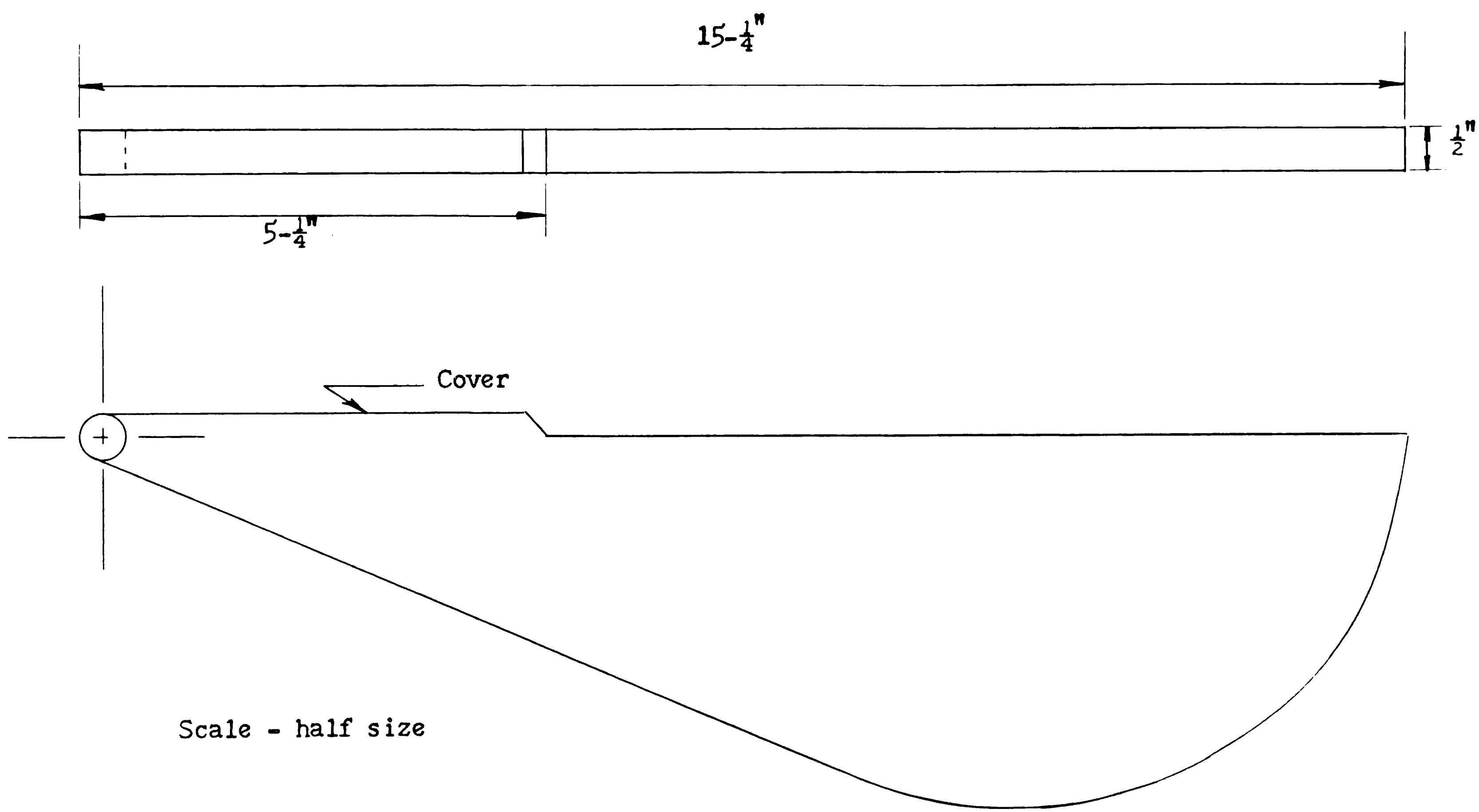


Figure 3. Shape of scoop obtained by computation

line of the pump was connected to a 2.5 gallon air tight lucite container in a Bernz-O-Matic, Tx - 2550 EE, portable electric refrigerator. Another line from this container was connected to the funnel located under the scoop assembly. As the scoop completed its revolution the pump created a vacuum in the air-tight container, pulling the sample into the box as the sampler scoop deposited it into the funnel. Thus, a very efficient means of collecting the sample in a cooled container was established. The flow diagram of the sampling procedure is illustrated in Figure 4. A picture of the sampler in operation at the East Lansing Sewage Treatment Plant is presented in Figure 5.

B. Apparatus - Description and Procedures

1. Warburg

The oxygen uptake rates of the primary effluent were determined in a twenty place, circular Warburg respiration apparatus using the techniques described by Umbreit, Burris and Stauffer (1945). The experiments were conducted in an atmosphere of air at a constant temperature of 20°C. The larger 125 ml reaction flasks were used which had the advantage of larger sample volume and hence less error. A sample volume of 50 ml was used in our experiments and the flasks were agitated with a shaking rate of 114 strokes per minute. The CO_2 evolved was eliminated by adding 1.5 ml of 10% KOH to the center well of each flask. A small fan shaped piece of Whatman #41 filter paper was used in the center well to increase the surface absorption area of the KOH. Brodies solution was used as the manometer fluid. Data were expressed in milligrams of oxygen consumed per liter of

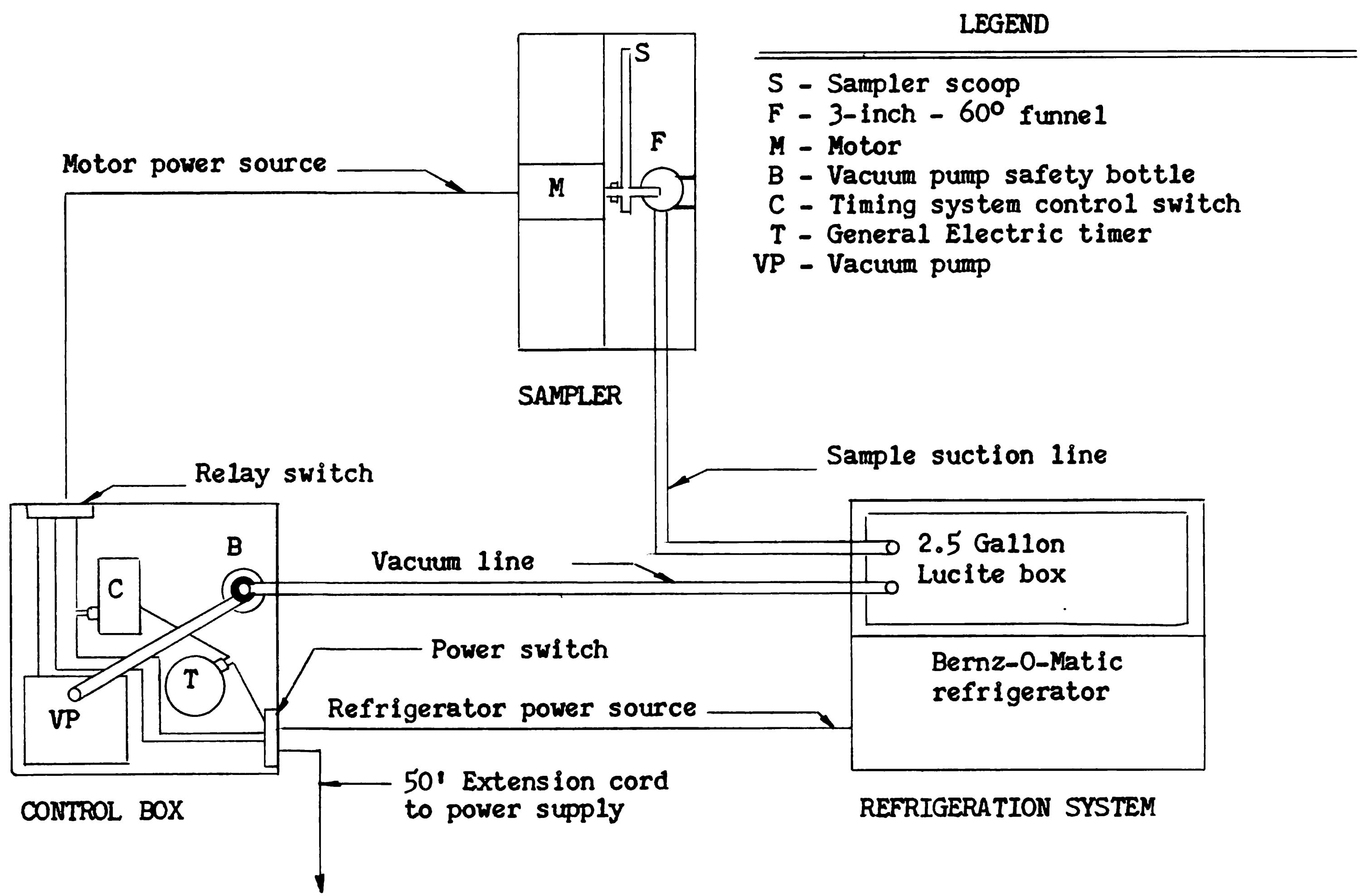


Figure 4. Flow diagram of sampler operation

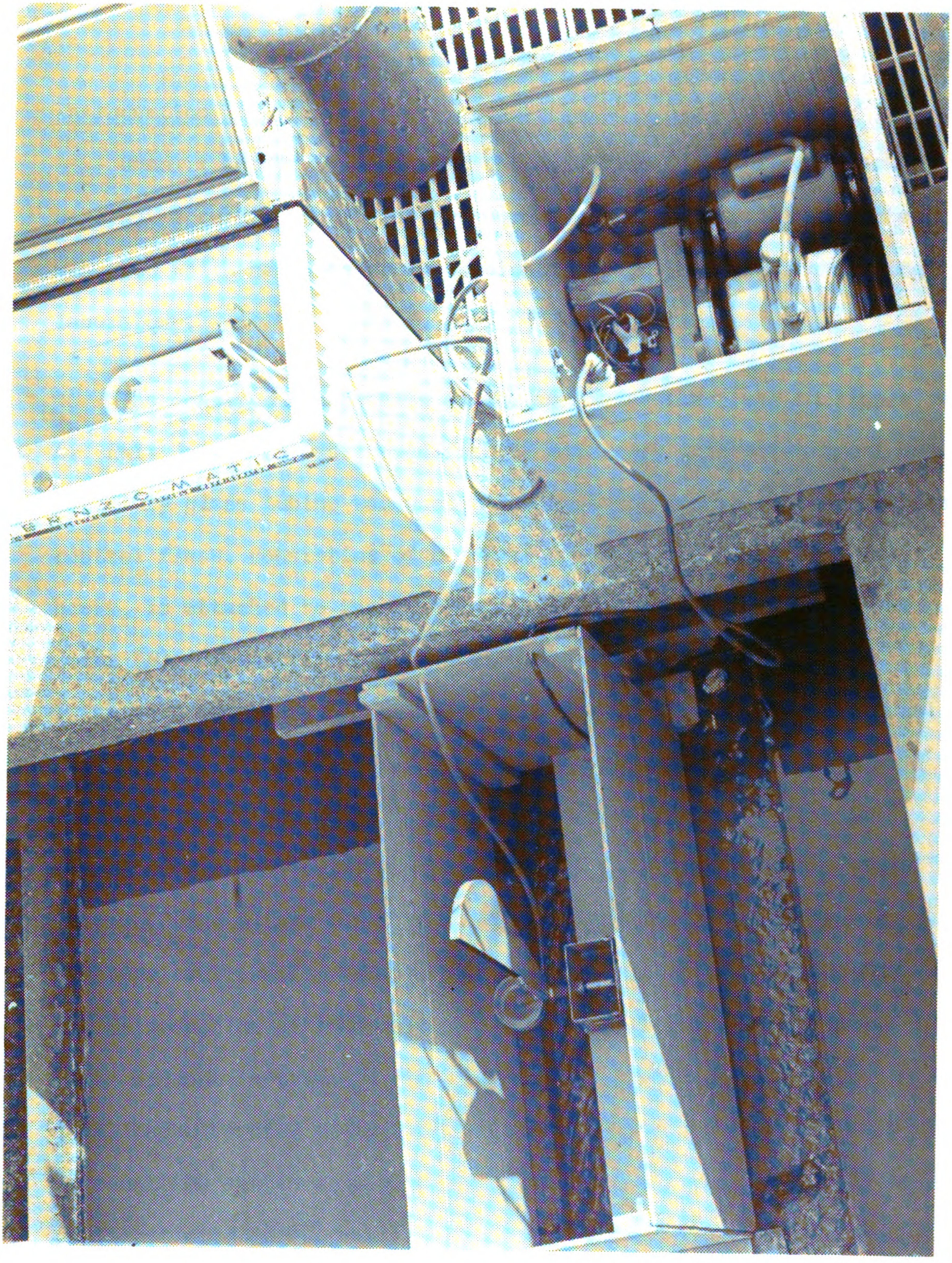


Figure 5. Sampler Operation at East Lansing Plant

sample per hour.

2. Differential Manometer

Introduction

When it became evident that the data from the Warburg apparatus were not accurate enough for our purposes, a differential manometer was used which had been newly developed in our sanitary engineering laboratory.

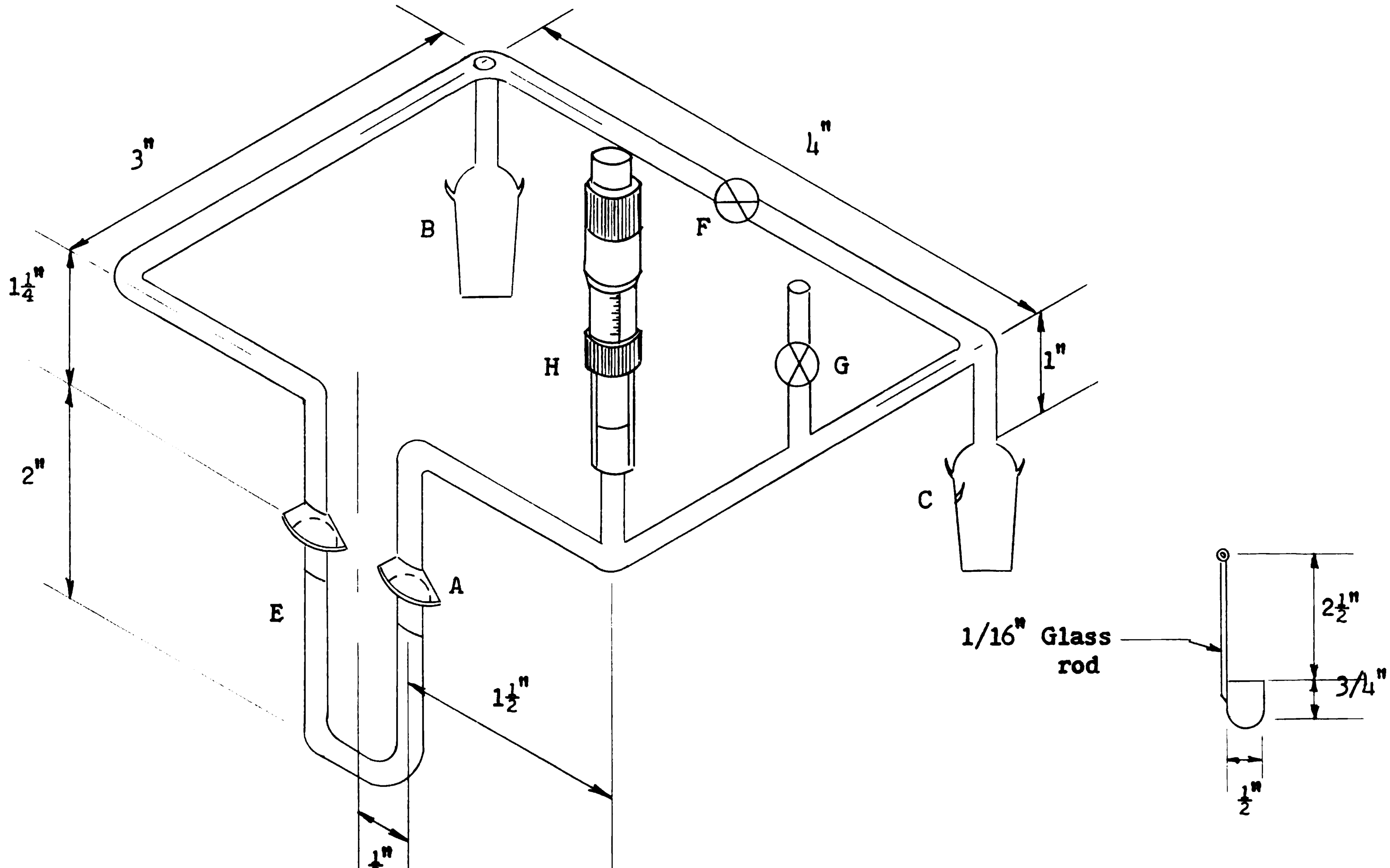
The construction details of the manometer are given in Figure 6. Ball joints (A) were used for the manometer connection and 1 mm capillary glass tubing was used throughout the system. A temperature control flask, consisting of a 300 ml round bottom flask with two hooks, is attached to the system at the 24/40 conical ground glass joint (B). A reaction flask, consisting of a 300 ml flat bottom flask with two hooks, is attached to the system at the 24/40 conical ground glass joint (C). A $\frac{1}{2}$" diameter glass KOH cup (D) is hung on the hook inside the joint (C). Paraffin oil was chosen for the manometer fluid because of its low density and inert nature. The valves (F and G) are glass valves with stopcock (F) allowing for pressure equilization between the two flasks and (G) allowing for the introduction of atmospheric air. The micrometer (H) is a RGI Roger Gilmont Instruments, Inc.) micrometer syringe. It has a total capacity of 2 ml in 0.002 ml divisions. It is advantageous to mount the system on a wood (or some other suitable material) base as illustrated in Figures 8 and 9. The entire system (including flasks and micrometer) cost about $75.00 to construct.

Principles of Operation

The Warburg is a constant volume respirometer in which changes in the amount of a gas are measured by changes in its pressure. The differential manometer is a constant pressure device in which changes in the amount of a gas are measured by changes in its volume.

The differential manometer operates in a closed atmosphere of air. As the oxygen is biologically utilized in the sample it is replenished with oxygen from the air above the sample. The CO_2 evolved is eliminated by absorption in KOH. As the oxygen is removed from the gas phase, a pressure difference is created which causes a corresponding change in the manometer fluid. The manometer fluid is brought back to its initial equilibrium point by introducing the micrometer plunger. The micrometer accurately measures the volume of the plunger inserted which is equal to the volume of oxygen consumed.

Since the differential manometer measured the decrease in the volume of oxygen in the gas phase rather than the partial pressure of the oxygen in solution, the rate of oxygen transferred into solution, governed by the stirring rate, is very important. The stirring rate must be held constant throughout the experiment, otherwise changes in stirring rates will be recorded as changes in oxygen consumption. Also, during periods of high oxygen demand the oxygen uptake rate may exceed the oxygen transfer rate and oxygen absorption from gas to liquid may limit the respiration measurements rather than the oxygen uptake of the bacteria. Thus, it is important that



D. Glass KOH cup

Figure 6. Construction details of Manometer

stirring be done at the maximum possible rate in order to increase the range of the manometer.

Subersible Brownwill Mag-Jet, air operated, magnetic stirrers were used with teflon-coated magnetized stirring bars (1" by 5/16"). These stirrers have the advantage that they can be operated under water and that they do not produce heat while running. A constant stirring rate was produced employing Kendall, model 30, pressure regulators in the air lines to regulate the downstream pressure to 10 psi. Needle valves were installed in the exit lines of the regulators making it possible to make small adjustments in the air flow rate. Thus, with a specific needle valve setting and a constant pressure of 10 psi, the stirring rates were held constant throughout the experiment.

Because the rate of oxygen transfer depends on, among other things, the rate of stirring, it was necessary to establish the stirring rates of each experiment. This was accomplished by holding the air pressure at 10 psi and measuring the RPM values of the stirring rods at different needle valve settings. The air flow rates at the different settings were measured with a Wet Test meter. The RPM values of the magnetic stirring bar were measured with a GR (General Radio Co.) Strobotac stroboscope, type No. 631-B. With the pressure held constant the stirring rod RPM values were plotted against the respective air flow rates as shown in Figure 7. This procedure was followed for each magnetic stirrer. The stirring rate of each subsequent experiment was determined by measuring the rate of air flow through a magnetic stirrer and referring to its stirring rate curve for the corresponding RPM value.

It was found that a stirring rate of 500-700 RPM with a 1" by 5/16" stirring bar produced about the maximum practical agitation for a 150 ml sample. Increasing the stirring rate beyond these values caused excessive splashing and frequently resulted in disengagement between stirring bar and the spinning magnet inside the Mag-Jet.

Procedure

The operation of the apparatus is relatively simple. Because the apparatus is new and has not previously been described in the literature, a detailed procedure is provided.

(1) Open both stopcocks

(2) Lubricate the ball joints with silicon grease.

(3) Add paraffin oil to the manometer until the bottom of the meniscus reaches the etch marks.

(4) Insert the manometer into the ball joint socket making sure that the grease seal is continuous, secure with clamps.

(5) Lubricate the conical joints with silicone grease.

(6) Add distilled water to the temperature control flask (equal to the amount being tested).

(7) Attach the temperature control flask to the system making sure that the grease seal is continuous, fasten together with two small springs.

(8) Add 1 ml 10% KOH to the glass cup, insert ($\frac{1}{2}$" wide-$1\frac{1}{2}$" length) piece of asbestose cloth, and hang cup on hook.

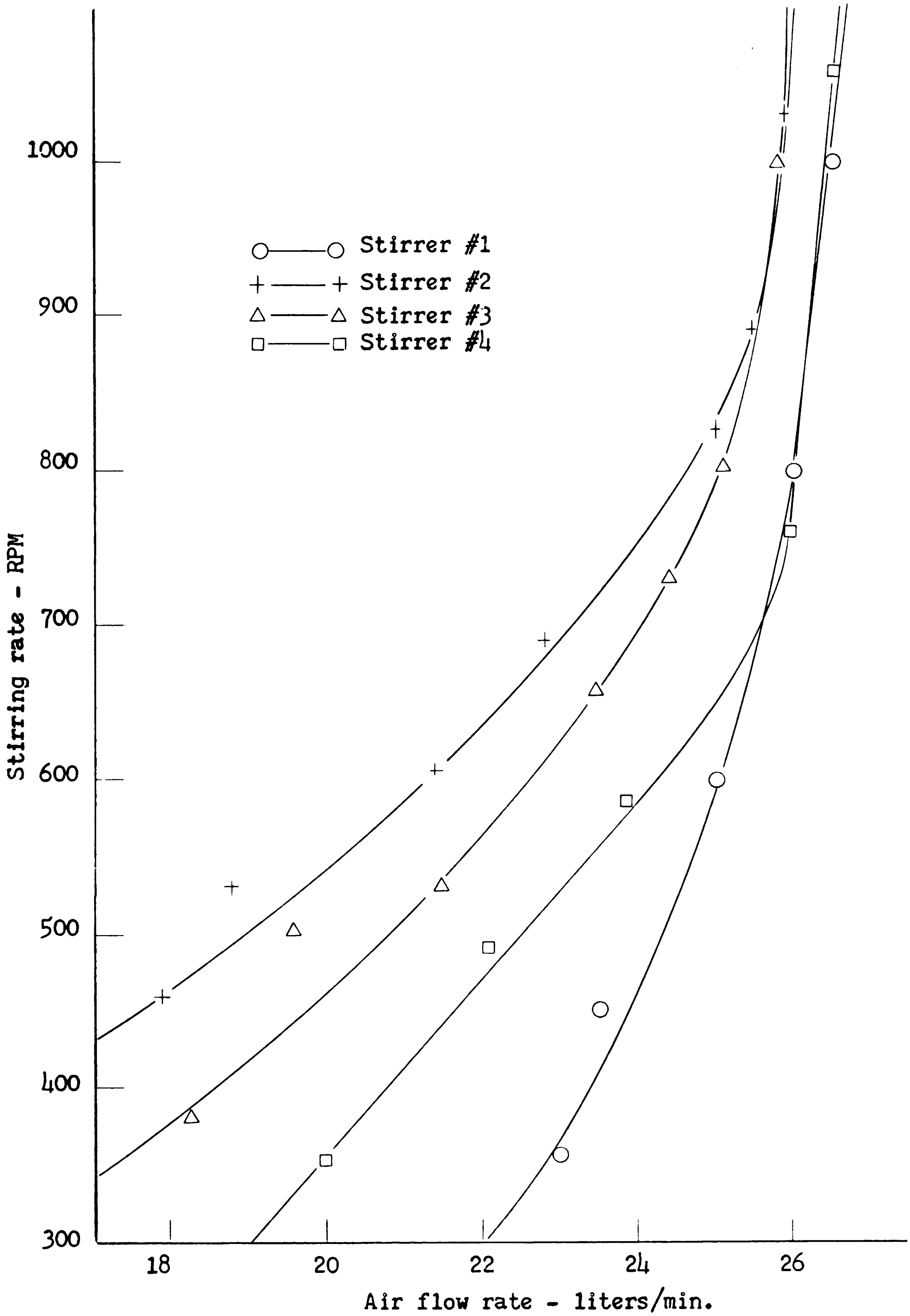


Figure 7. Stirring speed curves

(10) Pipette the desired amount of sample into the reaction flask.

(11) Attached the reaction flask to the system making sure that the grease seal is continuous, fasten together with two small springs.

(12) Place the manometer into a constant temperature water bath, adjust the micrometer to a zero setting, and begin stirring.

(13) After a 30 minute stabilization period, close the two stopcocks.

(14) After a specified time interval, bring the paraffin back to the equilibrium point by lowering the micrometer plunger, the volume of plunger inserted being equal to the ml of O_2 consumed. Repeat at regular time intervals.

(15) When it becomes apparent that the volume change would prevent a reading being made at the next time interval, open the two stopcocks (always open the stopcock separating the two flasks first), reset the micrometer to zero and then close the stopcocks. The reading may now be continued at the regular time interval.

(16) When the experiment is finished, stop stirring, open the two stopcocks, take the apparatus out of the bath, and remove the reaction flask. (It is not necessary to remove the temperature control flask and the manometer after each experiment).

After each experiment, the reaction flasks were removed from the manometer unit and rinsed several times with tap water. The flasks were then placed in a hot (125°F) soap solution containing 1 oz. HAEMO-SOL per gallon of water. After 24 hours, the flasks were removed from the HAEMO-SOL solution, rinsed several times with tap water, followed by several rinsings with distilled water, and dried in an inverted position.

For our experiments, a Waco lo-temp water bath (Wilkens-Anderson Co.) with a temperature control of ±0.1°C was used to set the temperature of the bath to 20°C. A sample volume of 150 ml was used except when a 400 ml reaction flask was used. In the latter case, a 200 ml sample was more suitable. Data were expressed in milligrams of oxygen consumed per liter of sample per hour. A photograph of the differential manometer is shown in Figure 8. Figure 9 illustrates the operational set up for the manometer in the laboratory.

C. Analytical Techniques

1. Biochemical Oxygen Demand

The standard five day BOD test as described in Standard Methods (1960) was used throughout the investigation. Dissolved oxygen determinations were made with the sodium azide modification of the standard Winkler test. The BOD bottles were completely submerged in a 20°C water bath for the five day incubation period.

2. Suspended Solids

The suspended solids were determined by filtration through a

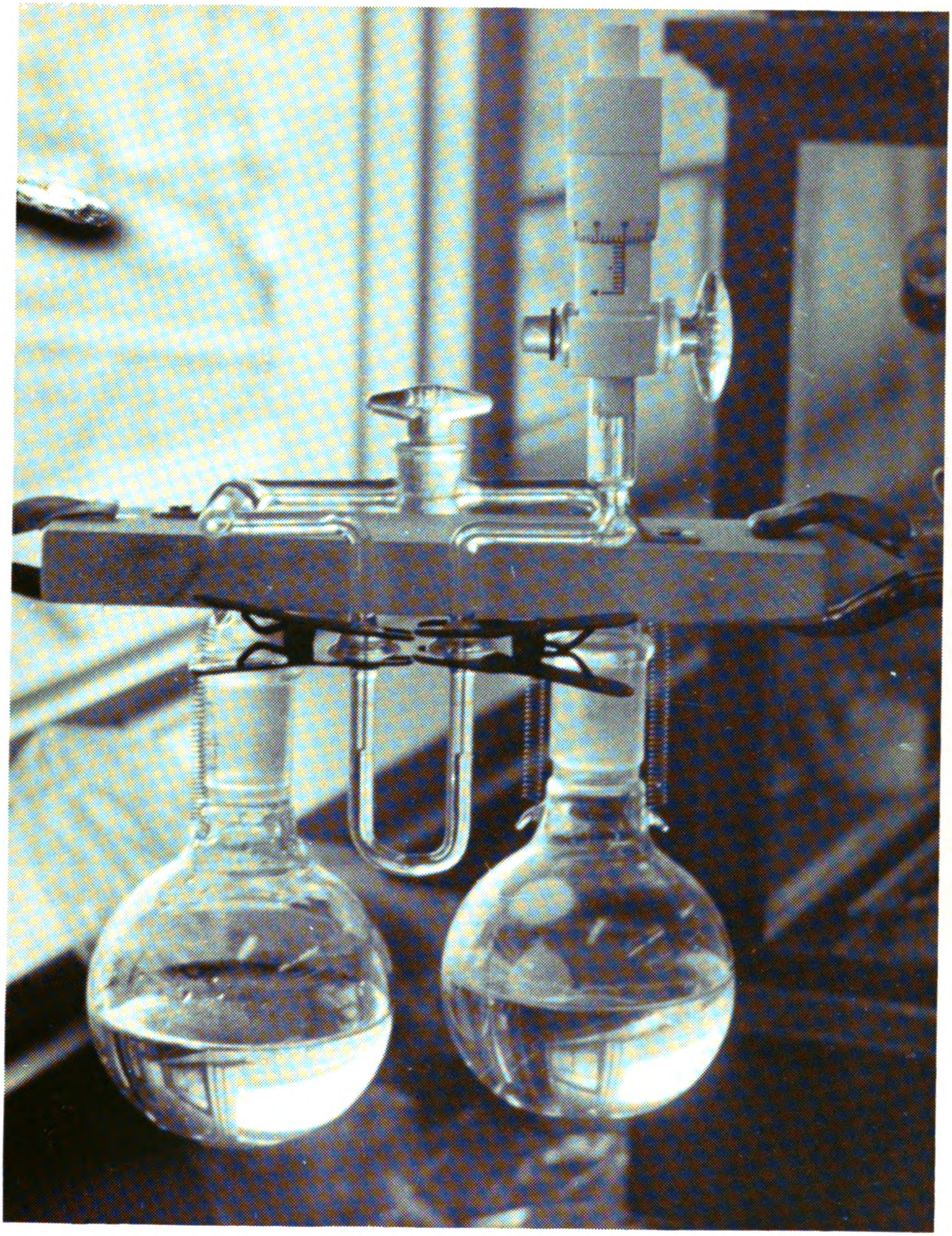


Figure 8. Differential Manometer

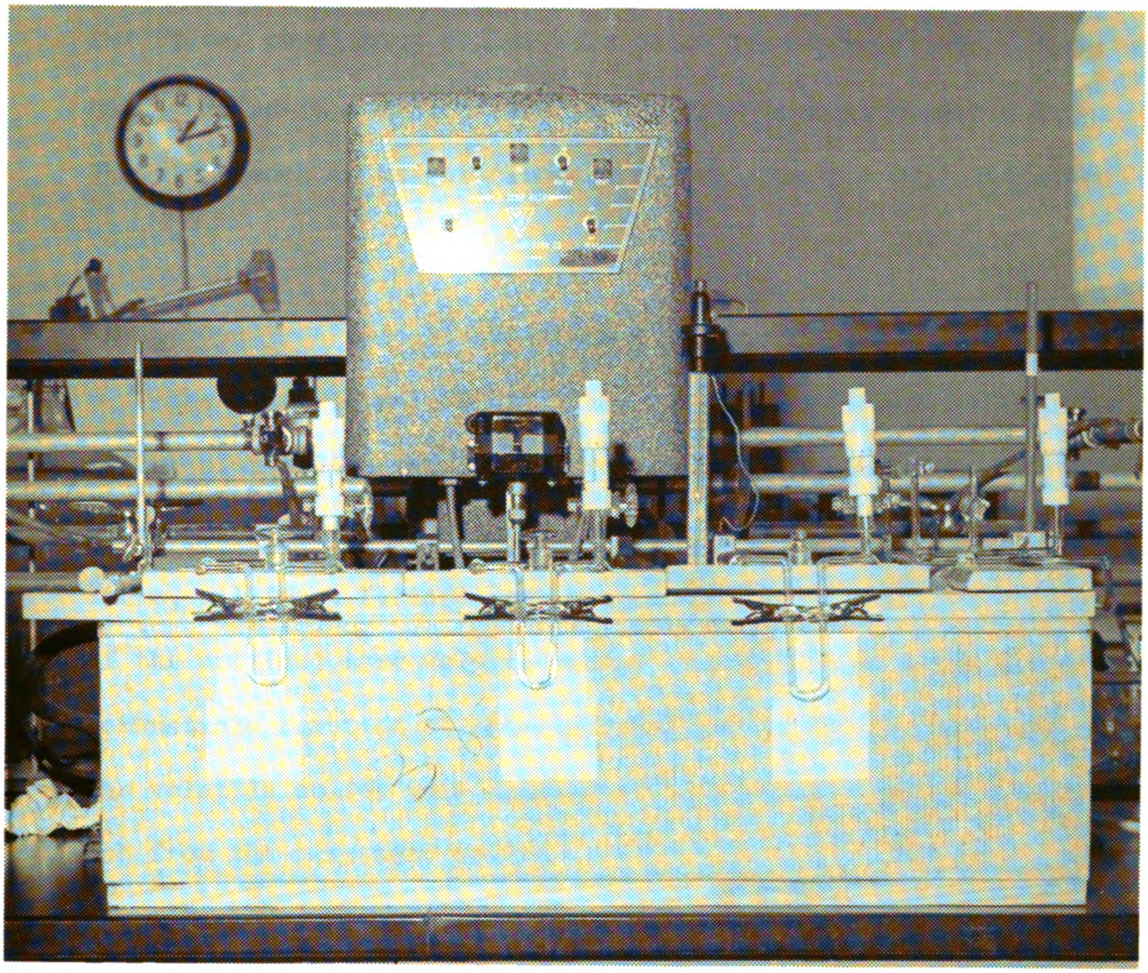


Figure 9. Operational Set Up for Differential Manometer

0.45 μ millipore membrane filter. The volume filtered was 50 ml. The membrane was dried in an oven at 103°C for one hour, cooled in a desiccator, and weighed to the nearest 0.1 mg. After filtration, the filter paper plus solids were dried in a oven at 103°C for one hour, cooled in a desiccator, and weighed to the nearest 0.1 mg. The difference in weight times 20 was recorded as mg/l suspended solids.

3. Volatile Solids

The volatile solids were determined by igniting the membrane filter plus solids left from the suspended solids test in a 600°C muffle furnace for 20 minutes. After ignition at the above temperature, millipore filters have a residual ash of less than 0.0001% of the original tare weight, making it possible to weigh the non-combustible fraction of material retained on the filters surface. The volatile solids were determined by substracting the residual ash from the suspended solids.

4. pH

The pH was determined before the test run with a Beckman Zeromatic, model H2, pH meter.

IV. EXPERIMENTAL RESULTS

The major portion of the data contained herein was collected from the East Lansing Sewage Treatment Plant. A somewhat smaller portion was collected from the Lansing, Mason and Williamston Sewage Treatment Plants. The primary purpose of these experiments was to demonstrate the range and applicability of the differential manometer for the measurement of microbial growth rates in sewage. As such, these results represent a preliminary investigation with this manometer into areas of interest to both the microbiologist and the sanitary engineer.

This thesis contains many statements concerning the measurement of the specific growth rate from a semi-log plot of respiration rates. Although this was at first an assumption, it was a logical one. The relationship between growth rate and respiration rate was investigated by Schulze and Lipe (1964). In their studies with a continuous flow culture of <u>Escherichia coli</u>, they found that the respiration rate was directly proportional to the specific growth rate. Herbert, as found in Schulze (1964), obtained similar results for <u>Aerobacter aerogenes</u>. Crieg and Hoogerheide (1941) also found the oxygen uptake of growing cultures to be directly proportional to bacterial content.

It was thereby concluded that increasing oxygen uptake rates per unit volume of culture indicate a proportional increase in the bacterial population. Since bacteria multiply exponentially, a semi-log plot of the oxygen uptake rates versus time should produce a straight line as long as the bacteria are growing at a constant rate. The slope of this straight line represents not only the rate of change in respiration but also the rate of change in cell numbers. The value of the slope should therefore be equal to the specific growth rate (k_1) of the bacteria present in the samples.

A. Comparison of Warburg and Differential Manometer Data

Initially, data for this thesis were to be obtained with the Warburg apparatus. It was felt that by using 50 ml samples in 125 ml reaction flasks instead of the 5 ml samples used by J. Dick (1964) in 15 ml flasks, more accurate data could be obtained. Caldwell and Langelier (1948) used the larger flasks successfully in their Warburg measurements of Biochemical Oxygen Demand. However, the investigations with a 50 ml sample volume did not produce good results. Most of the data collected in this manner were inconclusive without the aid of complicated statistics. On the other hand, the differential manometer produced data that could be analyzed to some degree of accuracy without the aid of statistics.

The following two experiments demonstrate a comparison of the data obtained from the Warburg and the differential manometer on parallel samples.

Experiment No. 1

Sample: Primary effluent, grab sample, East Lansing Plant, 11:00 A.M., July 7, 1964. A 50 ml portion of the sample was placed in a Warburg reaction flask and 200 ml were placed in the reaction flask of a differential manometer. The two flasks were attached to their manometers and placed in their respective water baths at 20°C. Data were collected at 30 minute intervals over a period of 10 hrs in the usual manner for each technique. A summary of the data is presented in Tables I and II. These tables are presented to demonstrate the manner in which the data were collected and handled. A semi-log plot of the oxygen uptake rate vs. time for the two methods is given in Figure 10. In later experiments the tables have been omitted and only the graphs are shown.

Experiment No. 2

A repeat of the above experiment was performed on January 18, 1965, using a grab sample obtained at 9:00 A.M.. A 50 ml portion of the sample was placed in a Warburg reaction flask and 150 ml were placed in the reaction flask of a differential manometer. The oxygen utilized with respect to time was measured in the same manner as outlined in experiment No. 1. A semi-log plot of the oxygen uptake rate vs. time for the two methods is given in Figure 11.

If corrected to standard conditions (0°C, 760 mm Hg) both curves should coincide. The curves in Figure 10 and 11 do not coincide, however. Part of the reason for this is that the Warburg data are presented at standard conditions while the differential manometer data are presented at 20°C and 740 mm Hg. If the differential manometer data are corrected to standard conditions, about half of the difference between the Warburg and differential manometer data can be made up. The reason for the remaining difference is not

TABLE I. Warburg data for experiment No. 1

Time	Interval	Thermal Barometer		Reaction Flask					
		Reading	Correction	Reading	Change	Actual Change	Flask Constant	O_2 Uptake	O_2 Uptake Rate
	(min.)	(mm)	(mm)	(mm)	(mm)	(mm)	$\frac{(mg/1\ O_2)}{mm}$	(mg/1)	(mg/1/hr)
1:30	-	149	-	161	-	-	0.2478	-	-
2:00	30	147	-3	151	10	8	0.2478	1.98	3.96
2:30	30	138	-9	133	18	9	0.2478	2.23	4.46
3:00	30	125	-13	111	22	9	0.2478	2.23	4.46
3:30	30	129	4	105	6	10	0.2478	2.48	5.96
4:00	30	134	5	95	10	15	0.2478	3.72	7.44
4:30	30	138	4	86	9	13	0.2478	3.22	6.44
5:00	30	135	-3	69	17	14	0.2478	3.47	6.94
5:30	30	135	0	51	18	18	0.2478	4.46	8.92
6:00	30	136	1	46	5	6	0.2478	1.49	2.98
7:00	60	152	16	45	1	17	0.2478	4.21	4.21
8:00	60	155	3	27	18	21	0.2478	5.20	5.20
8:00	-	150	-	161	-	-	0.2478	-	-
9:00	60	147	-3	138	23	20	0.2478	4.96	4.96
10:00	60	140	-7	113	25	18	0.2478	4.46	4.46
10:30	30	135	-5	101	12	7	0.2478	1.74	3.47

TABLE II. Differential manometer data for experiment No. 1.

Time	Interval (min.)	Micrometer Reading (ml O_2)	Change (ml)	O_2 Uptake (mg/1)	O_2 Uptake Rate (mg/1/hr)
1:30	-	0.000	-	-	-
2:00	30	0.365	0.365	2.61	5.22
2:30	30	0.790	0.425	3.04	6.08
3:00	30	1.277	0.487	3.49	6.98
3:30	30	1.795	0.518	3.71	7.42
3:30	-	0.000	-	-	-
4:00	30	0.566	0.566	4.05	8.10
4:30	30	1.300	0.734	5.25	10.50
4:30	-	0.000	-	-	-
5:00	30	0.618	0.618	4.42	8.84
5:30	30	1.140	0.522	3.74	7.48
6:00	30	1.540	0.400	2.86	5.72
6:00	-	0.000	-	-	-
7:00	60	0.967	0.967	6.91	6.91
7:00	-	0.000	-	-	-
8:00	60	1.042	1.042	7.45	7.45
8:00	-	0.000	-	-	-
9:00	60	0.722	0.722	5.52	5.52
10:00	60	1.458	0.686	4.90	4.90
10:00	-	0.000	-	-	-
10:30	30	0.290	0.290	2.07	4.14

EAST LANSING PRIMARY EFFLUENT

11:00 A.M., July 7, 1964
Suspended solids = 85 mg/1
BOD_5 = 135 mg/1
pH = 7.8

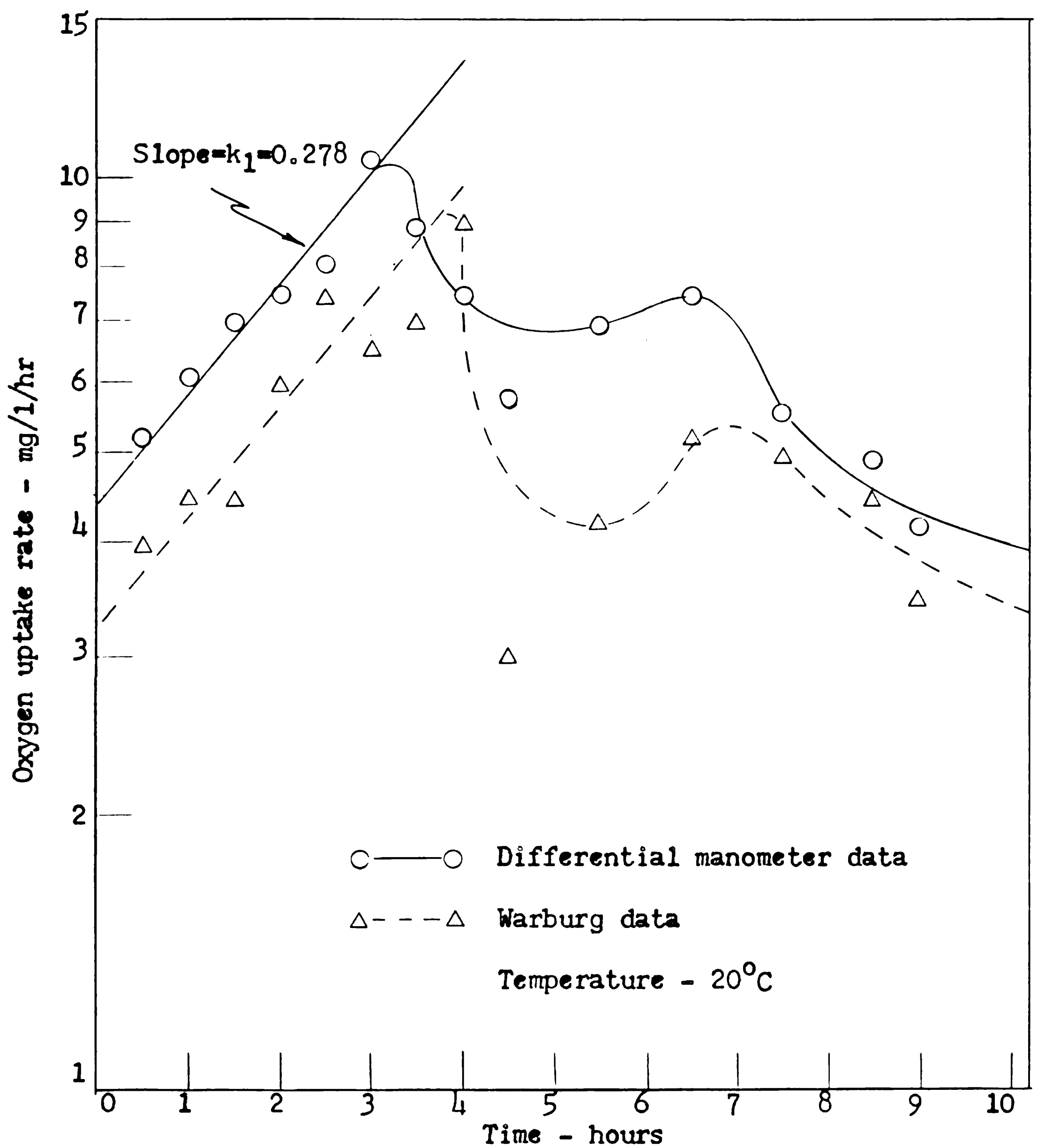


Figure 10. Experiment No. 1. Comparison of Warburg data with differential manometer data on a parallel sample

EAST LANSING PRIMARY EFFLUENT

9:00 A.M., January 18, 1965
Suspended solids = 140 mg/l
Volatile solids = 114 mg/l
BOD_5 = 168 mg/l
pH = 7.6

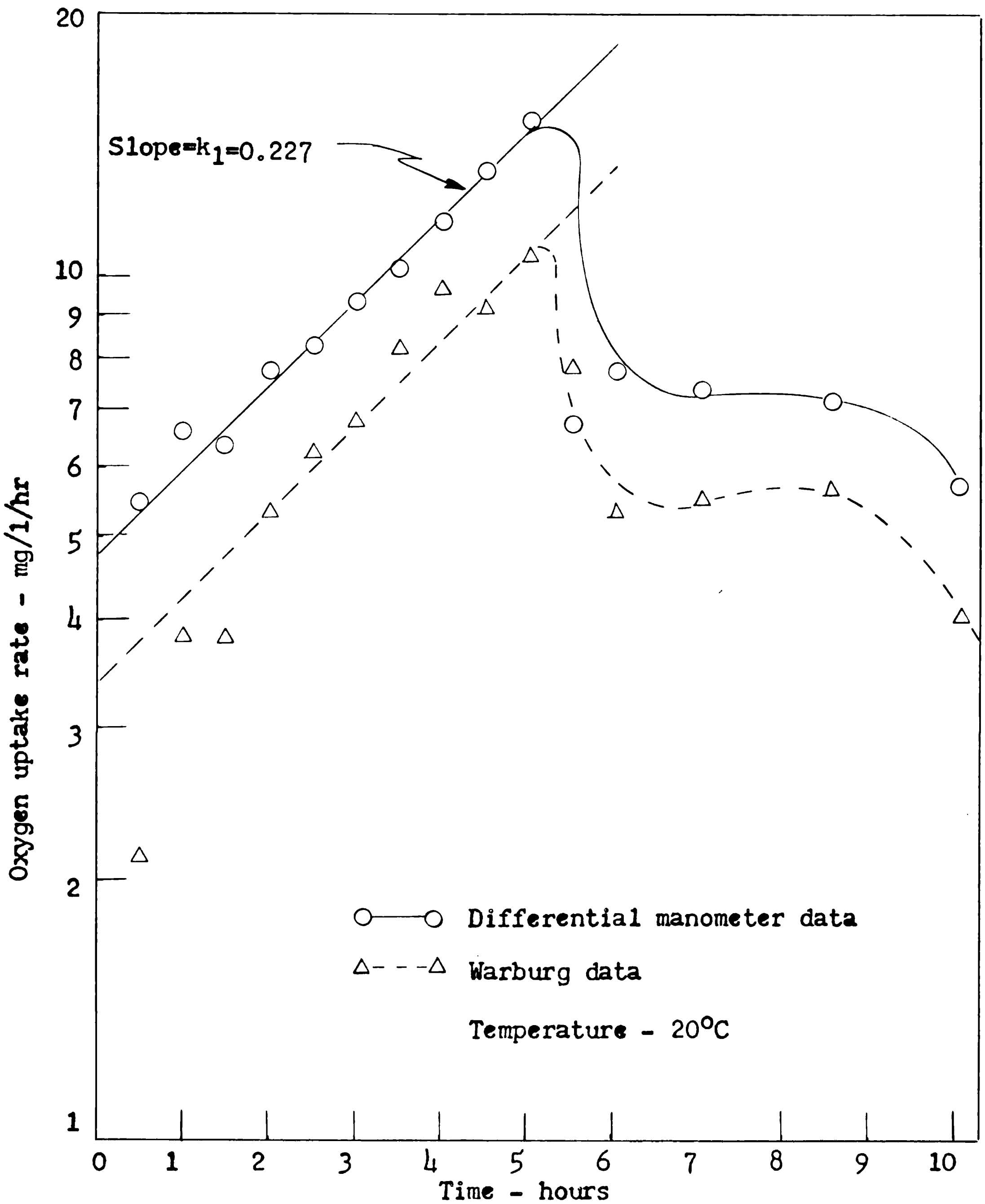


Figure 11. Experiment No. 2. Comparison of Warburg data with differential manometer data on a parallel sample

clear at this time.

Both experiments demonstrate that the shape of the curve produced by each method is very similar. However, the differential manometer produces a much more precise curve, especially in the straight portion. The growth rate found with the differential manometer was 0.278 per hour in experiment No. 1 and 0.227 per hour in experiment No. 2. Without the use of statistics no definite line can be drawn through the ascending portion of the Warburg curve and thus, an accurate determination of the growth rate cannot be made in this case.

The dashed lines shown for these data in Figure 10 and 11 represent a parallel to the differential manometer curve and emphasize the large variations of the Warburg measurements.

According to these results it was decided that the Warburg apparatus was not accurate enough for the relatively small oxygen uptake rates occurring in polluted wastes and that it would be preferable to use the differential manometer.

B. The Effect of Storage

Since many of the samples were to be collected and stored at 0-5°C for 24 hours, it was felt that it was necessary to establish the effect of this storage period on the oxygen uptake rate of the sample.

Experiment No. 3

On August 11, 1964, 9:30 A.M., a grab sample of primary effluent was obtained from the East Lansing Plant and split into two parts. A 200 ml portion was immediately placed into a differential manometer reaction flask, and the oxygen uptake was measured at 30 minute intervals for a period of 10 hours. The second portion was cooled to the range of 0-5°C and stored for 24 hours. After the storage period, the oxygen uptake rates were determined on a 200 ml sample in exactly the same manner as the preceding day. A semi-log plot of the oxygen uptake rate vs. time for the two days is given in Figure 12.

Experiment No. 4

The same experiment was repeated using a grab sample obtained at 9:00 A.M. on January 18, 1965. A 150 ml portion of the sample was immediately placed in a differential manometer reaction flask, and the oxygen utilized with respect to time was recorded over a 10 hour period. On the remaining portion of the sample oxygen uptake rates were determined after 24 hour storage at 0-5°C. A semi-log plot of the oxygen uptake rate vs. time for the two days is presented in Figure 13.

The most obvious effect of storage was the introduction of a lag phase. This was expected since the bacteria had to adapt to the 20°C temperature after 24 hours of metabolism at 0-5°C. Since the primary effluent was near 20°C when collected, this period of adaptation was not noticed in the samples tested before storage.

EAST LANSING PRIMARY EFFLUENT

9:30 A.M., August 11, 1964
Suspended solids = 85 mg/1
Volatile solids = 60 mg/1
BOD_5 = 125 mg/1
pH = 7.6

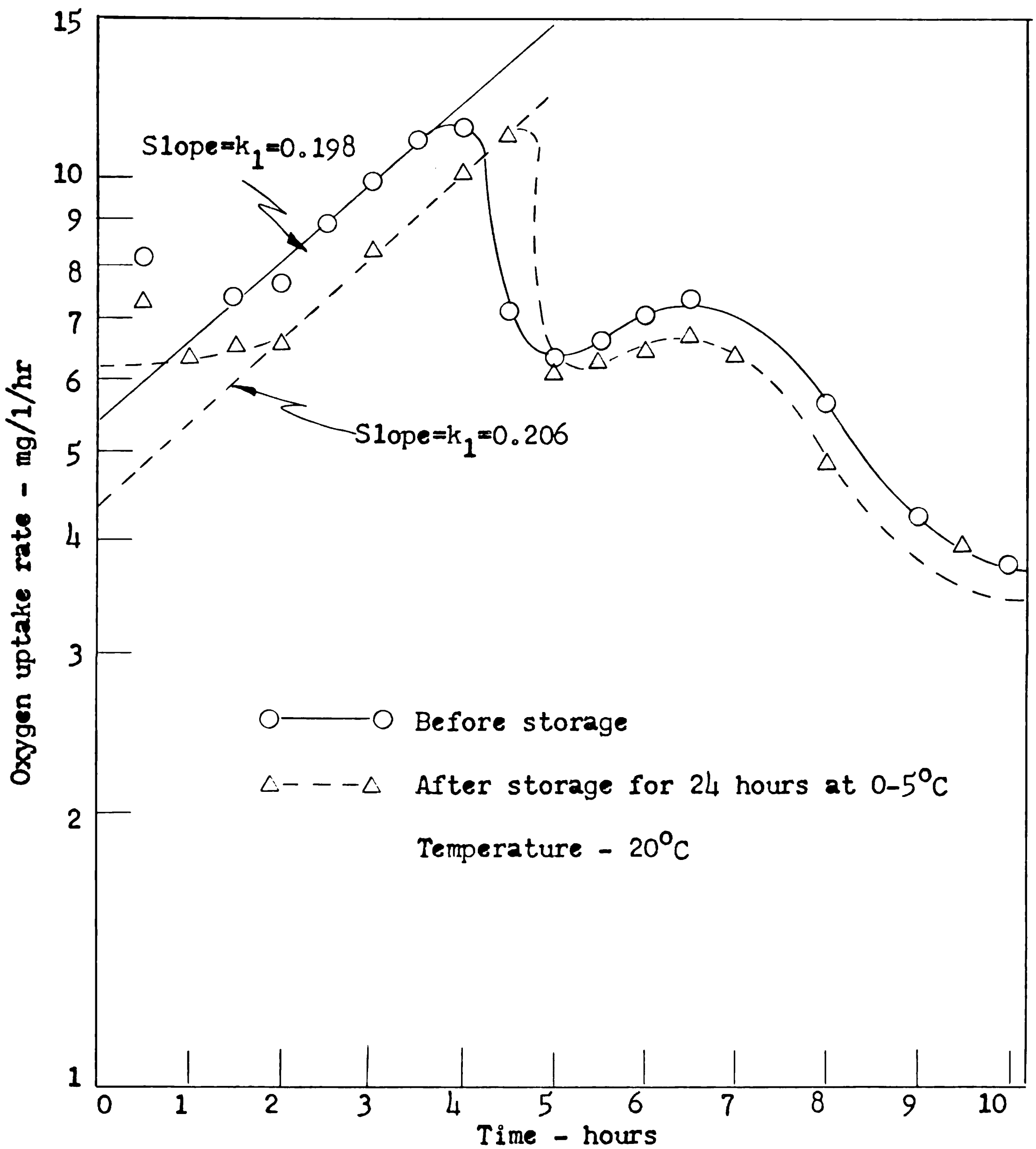


Figure 12. Experiment No. 3. The effect of storage at 0-5°C on a parallel sample

EAST LANSING PRIMARY EFFLUENT

9:00 A.M., January 18, 1965
Suspended solids = 140 mg/l
Volatile solids = 114 mg/l
BOD_5 = 168 mg/l
pH = 7.6

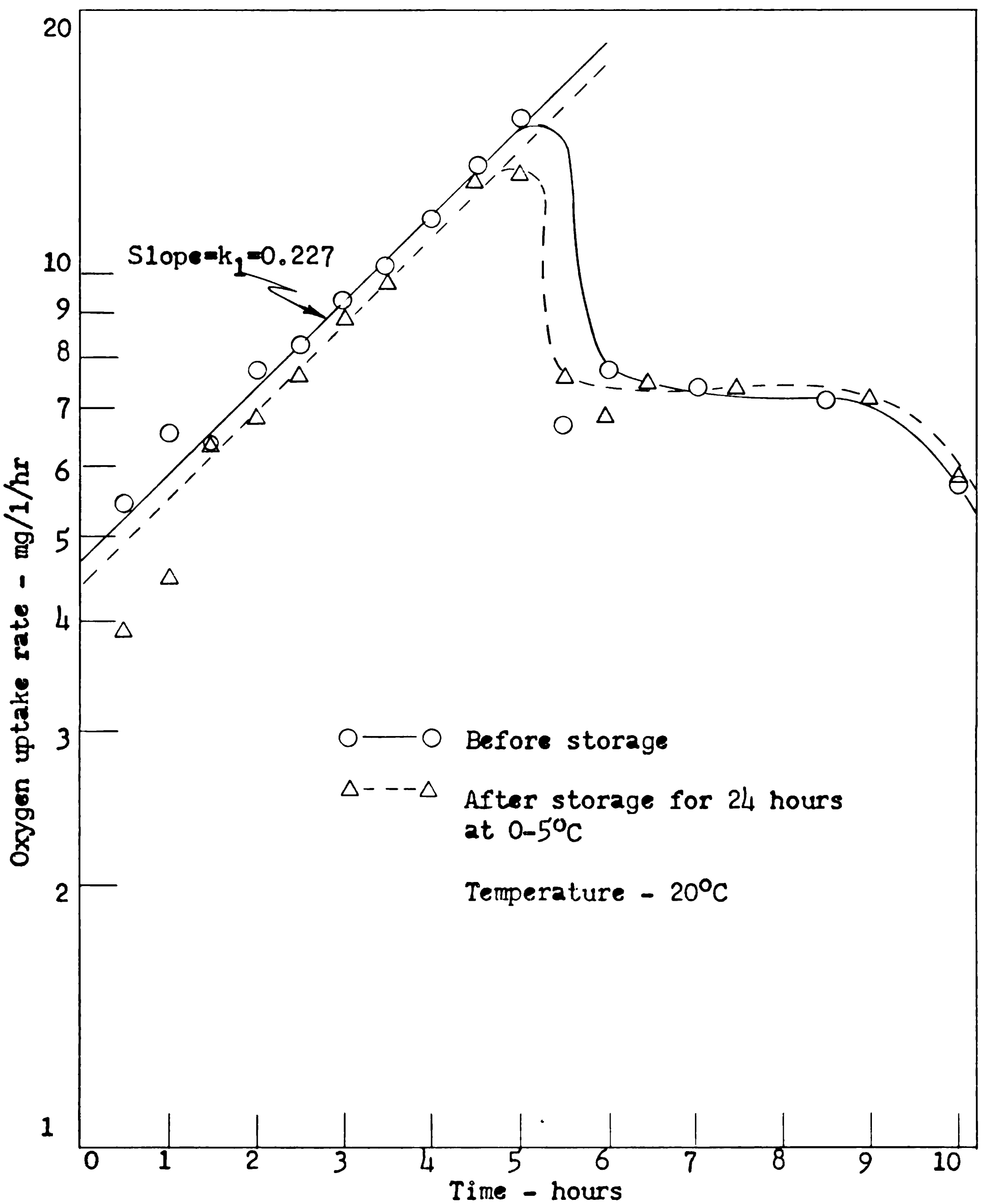


Figure 13. Experiment No. 4. The effect of storage at 0-5°C on a parallel sample

Experiment No. 3 shows a considerable lag phase. The maximum oxygen uptake was reached about one hour later than in the sample that was not stored. The lag in experiment No. 4 is very slight, but the first two points appear to be much lower than the rest of the data.

It is interesting to note that an identical maximum oxygen uptake rate of 11.5 mg/l/hr was obtained before and after storage in experiment No. 3. In experiment No. 4 the maximum oxygen uptake rate after storage was 2.0 mg/l/hr lower than the maximum rate of the first day. We would expect the maximum oxygen uptake rates to be about the same, and the reason for the difference in experiment No. 4 is not known at this time.

Both experiments demonstrate that the value for the slope of the straight portion of the curves, and therefore the value for the growth rate of the sample before storage was nearly equal to the growth rate of the sample after storage, $k_1 = 0.198$/hr, experiment No. 3, $k_1 = 0.227$/hr, experiment No. 4. Thus, it appears that the storage period had little or no effect on the specific growth rate of the bacteria.

C. Reproducibility of Data

The reproducibility of data obtained from the differential manometer was investigated by making parallel runs with primary effluent from the Lansing and East Lansing Sewage Treatment Plants.

Experiment No. 5

Sample: Primary effluent, grab sample, East Lansing Plant, July 23, 1964, 2:30 P.M. The sample was stored overnight. The next morning a 200 ml portion of the sample was placed in manometer #1 and a 150 ml portion was placed in manometer #2. The samples were run in parallel at 20°C. The oxygen utilized with respect to time was determined over a 10 hour period. A semi-log plot of the oxygen uptake rate vs. time for the duplicate samples is given in Figure 14.

Experiment No. 6

Sample: Primary effluent, grab sample, Lansing Plant, July 29, 1964, 10:00 A.M. The sample was stored overnight. The next morning two parallel runs were made. A 200 ml portion was placed in manometer #1 while a 150 ml portion was placed in manometer #2. A semi-log plot of the oxygen uptake rate vs. time for the duplicate samples is presented in Figure 15.

Both experiments indicate good reproducibility. A recurring feature of the manometer data was the high reading obtained for the first measurement. This is shown in both of the above experiments. Although the exact reason is not known, a possible explanation would be that when the manometers were first closed a certain amount of CO_2 was still left in the atmosphere of the vessel. The absorption of this CO_2 by the KOH would then cause an additional decrease in the partial pressure of the gas phase which is measured as O_2 consumption. In future experiments perhaps the introduction of

EAST LANSING PRIMARY EFFLUENT

2:30 P.M., July 23, 1964
Suspended solids = 76 mg/1
BOD_5 = 155 mg/1
pH = 7.5

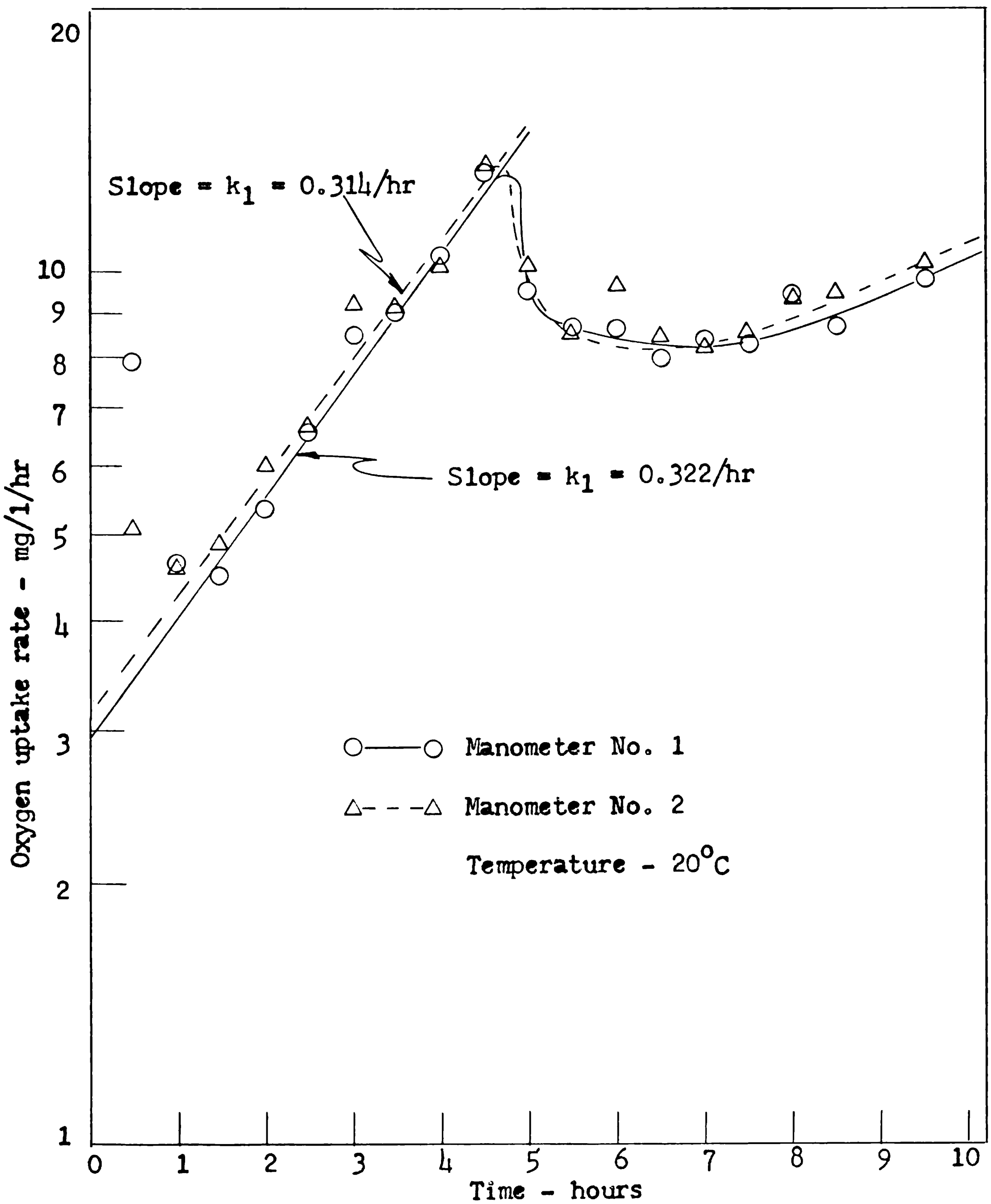


Figure 14. Experiment No. 5. Reproducibility of data on a parallel sample

LANSING PRIMARY EFFLUENT

10:00 A.M., July 19, 1964
Suspended solids = 163 mg/l
BOD_5 = 100 mg/l
pH = 7.5

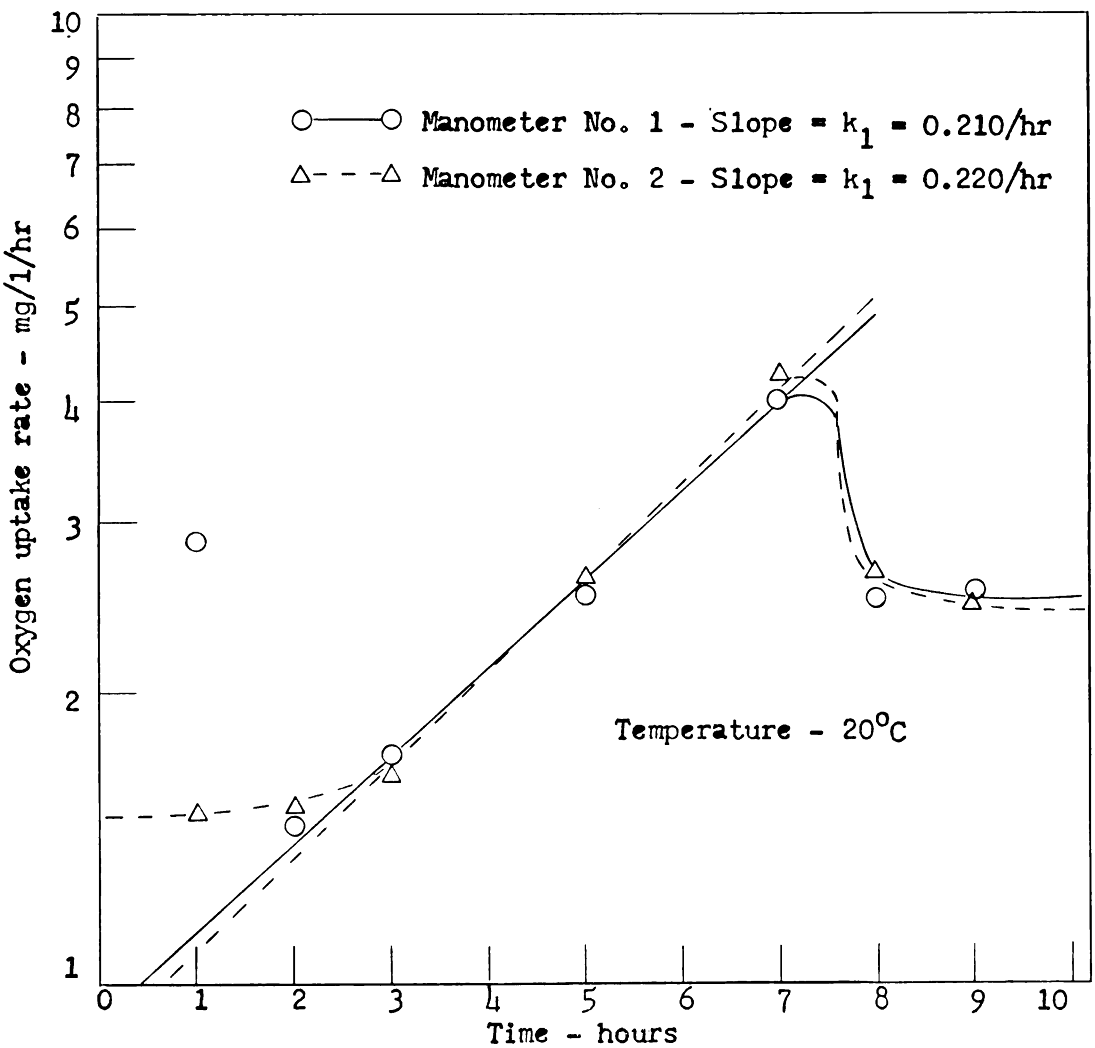


Figure 15. Experiment No. 6. Reproducibility of data on a parallel sample

a CO_2 free atmosphere would give better results. This could be accomplished by attaching an ascarite tube, filled with a carbon dioxide absorbing granular material such as Carorite or Ascarite, to the value which connects the manometer to the atmosphere.

The specific growth rates measured from the duplicate samples showed close agreement. It must be remembered that the data do not produce an exact straight line and that a small error will be introduced in the selection of the line to represent the given points. The values obtained in experiment No. 5 of $k_1 = 0.322$/hr for manometer #1 and $k_1 = 0.314$/hr for manometer #2 represent a difference of only 2.5%. In experiment No. 6 the values were $k_1 = 0.210$/hr for manometer #1 and $k_1 = 0.220$/hr for manometer #2 representing a difference of 4.5%. In this type of work, these values can be considered to be within the expected degree of accuracy.

D. Bacterial Growth Measurements

A comparison was made between the differential manometer and other established methods of growth measurement. The three methods used in obtaining these measurements were: (1) the differential manometer for measuring oxygen uptake rates, (2) the Bausch and Lomb Spectronic 20 colorimeter for measuring increase in turbidity as optical density, and (3) the suspended solids test for measuring the increase in cell concentration by weight. A 0.2% lactose broth solution was used as culture medium and primary effluent from the East Lansing Sewage Treatment Plant (grab sample, October 28, 1964) was used for seed material.

Experiment No. 7

A 100 ml portion of the 0.2% lactose broth plus 35 ml of primary effluent seed were placed into the reaction flask of a differential manometer. With the stop-cocks open, the manometer was placed in a $20^{O}C$ water bath and stirred with a magnetic stirrer for five hours. This gave the mixed flora of bacteria enough time to adapt to their new environment and reach a population which produced a measurable oxygen consumption. After the five hour incubation period, the stop-cocks were closed, and oxygen consumption readings were taken in the usual manner for a period of four hours. A semi-log plot of the oxygen uptake rates vs. time is given in Figure 16.

Experiment No. 8

A 100 ml portion of the 0.2% lactose broth plus 35 ml of primary effluent seed was placed into a 250 ml Erlenmeyer flask, placed in a $20^{o}C$ water bath, and stirred with a magnetic stirrer for five hours. After the five hour incubation period, optical density was measured with the Bausch and Lomb colorimeter at 30 minute intervals over a period of four hours. The data collected in this experiment are summarized in Table III. A semi-log plot of the optical density vs. time is given in Figure 17.

Experiment No. 9

A 200 ml portion of the 0.2% lactose broth plus 70 ml of primary effluent seed was placed into a 500 ml Erlenmeyer flask and aerated at $20^{o}C$ with a small diffuser for five hours. After the five hour

TABLE III. Turbidity data for experiment No. 8

Time	Interval (min.)	Optical Density (O.D.)
1:05	0	0.035
1:35	30	0.032
2:05	30	0.038
2:35	30	0.048
3:05	30	0.058
3:35	30	0.070
4:05	30	0.082
4:35	30	0.090

TABLE IV. Suspended solids data for experiment No. 9

Time	Interval (min.)	Wt. Paper (mg)	Wt. Paper +Solids (mg)	Wt. Solids (mg)	Volume Filtered (ml)	Suspended Solids (mg/l)
1:05	0	97.6	99.0	1.4	50	28
2:05	60	99.3	101.5	2.2	50	44
3:05	60	100.2	102.9	2.7	50	54
3:35	30	101.0	104.6	3.6	50	72
4:05	30	99.2	103.5	4.3	44	98

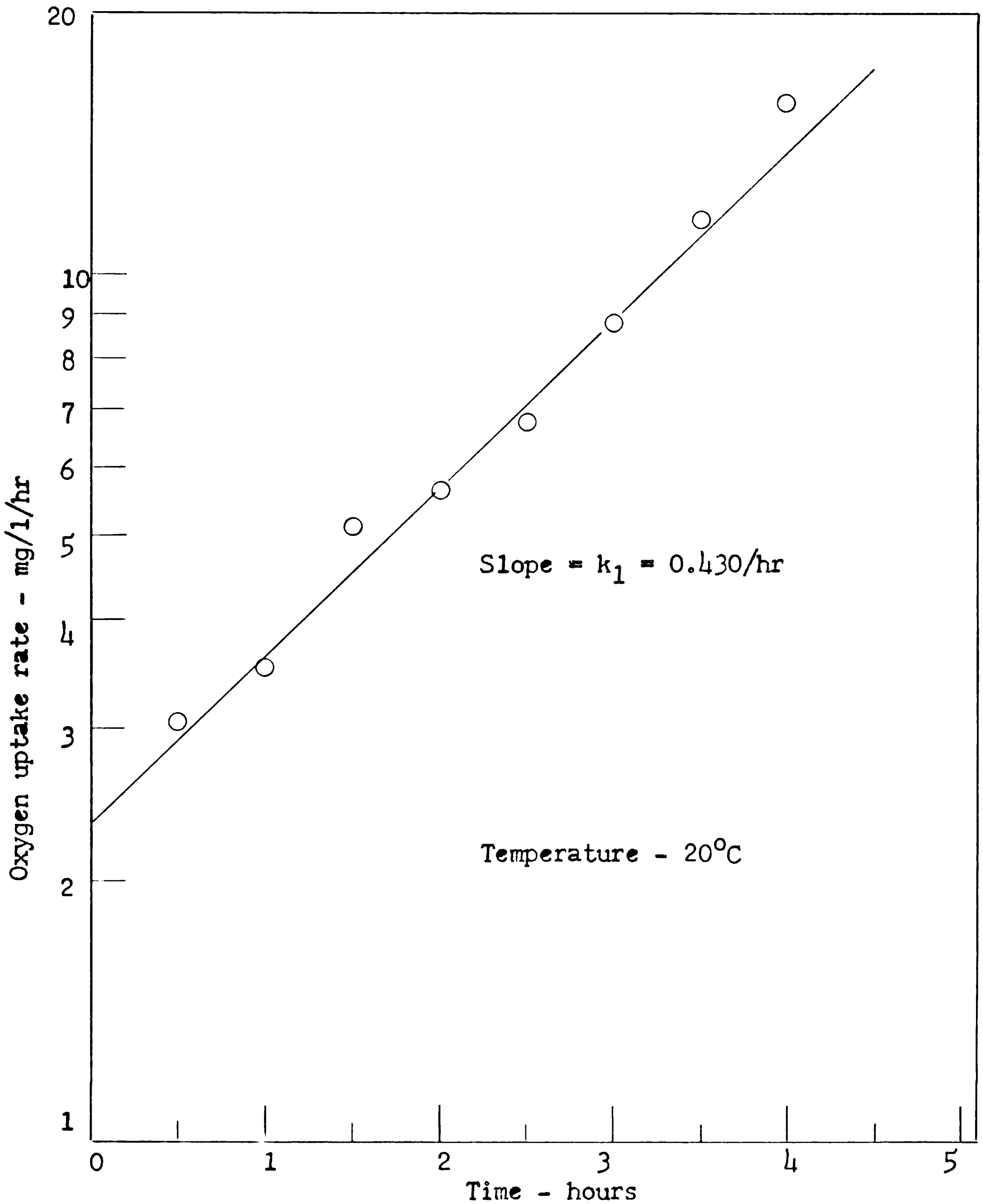


Figure 16. Experiment No. 7. Measurement of specific growth rate with differential manometer

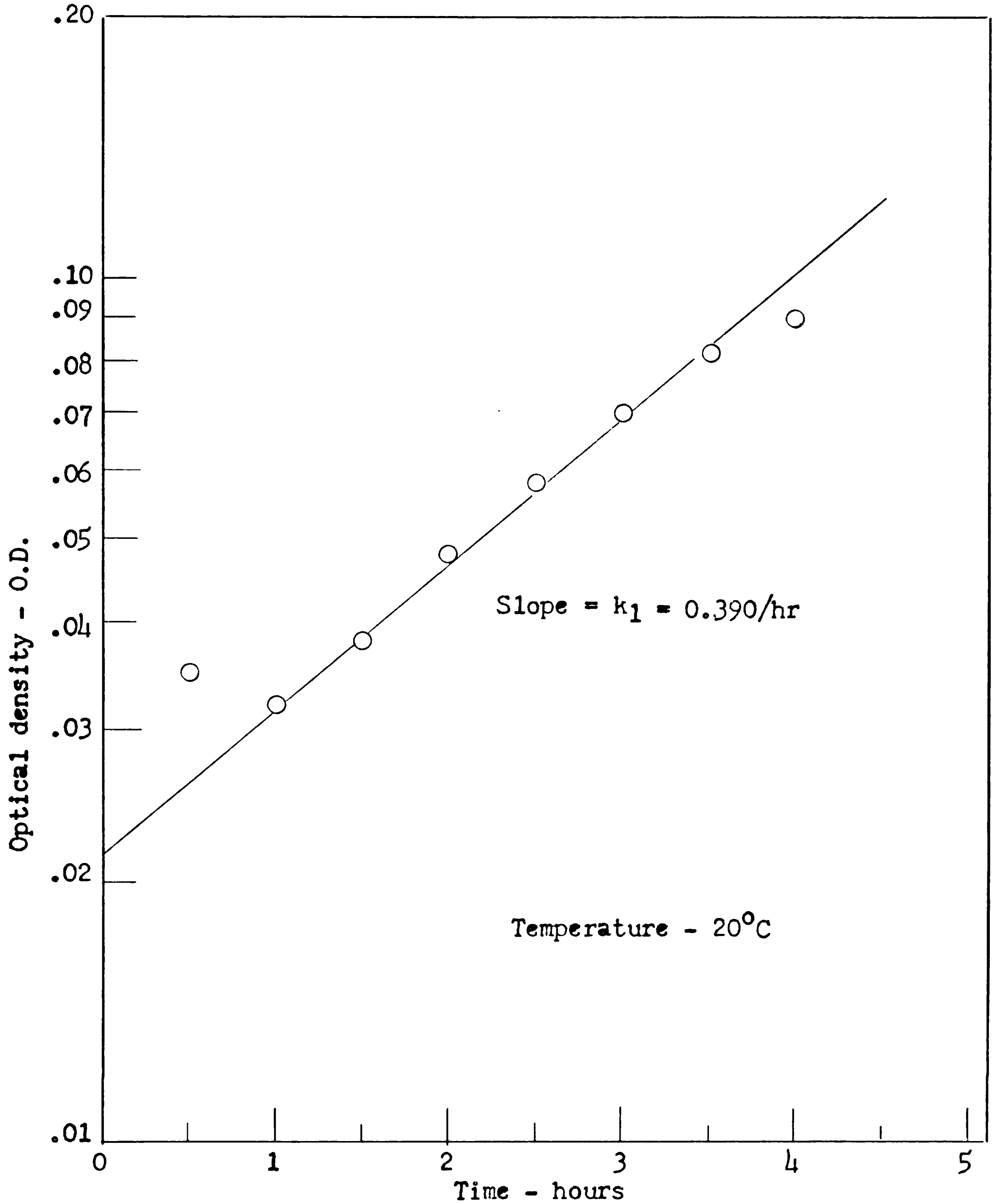


Figure 17. Experiment No. 8. Colorimetric measurement of specific growth rate (Bausch and Lomb, Spectronic 20)

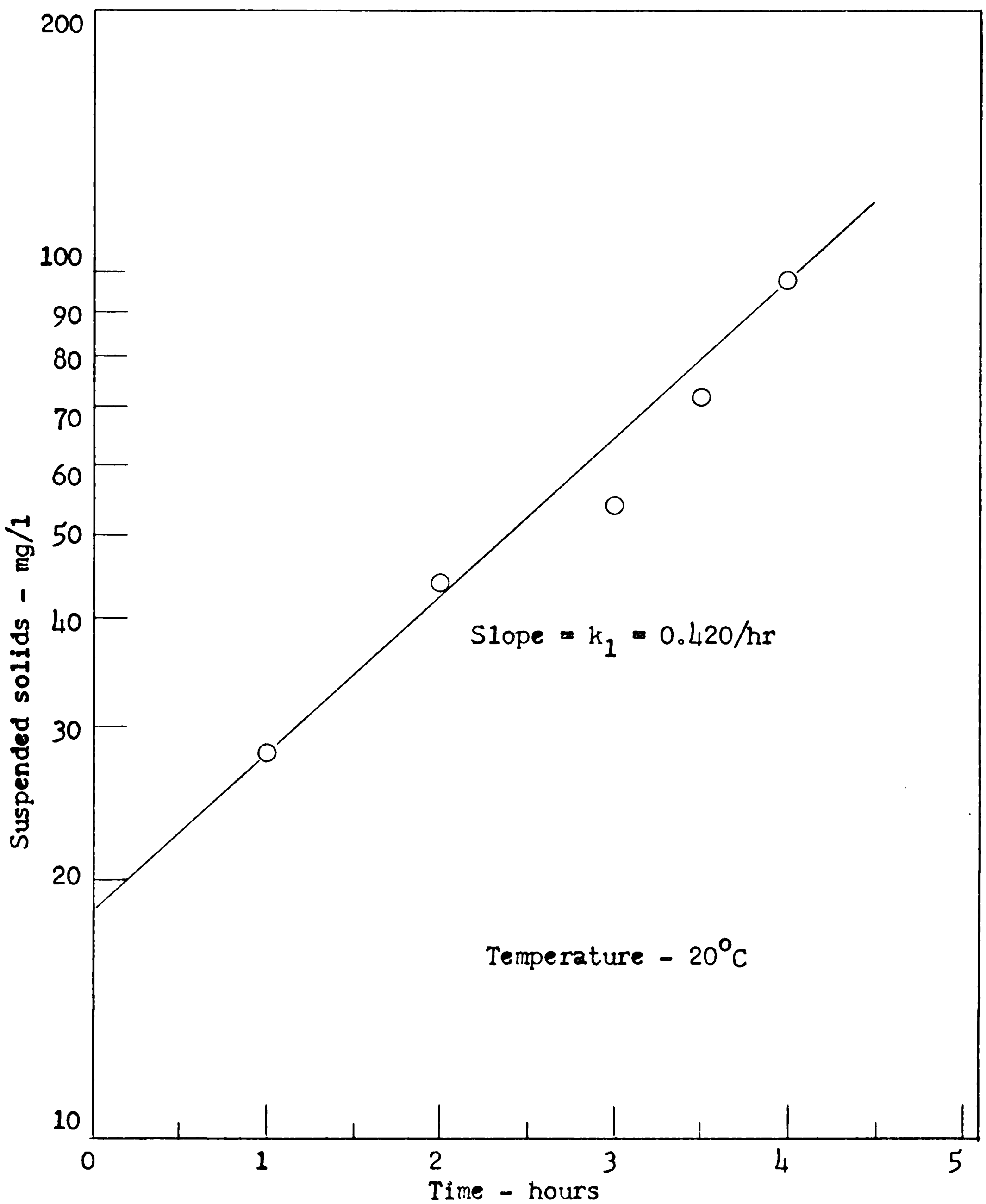


Figure 18. Experiment No. 9. Gravimetric measurement of specific growth rate

incubation period, suspended solids determinations were made at one hour intervals by filtering 50 ml of the culture through a 0.45 μ Millipore filter. The procedure outlined in Section III for suspended solids was followed. The data obtained in this experiment are summarized in Table IV. A semi-log plot of the suspended solids (cell concentration, mg/l) vs. time is given in Figure 18.

As indicated by the slopes obtained with each method, there was very good agreement in the results of the three experiments. The differential manometer, experiment No. 7, produced the highest growth rate, $k_1 = 0.430$/hr. Experiments 8 and 9 produced growth rates of 0.390/hr and 0.420/hr respectively.

An effort was made to keep the three experiments as representative of the other as possible. Thus, by keeping the primary effluent seed material well mixed, by using sterile lactose broth, and by using the same proportion of seed to media in each experiment, the environment and physiological conditions in each experiment were closely approximated. We realize, however, that we were not working with a pure culture, and that, in this sense, each experiment was not representative of the other.

Since gravimetric and optical measurements are established methods for evaluating the growth of bacteria, these experiments indicate that the differential manometer can also be used for this purpose. Of the three methods, the differential manometer seems to be more universal in its application. It is a closed system and oxygen consumption can be measured without disturbing the biological

balance of the sample. The effects of various nutrients and toxicants could be studied along with environmental factors such as pH and temperature.

E. The Effect of Initial Cell Concentration

Since the differential manometer measures the oxygen consumption of bacteria, it is important to know what effect variations in the initial cell concentration have on the oxygen uptake rate. The following experiment demonstrates this effect and illustrates the consequent changes which take place in the characteristics of the oxygen uptake rate curve.

Experiment No. 10

Sample: Primary effluent, grab sample, East Lansing Plant, 9:00 A.M., January 18, 1965. A small sample of return sludge was taken at the same time. A portion of the primary effluent was filtered through a Whatman No. 2 filter and 150 ml of the filtrate placed into a differential manometer reaction flask. A 150 ml portion of the original sample was placed into another reaction flask while 150 ml of the original sample plus 3 ml of the return sludge were placed into another. The three flasks were attached to their respective manometers and placed in a water bath at 20°C. The oxygen consumption was measured at 30 minute intervals for a period of 10 hours. The oxygen uptake rates vs. time were plotted on semi-log paper for the three curves as illustrated in Figure 19.

EAST LANSING PRIMARY EFFLUENT

9:00 A.M., January 18, 1965
Suspended solids = 146 mg/l
Volatile solids = 114 mg/l
BOD_5 = 168 mg/l
pH = 7.6

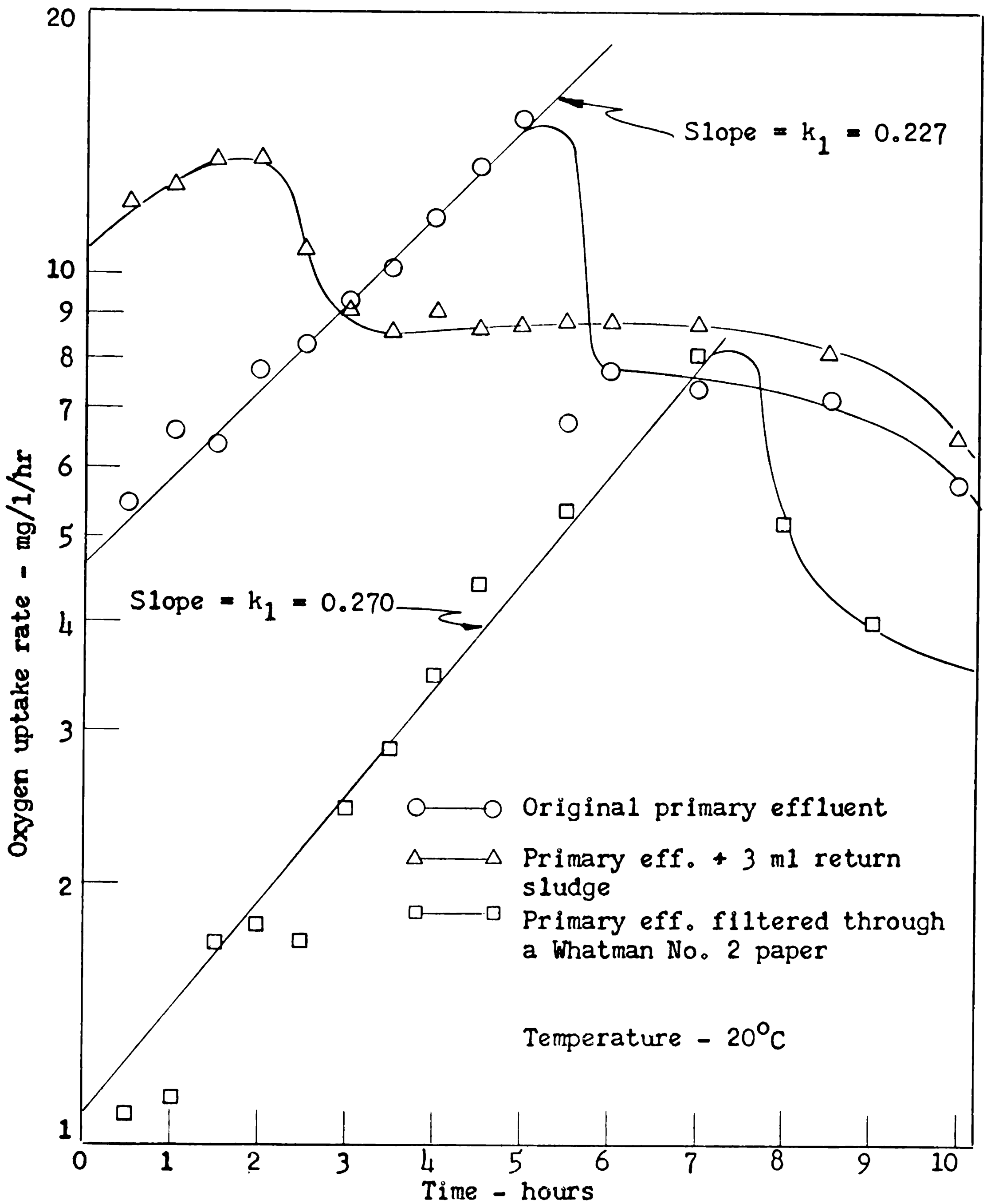


Figure 19. Experiment No. 10. Effect of initial cell concentration on characteristics of oxygen uptake curve

The first and most obvious effect of the initial population of bacteria is a change in the initial oxygen consumption rate. The larger the initial concentration of active cells, the higher the initial oxygen uptake rate.

The return sludge had a suspended solids concentration of 4800 mg/l. Therefore, the 3 ml addition to the original 150 ml of primary effluent represented an approximate addition of 100 mg/l suspended solids. This represents an increase of about 50 mg/l active cells if we assume that 50% of the suspended solids are active. The initial oxygen uptake rate increased from 5.5 mg/l/hr to 12 mg/l/hr. Obviously, this increase was due to an increase in cell numbers.

The filtered sample represented a decrease in the cell concentration. The observed effect was a decrease in the initial oxygen consumption rate from 5.5 mg/l/hr to about 1.1 mg/l/hr.

The initial cell concentration also had a marked effect on the maximum oxygen uptake rate, not only shifting the time of occurrance, but changing the rate as well. It took about five hours for the original sample to reach a maximum oxygen uptake rate of 15 mg/l/hr. The sample with the return sludge required two hours to reach a maximum rate of 13.5 mg/l/hr, while the filtered sample required seven hours to reach a maximum rate of 8 mg/l/hr.

It appears that the initial cell concentration can be increased to a point where the maximum oxygen uptake rate is reached instantaneously, thus completely eliminating the "typical" oxygen uptake rate curve represented by the original sample in Figure 19. It also seems that the higher the initial cell concentration, the higher the maximum oxygen uptake rate for a given substrate concentration. This is a result of the fact that the same amount of new cell material is produced for a given substrate concentration regardless of the initial cell concentration.

Experiment No. 10 does not completely demonstrate the latter statement. Although there was an increase in the maximum uptake rate from the filtered sample to the original sample, there was not a subsequent increase from the original sample to the sample with return sludge. The comparatively low rate obtained from the sample with return sludge may be caused by the poor condition of the sludge due to the fact that the East Lansing plant was consistently overloaded.

The k_1 values obtained from the graph were $k_1 = 0.227$/hr for the original sample and $k_1 = 0.27$ for the filtered sample. The reason for the slightly higher growth rate of the filtered sample is not clear at this time.

F. The Relation Between Substrate Concentration and Specific Growth Rate

The relationship between substrate concentration and growth rate of bacteria has been investigated by several authors (Monod, 1949; Hinshelwood, 1946; Novick, 1955; Herbert, Elsworth, and Telling, 1956; Schulze and Lipe, 1964). The results of these investigations indicate that the growth rate asymptotically approaches a maximum value as the substrate concentration increases. The purpose of the following experiments is to present a preliminary investigation of this relationship in sewage with the aid of the differential manometer.

Experiment No. 11

The composite sampler was set up at the East Lansing plant and samples proportional to the flow were collected every 30 minutes over the 24 hour period beginning at 6:00 A.M., September 15, 1964, and ending a 6:00 A.M., September16, 1964. During the same period, grab samples were collected at 6:00 A.M., 9:00 A.M., 12:00 Noon, 3:00 P.M., 6:00 P.M. and 12:00 Midnight. Since only four differential manometers were available, the 6:00 A.M., 9:00 A.M., and the 12:00 Noon samples were analyzed on September 15, 1964, while the other four samples were analyzed on September 16. The oxygen uptake rates for each sample were determined, plotted with respect to time on semi-log paper, and the growth rates measured. A summary of the results obtained from this experiment is given in Table V. Figure 20 illustrates the relationship obtained between BOD_5 and the corresponding growth rates.

Experiment No. 12

This experiment is a repeat of Experiment No. 11. The composite sampler was set up at the East Lansing Plant at 7:00 A.M. on September 24, 1964, and samples proportional to the flow were collected every 30 minutes until 7:00 A.M., September 25, 1964. Grab samples were taken during the same period at 7:00 A.M., 9:00 A.M.

12:00 Noon, 3:30 P.M., 7:00 P.M. and 12:00 Midnight. The samples were analyzed in the same manner as Experiment No. 11. A summary of the results obtained from this experiment is given in Table VI. Figure 20 illustrates the relationship obtained between BOD_5 and the corresponding growth rates.

Although there is inconclusive evidence to demonstrate that the growth rate is asymptotically approaching a maximum value as the substrate increases (BOD_5), the growth rate did increase with increasing BOD_5, and does appear to be leveling off at the higher BOD_5 values (Figure 20). This indicates that the specific growth rate is a function of the substrate concentration.

The relationship between growth rate and substrate concentration is different for the two experiments. The rate of increase in growth rate for Experiment No. 11 is much lower than the rate of increase in Experiment No. 12. The growth rate for a given substrate concentration is also lower in Experiment No. 11. This indicates that the growth rate is also a function of the composition of the substrate, and that some materials are metabolized fasten than others. Thus, especially in sewage where the type and composition of the organic matter varies considerably, it is possible to obtain a relatively wide range of growth rates for a given BOD_5. With this in mind, it becomes increasingly difficult to determine the relation of substrate concentration and growth rate in sewage.

TABLE V. Growth rate measurements for experiment No. 11

Date	Suspended Solids (mg/1)	Volatile Solids (mg/1)	pH	BOD_5 (mg/1)	Max. O_2 Uptake Rate (mg/1/hr)	Specific Growth Rate (hr^{-1})
9/15/64	76	42	8.1	165	10.8	0.332
9/15/64	88	68	7.7	120	11.8	0.308
9/15/64	66	40	7.6	102	9.5	0.272
9/15/64	80	60	7.6	111	11.9	0.289
24 hour composite 9/15/64-9/16/64	94	72	8.0	123	9.8	0.312

TABLE VI. Growth rate measurements for experiment No. 12

Date	Suspended Solids (mg/1)	Volatile Solids (mg/1)	pH	BOD_5 (mg/1)	Max. O_2 Uptake Rate (mg/1/hr)	Specific Growth Rate (hr^{-1})
9/24/64	46	38	7.7	69	8.5	0.185
9/24/64	66	54	7.7	90	10.6	0.277
9/24/64	58	34	7.8	132	15.5	0.388
9/24/64	96	66	7.7	108	12.3	0.365
9/24/64	86	68	7.5	90	12.0	0.275
9/24/64	78	58	7.7	98	13.6	0.321
24 hour composite 9/24/64 - 9/25/64	94	66	8.0	96	10.5	0.332

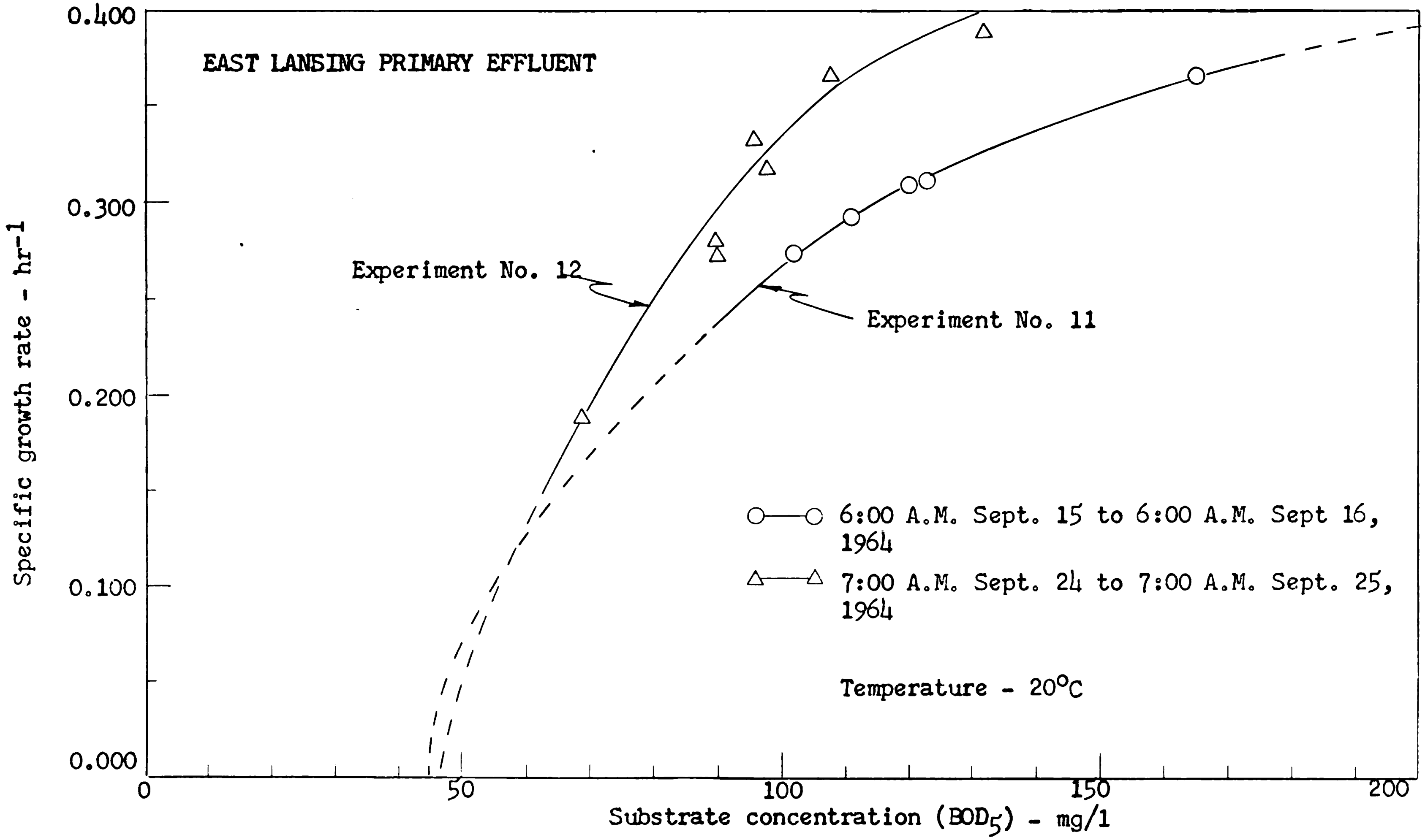


Figure 20. Relation between substrate concentration (BOD_5) and specific growth rate (k_1) for a 24 hour period

Growth rates were measured at the East Lansing Plant from July, 1964 to January, 1965. Both grab and composite samples were analyzed. Some samples were stored overnight while others were analyzed on the day of collection. Table VII summarizes the results found in these experiments and Figure 21 is a plot of k_1 versus BOD_5. The plot shows that no definite relationship between k_1 and BOD_5 can be established by these random samples. This is probably due to the variations in the composition of the organic material in the primary effluent. The plot does, however, contain information about the maximum value of k_1 which was measured during the period, $k_m = 0.39/hr$.

The BOD in this investigation was measured as standard five day BOD and, therefore, it represents dissolved, colloidal, and particulate BOD. Since substrate in colloidal and particulate form must be hydrolyzed and made soluble by exoenzymes before it can enter into the cells, it is not immediately available to the bacterial cells. The oxygen uptake measurement in the differential manometer reflects the cell metabolism due to dissolved substrate. Perhaps this is the reason why the growth rate curves in Figure 20 appear to reach zero at a total BOD_5 of around 40-50 mg/1, instead of passing through the origin. The dissolved BOD could be approaching zero at this concentration. In future investigations of this type the BOD determinations should be made on the original and on filtered samples.

TABLE VII. East Lansing growth rate measurements from July, 1964 to January, 1965

Date	Suspended Solids (mg/l)	Volatile Solids (mg/l)	pH	BOD_5 (mg/l)	Max. O_2 Uptake Rate (mg/l/hr)	Specific Growth Rate (hr^{-1})
7/7/64	85		7.8	135	10.5	0.278
7/8/64	130		7.7	165	10.8	0.268
7/15/64	82		7.7	110	10.0	0.305
7/23/64	76		7.5	155	13.0	0.320
8/11/64	85	60	7.6	125	11.5	0.198
8/17/64	93	55	7.6	110	9.8	0.244
9/15/64	76	43	8.2	165	10.8	0.332
9/15/64	88	68	7.7	120	11.8	0.308
9/15/64	66	40	7.6	102	9.5	0.272
9/15/64	80	60	7.6	111	11.9	0.289
24 hour composite 9/15/64 - 9/16/64	94	72	8.0	123	9.8	0.312
9/17/64	72	40	7.5	87	10.0	0.252
9/24/64	46	38	7.7	69	8.5	0.185
9/24/64	66	54	7.7	90	10.6	0.277
9/24/64	58	34	7.6	132	15.5	0.388
9/24/64	96	66	7.7	108	12.3	0.365
9/24/64	86	68	7.5	90	12.0	0.275
9/24/64	78	58	7.7	98	13.6	0.321
24 hour composite 9/24/64 - 9/25/64	94	66	8.0	96	10.5	0.332
9/30/64	96	68	7.5	150	23.0	0.294
9/30/64	120	90	7.6	171	25.5	0.379
1/18/65	140	114	7.6	168	15.0	0.227

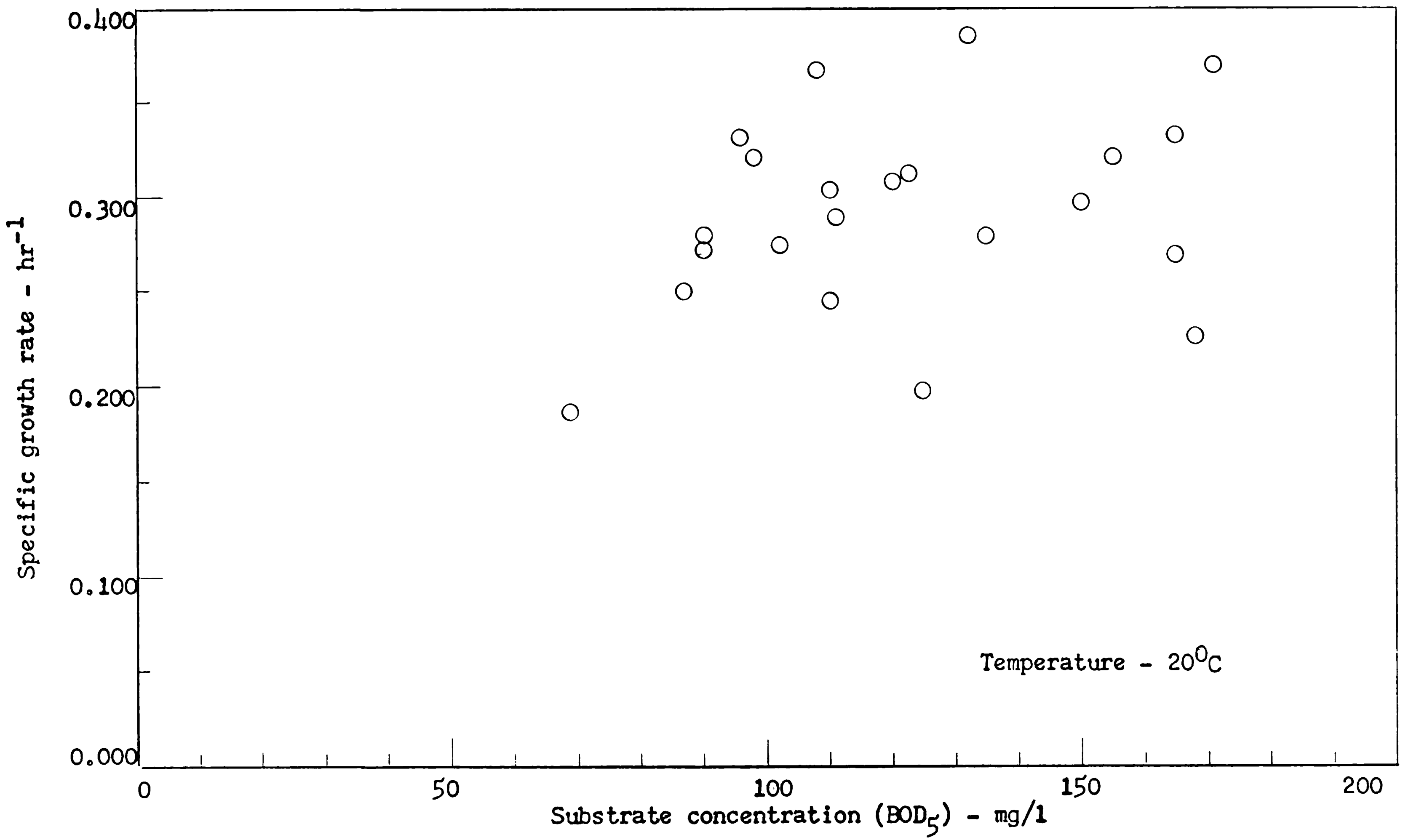


Figure 21. Plot of substrate concentration (BOD_5) versus specific growth rate (k_1) for the East Lansing Sewage Treatment Plant from July, 1964 to January, 1965; all samples

G. Oxygen Uptake Rate Curves from Lansing, Mason, and Williamston

In addition to the East Lansing samples, a number of samples from the primary effluent of the Lansing, Mason and Williamston Sewage Treatment Plants were tested. The results of these experiments are shown and discussed in the following section.

Experiment No. 13

Sample: Primary effluent, grab sample, Lansing Plant, September 17, 1964, 11:00 A.M. The sample was stored overnight. The next morning a 150 ml portion of the sample was placed in a differential manometer reaction flask. The oxygen consumption was measured at 20°C for a period of 10 hours. The oxygen uptake rates vs. time were plotted on semi-log paper as illustrated in Figure 22.

The oxygen uptake rate curve shown in Figure 22 is a typical example of several other curves obtained from the Lansing primary effluent. Table VIII summarizes the data from four experiments.

The growth rate found in experiment No. 13 was 0.160/hr at a BOD_5 of 120 mg/l. Evidently the Lansing primary effluent is not as favorable for bacterial growth as the East Lansing primary effluent. A BOD_5 of 120 mg/l at the East Lansing Plant resulted in a growth rate of 0.308/hr (Table VII). Since the Lansing Plant treats a large quantity of industrial waste, this is not surprising.

LANSING PRIMARY EFFLUENT

11:00 A.M., September 17, 1964
Suspended solids = 106 mg/1
Volatile solids = 74 mg/1
BOD_5 = 120 mg/1
pH = 8.0

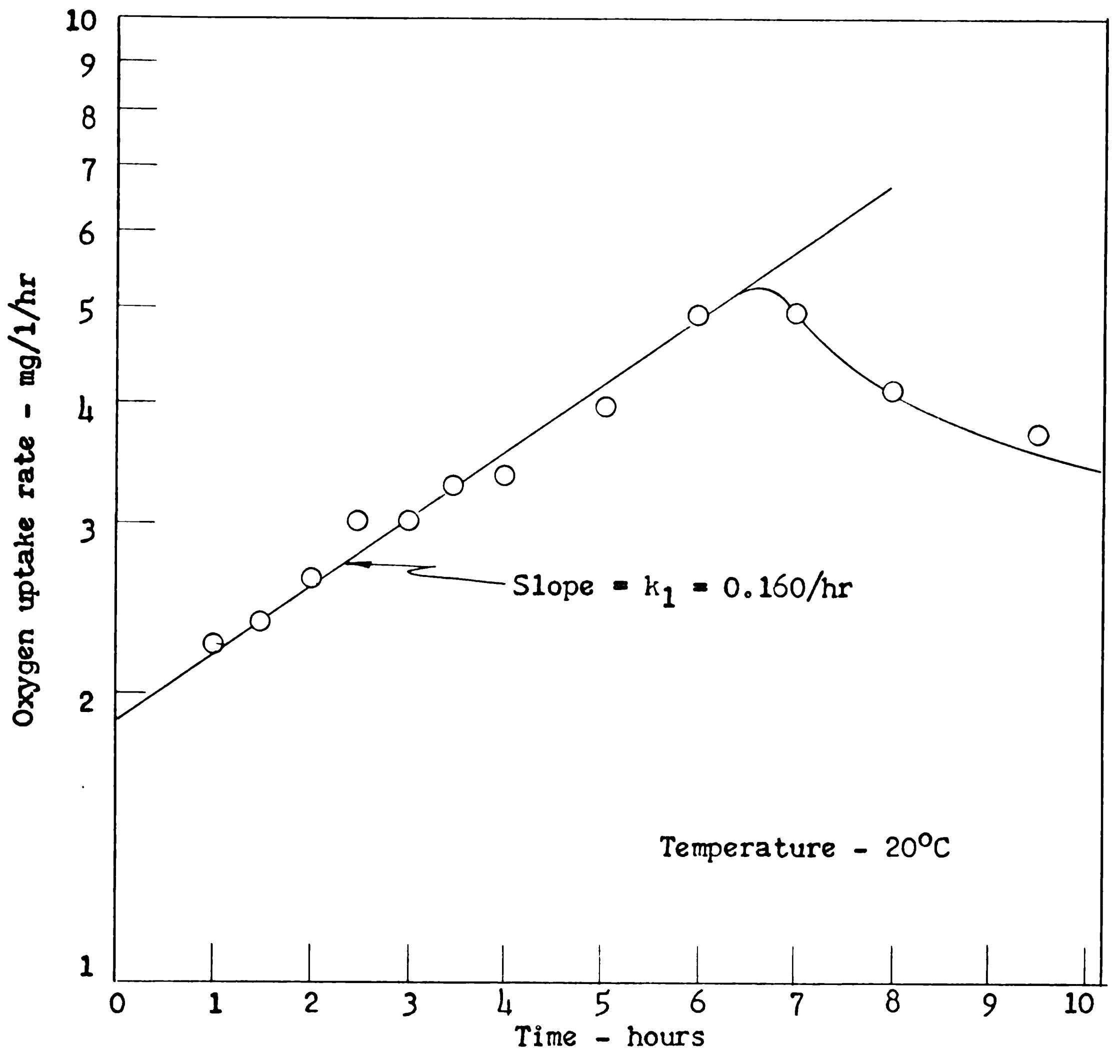


Figure 22. Experiment No. 13. Typical oxygen uptake rate curve from Lansing primary effluent

TABLE VIII. Lansing growth rate measurements

Date	Suspended Solids (mg/l)	Volatile Solids (mg/l)	pH	BOD_5 (mg/l)	Max. O_2 Uptake Rate (mg/l/hr)	Specific Growth Rate (hr^{-1})
7/29/64	163		7.5	100	4.2	0.210
8/5/64	82		8.0	70	4.0	0.215
8/11/64	126	81	7.3	80	5.0	0.231
9/17/64	106	74	8.0	120	5.0	0.160

The maximum oxygen uptake rate of 5.0 mg/l/hr is consistent with other experiments (Table VIII.). Usually, the values were in the 4.0 - 5.0 mg/l/hr range. It required about 7 to 8 hours to reach the maximum oxygen uptake rate (Figure 22). This was also very typical of the Lansing sewage. The East Lansing sewage usually required from 3 to 5 hours to reach the maximum rate.

Experiment No. 14

A grab sample was obtained from the Mason, Michigan plant at 3:00 P.M. on July 30, 1964. The sample was stored overnight. The next morning a 150 ml portion of the sample was placed into a differential manometer reaction flask and analyzed at 20°C for oxygen consumption. A second grab sample was obtained at 10:00 A.M. on August 19, 1964 and analyzed in the same manner. A semi-log plot of the oxygen uptake rate vs. time for the above data is presented in Figure 23.

Initial trial samples from the Mason plant regularly produced curves such as curve No. 1 in Figure 23, i.e. not a "typical" oxygen uptake curve. There was no period of constant logarithmic growth.

MASON PRIMARY EFFLUENT

3:00 P.M., July 30, 1964
Suspended solids = 275 mg/1
BOD_5 = 165 mg/1
pH = 7.7

10:00 A.M., August 19, 1964
Suspended solids = 58 mg/1
Volatile solids = 39 mg/1
BOD_5 = 110 mg/1
pH = 7.9

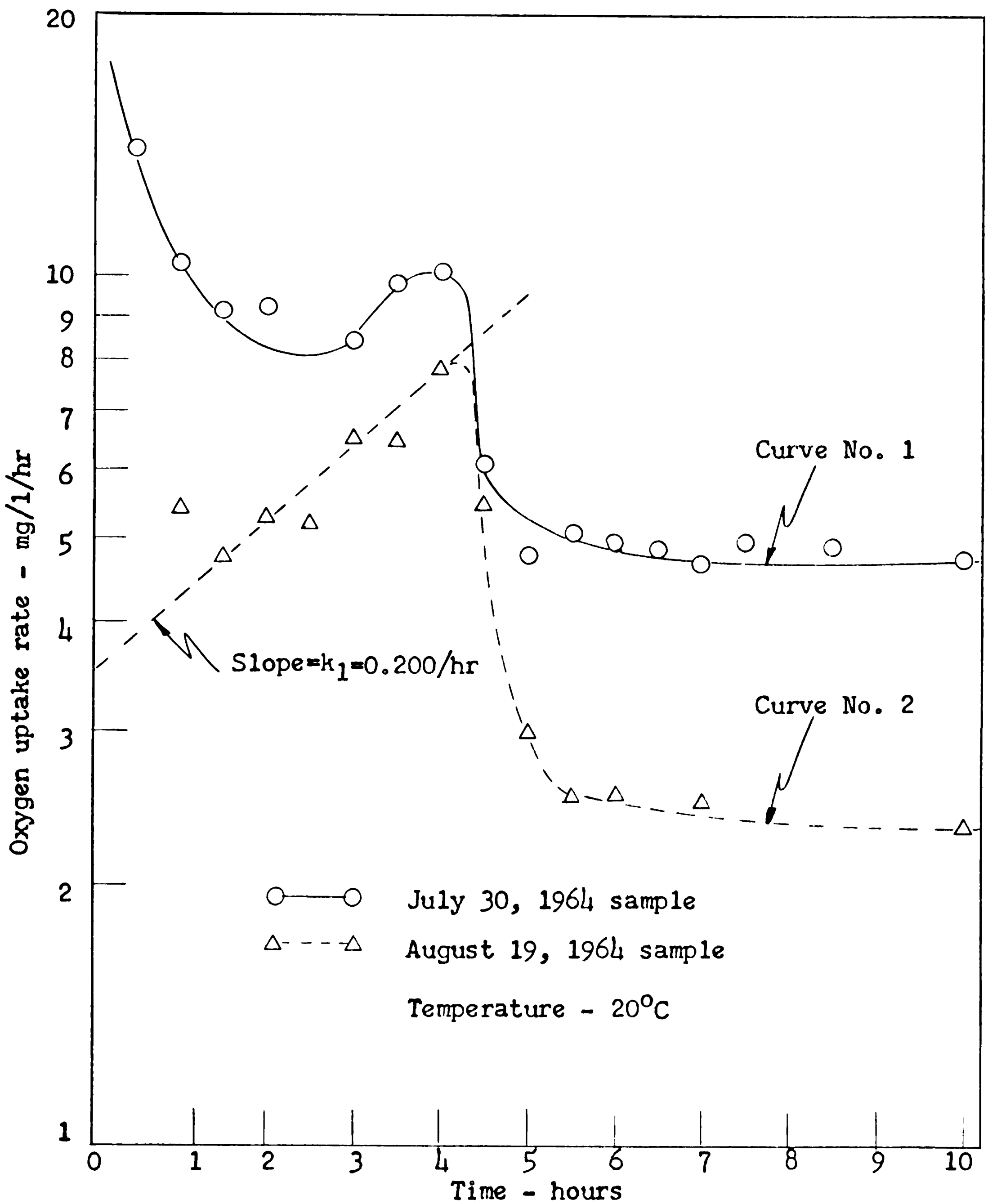


Figure 23. Experiment No. 14. Oxygen uptake rate curves for Mason Sewage Treatment Plant

This was surprising since the BOD_5 was always well above 100 mg/l. A possible explanation was the high suspended solids concentration (275 mg/l). The primary effluent suspended solids concentration in the East Lansing Plant very seldom measured above 100 mg/l (Table VII). In questioning the plant operator about this, it was found out that he was having difficulty with the sludge withdrawal system in the primary tanks. Since excess sludge was wasted through the primary tanks, there was a build-up in suspended solids and the tank was actually becoming septic. Once this problem was solved, the suspended solids concentration decreased and a "typical" curve such as curve No. 2 in Figure 23 was obtained.

The high concentration of suspended solids in the earlier samples probably also represented a high concentration of bacteria. The effect of the initial cell concentration was discussed in Part E of this thesis. The shape of curve No. 1 can therefore be explained on the basis of a large initial cell concentration.

The August 19 sample reached a maximum oxygen uptake rate of 8.0 mg/l/hr after four hours. The specific growth rate was 0.20 per hour at a BOD_5 of 110 mg/l. This seems to be comparable to the type of curve obtained from the East Lansing Plant.

Experiment No. 15

A grab sample was obtained from the primary effluent of the Williamston, Michigan Plant at 11:00 A.M. on September 21, 1964. At the same time a grab sample was taken from the incoming raw

WILLIAMSTON PRIMARY EFFLUENT

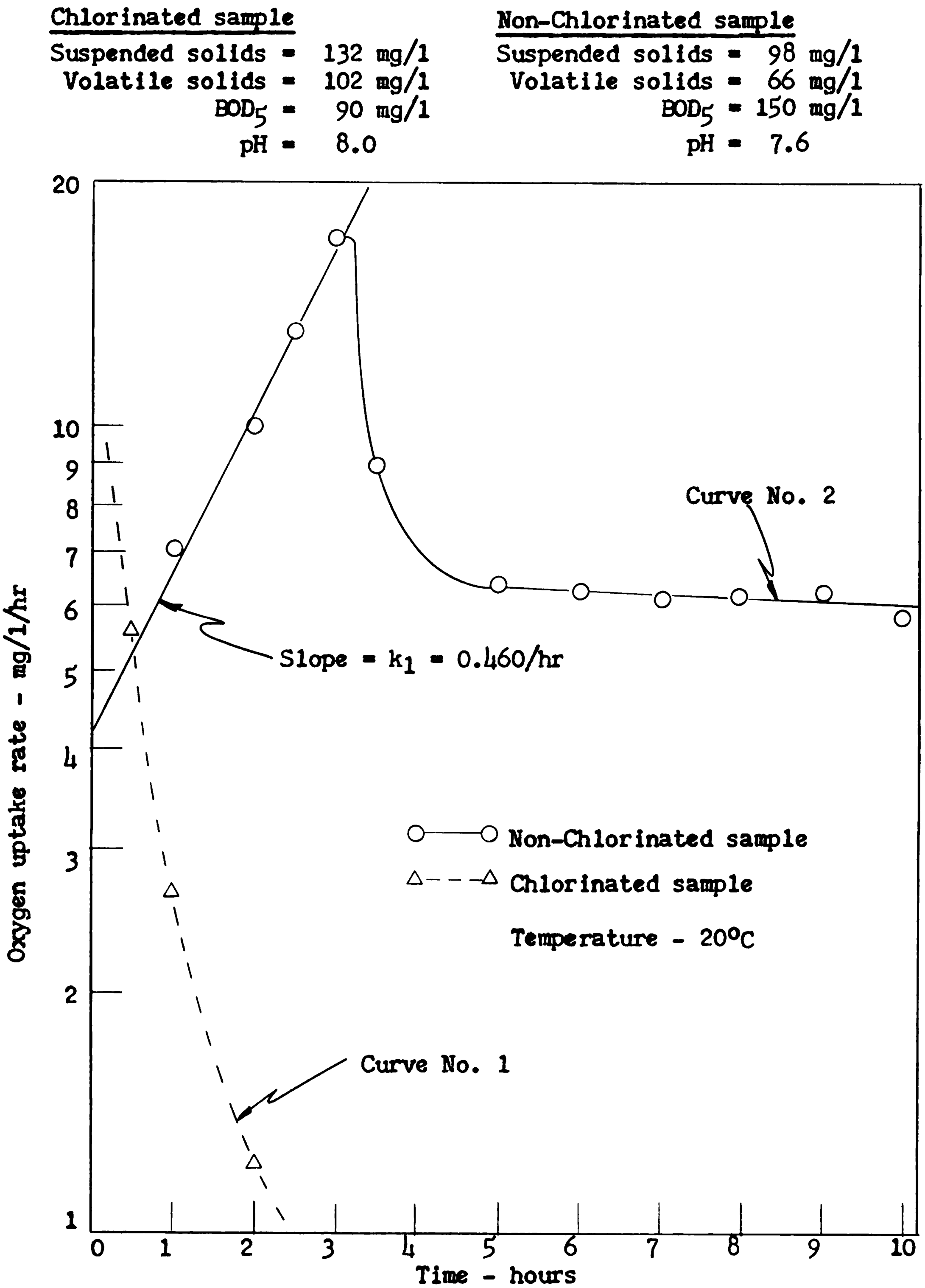


Figure 24. Experiment No. 15. Oxygen uptake rate curves for the Williamston Sewage Treatment Plant

sewage. Upon returning to the lab the raw sewage was allowed to settle for 30 minutes. After the settling period, the supernatant was siphoned off. A 150 ml portion of the supernatant was placed into a differential manometer reaction flask and 150 ml of the primary effluent was placed into another reaction flask. The two samples were at 20°C for oxygen consumption. A semi-log plot of the oxygen uptake rates vs. time for the two samples is given in Figure 24.

A raw sewage sample was used for this experiment because at the Williamston plant the sewage was chlorinated just before it entered the primary tank. This was done because there were no facilities for a chlorine holding tank.

The chlorinated primary effluent samples demonstrated a rapidly decreasing oxygen uptake rate such as shown in curve No. 1, Figure 24. In a matter of 2-3 hours, the uptake rates decreased essentially to zero. The surprising thing was that there was any oxygen uptake at all. Some of the samples were stored for almost 24 hours at 0-5°C.

The settled raw sewage, on the other hand, produced a "typical" oxygen uptake curve such as shown in curve No. 2, Figure 24. The growth rate was 0.460/hr for a BOD_5 of 150 mg/1. This was actually the highest growth rate measured and it indicates a high degree of treatability for the Williamston sewage. The maximum oxygen uptake rate of 17.0 mg/1/hr was reached in three hours.

V. DISCUSSION

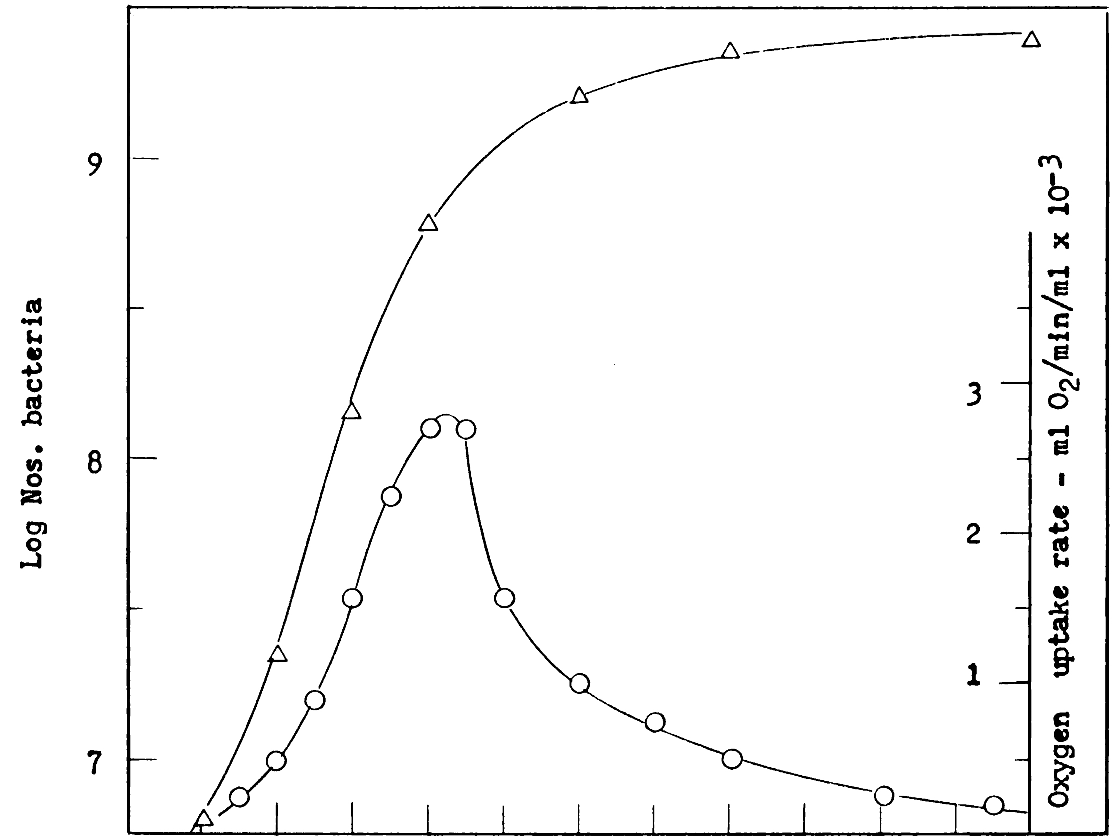
The low oxygen uptake rates in sewage do not create an oxygen demand large enough to be measured accurately in a Warburg apparatus. On the other hand, the differential manometer has many desirable features which make the device useful in the waste treatment field.

Basically, the differential manometer has three advantages over the Warburg apparatus: (1) large sample volumes can be analyzed, making it possible to study more representative samples and to increase the amount of oxygen obsorption; (2) flask contents are stirred with a magnetic stirring rod, making it possible to keep dissolved oxygen in larger samples; and (3) the amount of gas exchanged is measured directly in terms of ml O_2 consumed, thus eliminating the necessity for calibrating the apparatus.

It is interesting to note that Clifton (1937) obtained oxygen uptake rate curves in 1937 similar to those found in this investigation. Using standard Warburg techniques, Clifton obtained oxygen uptake rate curves for A. aerogenes, E. typhi and E. coli growing in a 1.0 per cent peptone solution at 37.5°C. He worked with a high initial cell concentration (8×10^6 cells/ml). The maximum oxygen uptake rate measured for A. aerogenes was 240 mg/l/hr (at standard conditions). This is about 20 times higher than the maximum oxygen rates of 10-15 mg/l/hr found in this investigation.

Clifton also made plate counts from duplicate control cultures. Thus, he was able to follow the increase in cell numbers along with

Figure 25. Time-growth, time-oxygen uptake rate relationships observed during growth of <u>A. aerogenes</u> in a 1.0 per cent peptone solution at 37.5°C, from Clifton, 1937.



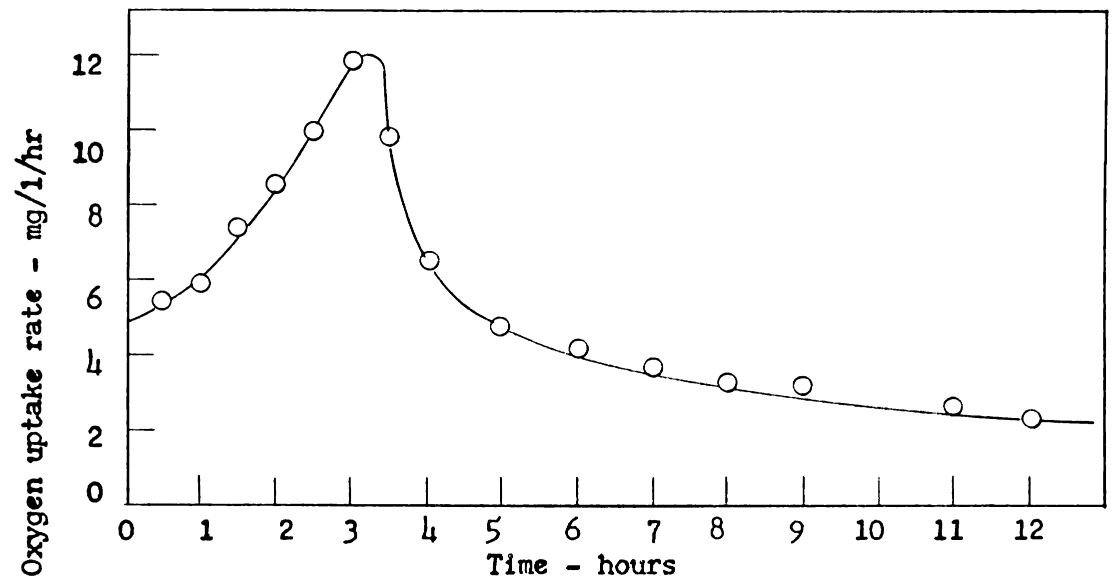


Figure 26. Time-oxygen uptake rate curve from East Lansing primary effluent with BOD_5 = 120 mg/1 at 20°C, September 15, 1964; with differential manometer

the corresponding oxygen uptake rates. With this type of analysis a better understanding of the oxygen uptake rate curve is possible. Figure 25 demonstrates the time-growth, time-oxygen uptake rate relationships found by Clifton for A. aerogenes. Figure 26 shows a comparable time-oxygen uptake rate curve obtained by differential manometer for the East Lansing Primary Effluent.

Except for a difference in scale, the oxygen uptake rate curves in Figure 25 and 26 are very similar. The oxygen uptake rates appear to rise exponentially until a maximum value is reached and then decrease to a low endogenous rate. Even though the oxygen uptake rate decreases very rapidly after the maximum rate is reached, the bacteria in the culture are still growing (Figure 25).

There appear to be two processes taking place in sewage which affect the rate of oxidation as measured with the differential manometer. First, there is exponential growth due to the presence of available substrate. Simultaneously the oxygen uptake rate increases exponentially. Second, when the substrate has been nearly depleted the growth rate decreases continually and the oxygen uptake rate per cell decreases proportionally.

At these levels of substrate concentration where the growth rate is a function of substrate concentration, we cannot actually measure a constant growth rate with the differential manometer. But, because we deal with low cell concentrations in sewage, the initial decrease in the amount of available substrate is small. Thus, in the first few hours of the experiment the oxygen uptake rate appears to be increasing exponentially. However, as the substrate concentration is further decreased and the bacterial population increased, the balance shifts in the other direction. The oxygen uptake rate per cell decreases faster than the increase in oxygen uptake rate due to growth. Thus, we have a decrease in the oxygen uptake rate with time, even though the bacteria may still be growing.

VI. CONCLUSIONS

1. The differential manometer is more accurate than the Warburg apparatus for oxygen consumption measurements in sewage.

2. Reproducible results can be obtained from differential manometer measurements.

3. Samples can be stored at 0-5°C for 24 hours.

4. The differential manometer can be used to evaluate the growth rate of bacteria in sewage.

5. The maximum hourly respiration rate per unit volume is related to the initial BOD and cell concentration.

6. The specific growth rate is a function of the available substrate composition and concentration.

7. A bio-degradable sewage can be represented by the "typical" time-oxygen uptake rate curve. The "typical" curve is indicative of bacteria growth.

8. The maximum growth rate value k_1 at 20°C was found to be approximately 0.39 per hour for East Lansing, Michigan, primary effluent.

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