WARBURG STUDIES OF THE OXIDATIVE ACTIVITIES OF ACTIVATED SLUDGE SUBJECTED TO VARIED CONCENTRATIONS OF GREASE

by

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I. INTRODUCTION

Grease is a natural component of sewage and some industrial wastes and has been the source of certain operational difficulties in waste water treatment systems since their inception. Grease has been responsible for the clogging of sewer lines, fine screens, dosing siphons, and trickling filter nozzles as well as adding to the biochemical oxygen demand (BOD) of the waste and disrupting anaerobic digestion units.

Grease, which includes fats, oils, waxes, and high molecular-weight fatty acids, is usually in the colloidal form in sewage since few of these compounds are water soluble and are usually introduced through a washing process. Historically, grease treatment has consisted of removal of the naturally destabilized grease from the waste water, either at its source by means of a grease trap, or at the treatment plant by various skimming mechanisms. Where grease constitutes especially difficult operating problems, removal is attempted by various mechanical and/or chemical means which are designed to destabilize the emulsion and cause the grease to float on the water's surface where it can be removed by skimming.

The grease thus removed is disposed of by various methods which include burial, incineration, and anaerobic

digestion. Anaerobic digestion is the least desirable method since grease acts as a matrix for the formation of scum layers and also causes a depression of the pH in the unit resulting in digestor failure.

Sewage grease is made up predominantly of animal and vegetable fats and oils, and therefore should be readily metabolized by the organisms utilized in biological waste treatment systems. Many bacteria dissimilate fats by the process of lipolysis, employing enzymes known as lipases. Such a scheme is illustrated below.

$$\text{FAT (LIPID)} + H_2O \xrightarrow[\text{LIPASE}]{} \text{GLYCEROL} + \text{FATTY ACID(S)}$$

It has been almost universally accepted that sewage grease is detrimental to aerobic treatment systems and cannot be treated effectively in these systems. The object of this investigation was to explore aerobic degradation of a natural sewage grease by the organisms of activated sludge. Manometric techniques were utilized to measure the oxygen consumed and the rate of consumption with various concentrations of grease.

If experimental data indicate that grease is readily oxidized by the organisms of activated sludge, the costly construction of flotation and coagulation units may be avoided by utilizing activated sludge units, with some possible modification, with a considerable savings in economy of design and operation.

II. REVIEW OF LITERATURE

The term grease applies to a wide variety of organic substances that are extracted from aqueous solution or suspension by hexane. Hydrocarbons, esters, oils, fats, waxes, and high molecular-weight fatty acids are the major materials included in this category (1, 9). All these materials have a "greasy feel" and are associated with the multifaceted difficulties in waste treatment related to grease.

Grease is a natural component of sewage having several origins of introduction: a) fecal material, which contains approximately 225 mg/l grease per gram of fecal material; b) kitchen slops, garbage, and household waste waters; c) manufacturing wastes, such as meat and poultry processing plants, package food processing plants, and refinery wastes; and d) garage and automobile washing plants (2, 3, 7). With the increased use of home garbage disposals, the total grease content of sewage has increased considerably. Watson et al (17) indicate there is an average per capita increase of 35 per cent in the grease content of sewage in homes after the installation of garbage disposals.

Grease presents many problems in the field of sewage and industrial waste treatment. The major problems are:

clogging of fine screens; formation of unsightly scum on the surface and walls of sedimentation basins; increased biochemical oxygen demand (BOD); clogging of trickling filter nozzles; disruption of anaerobic digestion by causing a depression of the pH and by serving as a matrix for the formation of scum blankets; and inhibiting oxygen transfer to biological cells in aerobic treatment (7, 10).

Grease usually reaches the treatment plant in particulate form. Heukelekian and Balmat (6) reported that the major portion of grease is particulate and consists of 19.13 per cent settleable, 23.85 per cent supercolloidal and 51.48 per cent colloidal particles. Since the major portion of the grease is found in the colloidal form, it may be acted upon readily by microorganisms.

It is generally assumed that the saponifiable or fatty matter portion of the grease is most readily metabolized by microorganisms. Bowerman and Dryden (2) in a study at Los Angeles reported that 90 to 95 per cent of the grease was in the form of saponified fatty acids or insoluble soaps. Heukelekian (6) reported that glyceride fatty acids comprise the major portion of the total grease. Mahlie (7) indicated that the major constituents of grease are soaps and glycerides.

Considerable effort has been expended in studying anaerobic degradation of grease (7) while very little

mention is given to aerobic degradation. There is very little information in the literature concerning the aerobic degradation of grease, and this appears to be contradictory. Mahlie (7) indicated that grease will disrupt activated sludge and trickling filters. Fullen and Hill (3) doubted the degradability of grease under normal circumstances since it is not soluble to any great extent. Viswanathan et al (14, 15, 16) reported that the fatty matter portion of grease is readily degradable by activated sludge. Comparing three treatment systems, biological filters, septic tanks, and activated sludge, Viswanathan et al (14) reported that activated sludge removed grease with great rapidity and that the effluent was free from grease after 18 days, while the biological filters and anaerobic systems still contained considerable grease. In a later report using a batch activated sludge process and natural sewage, Viswanathan et al (14) noted an absence of grease in the effluent from the activated sludge system after three days. Further experimentation is necessary to define the oxidative behavior of organisms of activated sludge in the presence of grease before it can be concluded that activated sludge can or cannot be considered an effective treatment method for grease.

III. MATERIALS AND METHODS

A. Specialized Materials

1. Extraction Apparatus, Pyrex, Micro Soxhlet. Used in grease extraction.

2. Extraction Thimbles, Whatman, Micro. Used in grease extraction.

3. Filter Glass Fiber, Reeve Angel. Used in suspended and volatile solids determination.

4. Filter Paper, Whatman, No. 40. Used in grease determination.

5. Warburg Apparatus, Precision Scientific Company. Used to measure the oxygen uptake of the waste.

6. Alkyl Benzene Sulfonate, Soap and Detergent Association. Used in substrate.

7. Alumina Activated, Matheson Coleman and Bell, Chromatographic Grade, 80-200 Mesh. Used to determine hydrocarbon and fatty matter content of grease.

8. Ammonium Chloride, (NH_4Cl), Allied Chemical, A.S.C. Used in buffer for substrate.

9. Bacto-Dextrose, Difco Laboratories. Used in substrate.

10. Bacto-Peptone, Difco Laboratories. Used in substrate.

11. Bacto-Yeast Extract, Difco Laboratories, Dehydrated. Used in substrate.

12. Dipotassium Hydrogen Phosphate, (K_2HPO_4), Fisher, A.S.C. Used in buffer for substrate.

13. Potassium Dihydrogen Phosphate, (KH_2PO_4), Fisher A.S.C. Used in buffer for substrate.

14. Sodium Phosphate, ($Na_2HPO_4 \cdot 7H_2O$), Baker, Reagent Grade. Used in buffer for substrate.

15. Chemicals and other materials for tests according to Standard Methods (1).

B. Methods

1. Procedure for Obtaining Grease Source.

The grease used in this investigation was obtained from the grease flotation unit at Strouble's Creek Sewage Treatment Plant which is owned by the Blacksburg-V.P.I. Sanitation Authority. In order to utilize the grease in its pure form, the grease was extracted with hexane. This was done by acidifying the grease to pH 1 in an aqueous suspension with concentrated hydrochloric acid (HCl). Hexane was added to the

aqueous suspension and mixed for two hours on an automatic shaker. The mixture was then allowed to become quiescent. The hexane was decanted and filtered through Whatman No. 40 filter paper. Filtering was facilitated by a Buchner funnel and a vacuum pump. The residue remaining after filtering was returned to the container with the original grease. The hexane was distilled at 85°C over a water bath. Hexane was recovered for reuse while the grease was collected and refrigerated at 10°C. The above process was repeated on the original grease sample until no more grease could be recovered from the hexane.

2. Laboratory Bench Activated Sludge Unit.

The activated sludge unit, Figure 1, consisted of an aeration tank, settling tank, sludge return pump, and substrate feed pumps. Substrate (A) was fed at the rate of 167 ml per hour. Grease emulsion (substrate B) was fed at the rate of 10 ml per hour. The aeration tank had a working volume of 4 liters and a detention time of 24 hours at the normal substrate feed rates. Diffused air released near the bottom provided aeration and some mixing. A magnetic mixer was used to insure thorough mixing of the aeration tank contents. Mixed liquor

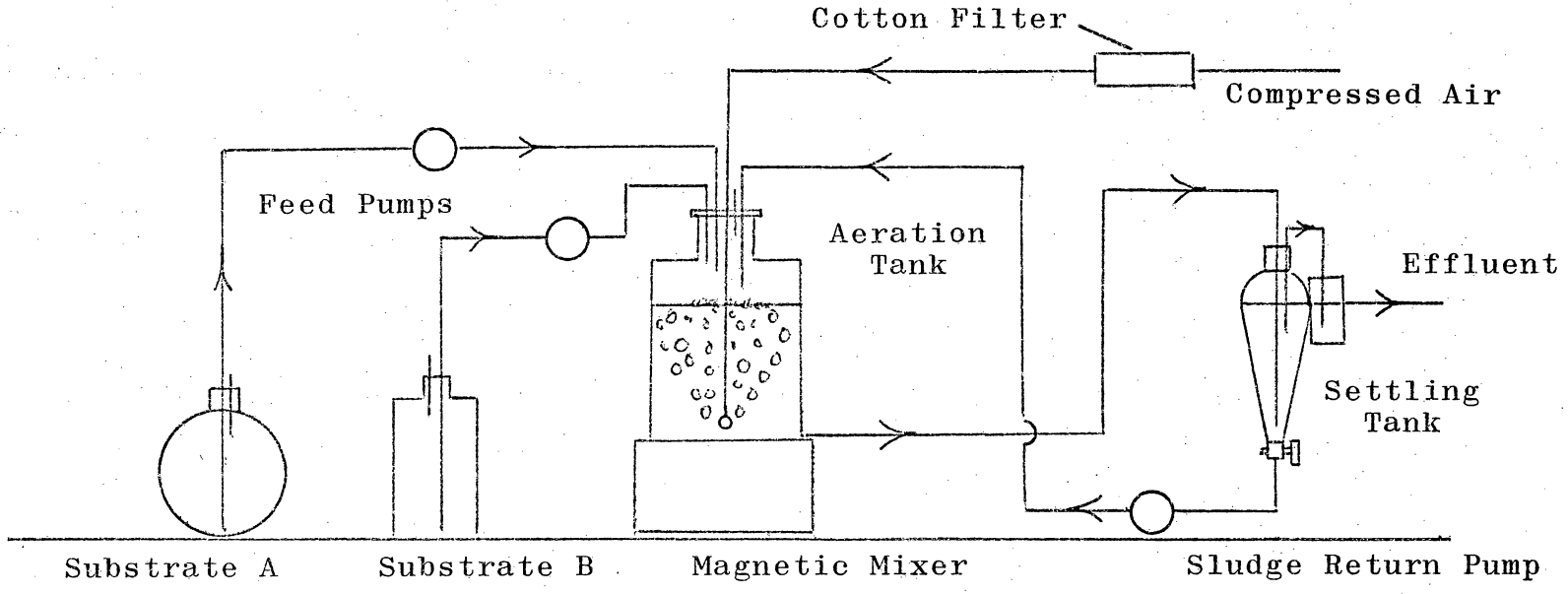

Figure 1. Activated Sludge Bench Unit.

passed by gravity to a 1000 ml separatory funnel which acted as a settling tank. The settling tank has a working volume of 750 ml which provided 4.5 hours detention at normal flow rates. Supernatant was continuously removed from the settling tank by a siphon to a constant head tank. Effluent was discharged to waste. Settled sludge was returned to the aeration tank by means of a pump rated at 416 ml per hour, which provided a recirculation ratio of 2.5 to 1. The unit was originally seeded with sludge obtained from the aeration tank at the Roanoke, Virginia municipal sewage treatment plant.

3. Substrates.

Substrate A was prepared every third day in a 12 liter pyrex bubble, and autoclaved to prevent decomposition prior to being pumped to the aeration tank. Table I shows the composition of substrate A.

Substrate B (a grease emulsion) was prepared by dilution of a stock grease emulsion with distilled water to a concentration of 167 mg/l. This provided a feed concentration of 10 mg/l per day in the aeration tank.

TABLE I

COMPOSITION OF SUBSTRATE A

Constituent	Quantity
Peptone	200 mg/l
Dextrose	50 mg/l
Yeast Extract	40 mg/l
ABS	2 mg/l
Phosphate Buffer	25 ml/l
Tap Water	200 ml/l
Distilled Water	To Volume

Substrate A and substrate B combined had a biochemical oxygen demand of approximately 250 mg/l.

The grease used in substrate B had a fatty matter content of 93.4 per cent and a chemical oxygen demand of 10.6 mg/l per mg of grease.

4. Hydrocarbon and Fatty Matter Content of Grease.

The methods and procedure for testing grease for hydrocarbon and fatty matter content were those contained in the twelfth edition of Standard Methods (1).

5. Total Suspended Matter (Nonfiltrable Residue).

The methods and procedure for determining suspended solids were those contained in the twelfth edition of Standard Methods (1) with the exception of the asbestos mat filter for the Gooch crucible. A glass fiber filter was used in its place.

6. Volatile Suspended Matter.

The methods and procedure for determining volatile suspended solids were those contained in the twelfth edition of Standard Methods (1).

7. Oxygen Demand (Chemical).

The methods and procedure for determining

the chemical oxygen demand of grease were those contained in the twelfth edition of Standard Methods (1). An emulsion of 100 mg/l grease was tested for C.O.D.

8. Sludge Volume Index.

The method and procedure for determining the sludge volume index of activated sludge was that contained in the twelfth edition of Standard Methods (1).

9. Preparation of Stock Grease Emulsion.

Natural sewage grease previously extracted and stored at 10°C was liquified by placing the container in a hot water bath. One ml of the shaken liquified grease was added to a Waring blender containing 250 ml of distilled water and blended for one minute. The emulsion was then analyzed for grease content.

10. pH.

A glass-calomel electrode (Leeds and Northrop) pH meter was used to measure the pH value of the samples directly.

11. Grease Extraction.

Grease was extracted by a modified Soxhlet technique. For detailed procedure see Appendix A.

12. Cleaning of Warburg Reaction Flasks.

The method used for cleaning the reaction flasks was that suggested by McKinney (8). See Appendix B for detailed procedure.

13. Calibration of Warburg Reaction Flasks.

The reaction flasks were calibrated by the water method as suggested by McKinney (8). Formulation for calculating flask constants were those of Umbriet (13). A sample calculation is shown in Appendix C.

14. Preparation of Warburg Reaction Flask for Use.

Prior to the reaction flasks reuse, the center wells were greased with a petroleum based lubricant, such as stopcock grease, to prevent the creep of potassium hydroxide into the microbial solution under study. Greasing the center well was facilitated by the use of a long handled swab. The 20 per cent potassium hydroxide solution used was injected into the center well with a 1 ml syringe.

15. Preparation and Addition of Activated Sludge and Substrates to Warburg Flasks.

A thoroughly mixed 1000 ml sample was withdrawn from the aeration tank of the laboratory bench activated sludge unit. A sample of the

sludge to be used in the respirometer was analyzed for total suspended solids, volatile suspended solids, and sludge volume index. Samples of the sludge were also observed under the microscope to determine gross floc characteristics and protozan activity. Since the activated sludge used in the experiment was subjected to a 50 per cent dilution in the Warburg flask, it was first concentrated to 4000 mg/l suspended solids by decanting the supernatant after sludge settling. Standard grease emulsions were prepared as previously described in this chapter. The activated sludge was blended for 10 seconds in a Waring blender to insure homogeneity before being added to the Warburg flasks via a 10 ml tilting dispenser. Substrates and distilled water were added via pipette to establish a final flask volume of 20 ml.

IV. EXPERIMENTAL RESULTS

Experiment I. Effect of Various Concentrations of Grease on the Organisms of Activated Sludge.

The object of this experiment was to study the effects of varied grease concentrations on the oxygen demand exerted by activated sludge. Measurements of the sludge oxidative activities were performed using the Warburg respirometer technique. A run of four days was employed in this test. The respirometer was operated at 20°C with a shaker rate of 60 cycles per minute. All samples were run in triplicate including the control.

Activated Sludge

The activated sludge used in this experiment had the following characteristics: total suspended solids, 1600 mg/l; volatile suspended solids, 1550 mg/l; per cent volatile solids in suspended solids, 97; sludge volume index, 200; color, golden brown; and large numbers of stalked ciliates and rotifers. This sludge had been acclimated to substrates A and B for approximately three and one-half months and had reached a steady state condition.

Substrate

A standard grease emulsion of 800 mg/l grease was prepared as outlined in the chapter "Materials and Methods." Table II shows the flask additions of activated sludge, grease emulsion, and distilled water, and the resulting

flask concentration. Concentrations of 100, 200, 300, and 400 mg/l grease were used in this experiment.

BOD Values

Gross cumulative BOD values are shown graphically in Figure 2 and net cumulative BOD values are shown graphically in Figures 3 through 6. Data points for both gross and net BOD are an average of the triplicate values for each concentration. Gross BOD values are those obtained directly by multiplying the millimeters change in pressure in the reaction flask by the flask constant. Net BOD values are those obtained by subtracting the gross values of BOD for a given sample from the BOD of the control. Net BOD represents the BOD of the substrate tested.

K_{10} and L Values

The Thomas Graphical Method (12) was used to determine the velocity constant (K_{10}) and ultimate BOD (L) for each stage of net BOD exertion, as well as determining the beginning and end of each stage. These results are shown in Table III. See Appendix D for a sample for application of the Thomas Graphical Method.

Results

The cumulative net BOD curves (Figures 3, 4, 5, 6) indicate that the grease was readily metabolized by the

organisms of activated sludge. Slight "lag" periods were observed in the 200, 300, and 400 mg/l grease samples. This could have resulted from an initial high food to microorganism (F/M) ratio, and is characterized by a concave upward curve. All four concentrations of grease exhibited a plateau when evaluated as net BOD exertion. These plateaus varied in magnitude in a sequential manner, the most pronounced found in the 100 mg/l grease concentration curve, and the least obvious in the 400 mg/l grease concentration curve.

TABLE II

FLASK ADDITIONS AND RESULTING CONCENTRATIONS
EXPERIMENT I

Flask	Activated Sludge ml Added	Activated Sludge Concentration mg/l	Grease ml Added	Grease Concentration mg/l	Distilled Water ml
1	10	2000	0	0	10.0
2	10	2000	0	0	10.0
3	10	2000	0	0	10.0
4	10	2000	2.5	100	7.5
5	10	2000	2.5	100	7.5
6	10	2000	2.5	100	7.5
8	10	2000	5.0	200	5.0
9	10	2000	5.0	200	5.0
11	10	2000	5.0	200	5.0
12	10	2000	7.5	300	2.5
13	10	2000	7.5	300	2.5
14	10	2000	7.5	300	2.5
15	10	2000	10.0	400	0
16	10	2000	10.0	400	0
17	10	2000	10.0	400	0

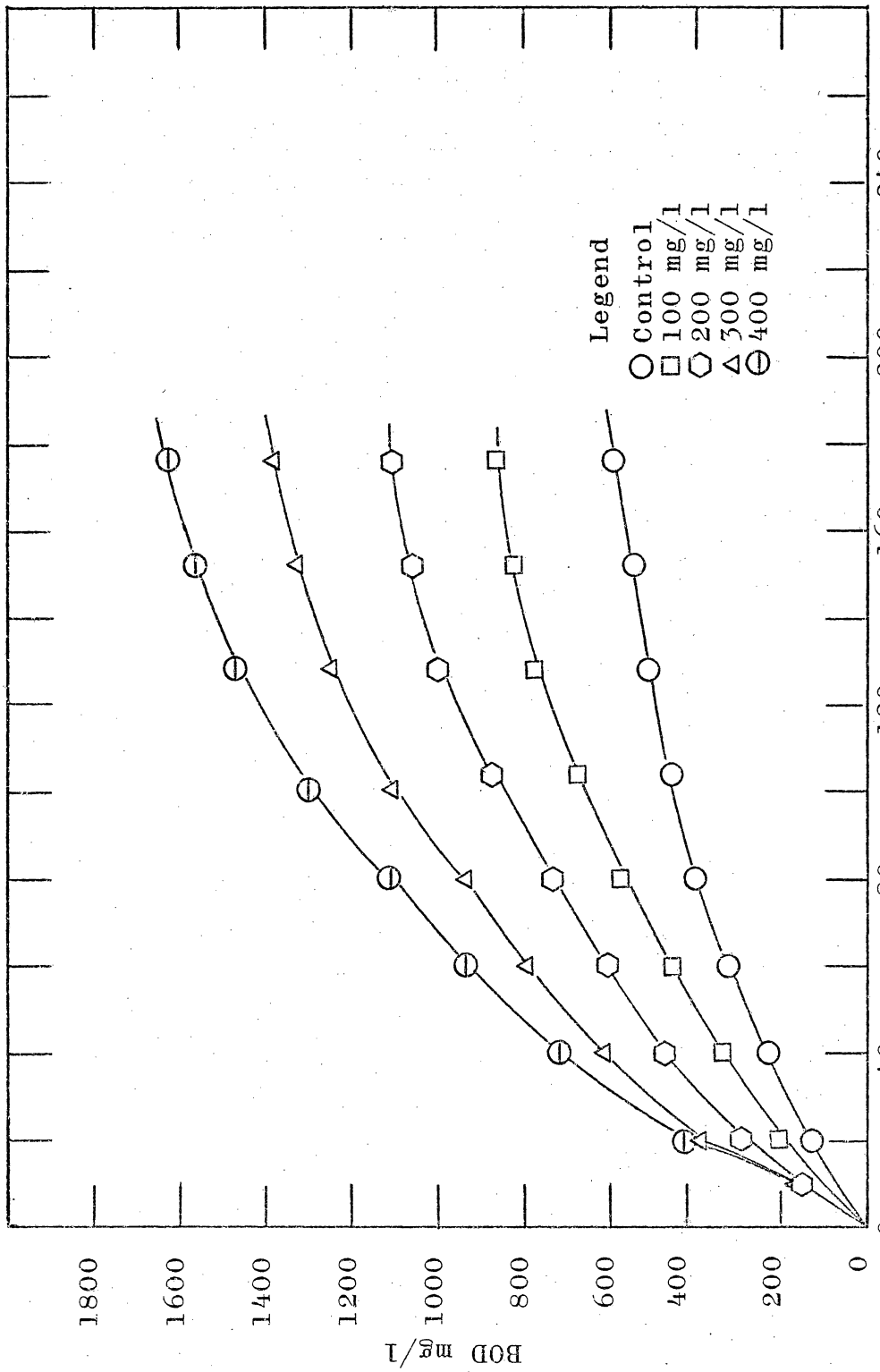

Figure 2. Gross BOD - Grease - Experiment I.

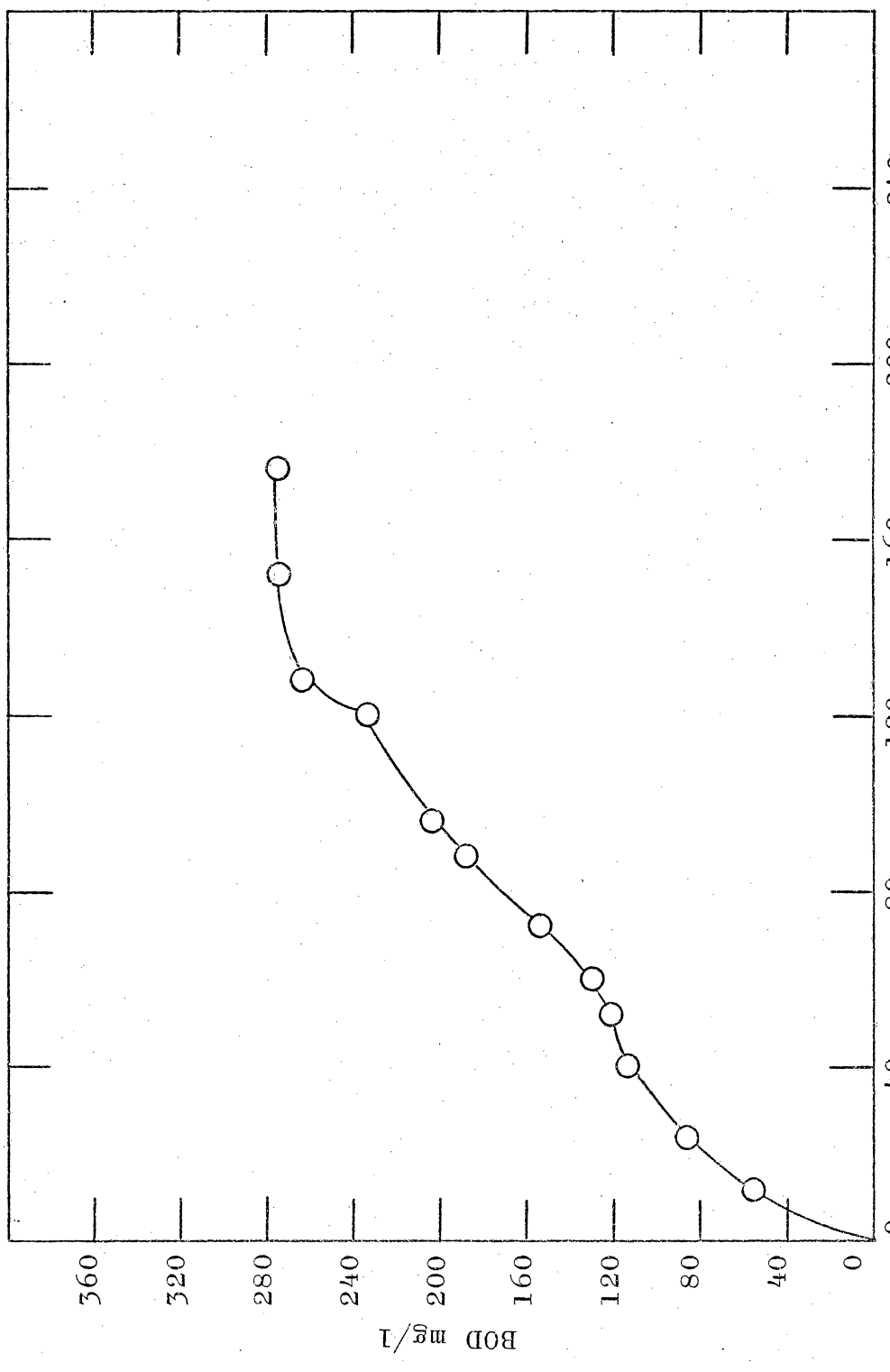

Figure 3. Net BOD - 100 mg/l Grease - Experiment I.

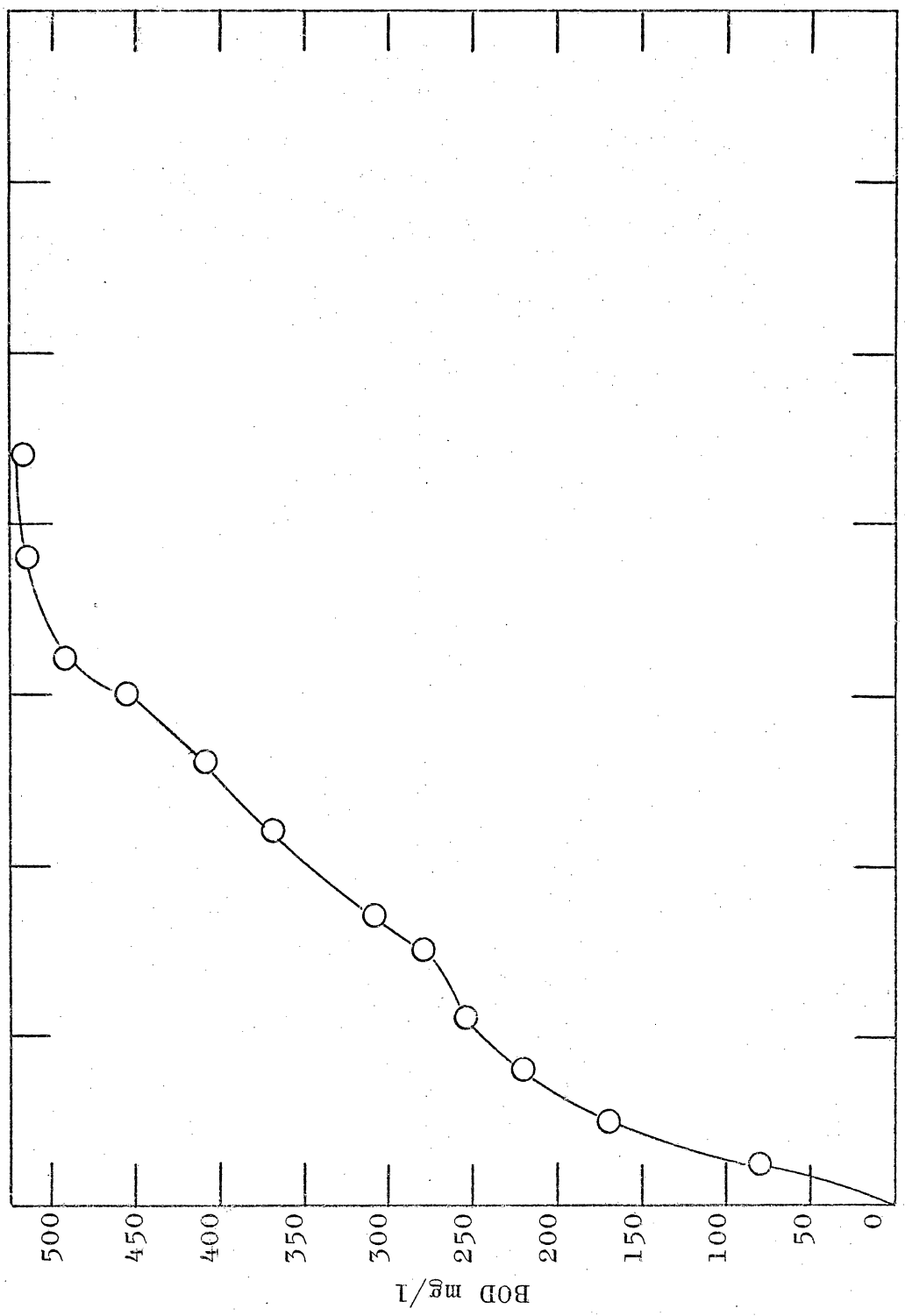

Figure 4. Net BOD - 200 mg/l Grease - Experiment I.

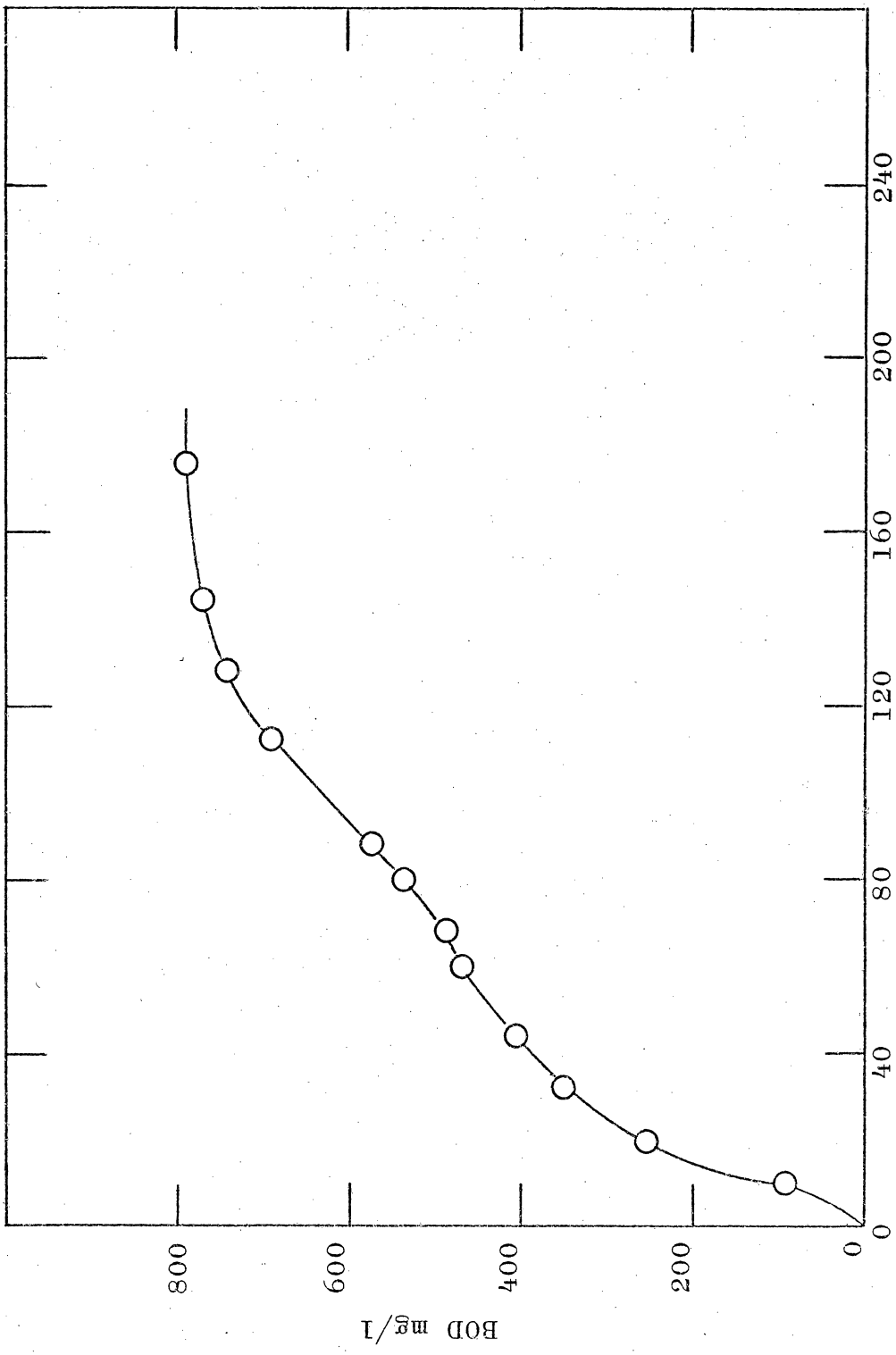

Figure 5. Net BOD - 300 mg/l Grease - Experiment I.

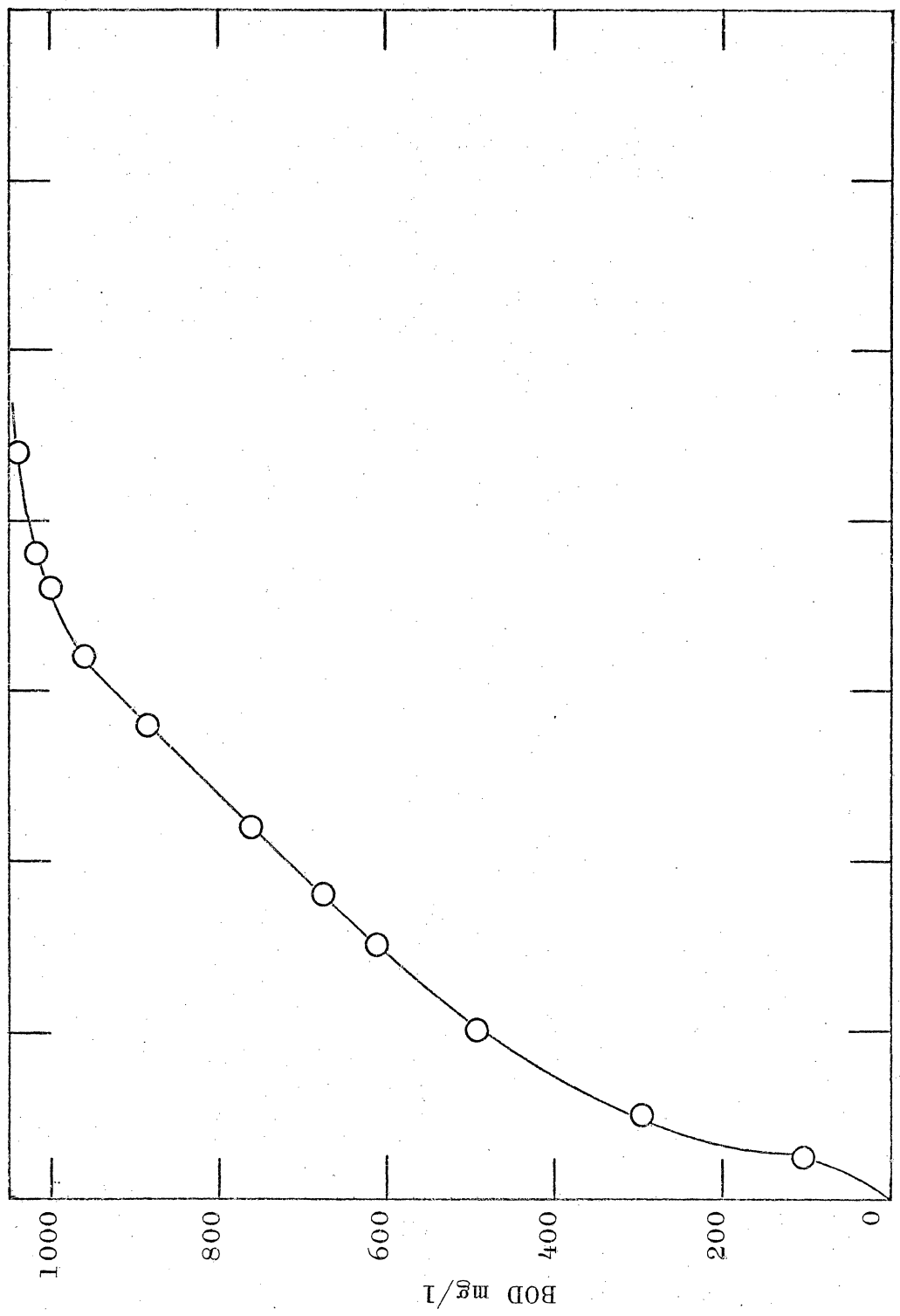

Figure 6. Net BOD - 400 mg/l Grease - Experiment I.

TABLE III

VELOCITY CONSTANTS (K_{10}) AND ULTIMATE BOD(L)
EXPERIMENT I

Control	Grease mg/l	Stage of BOD	K_{10}	L mg/l
Yes	0	1	0.122	615
Yes	0	2	0.063	868
Yes	0	3	0.200	185
	100	1	0.485	132
	100	2	0.165	192
	200	1	0.443	323
	200	2	0.133	348
	300	1	0.487	519
	300	2	0.109	661
	400	1	0.424	694
	400	2	0.258	274

Experiment II. Comparison of BOD Curves Generated By Varied Concentrations of Glucose and Varied Concentrations of Grease.

The objective of this experiment was to determine whether the diphasic nature of the BOD curve generated by activated sludge when subjected to varied concentrations of grease would occur when a sample of the same activated sludge was subjected to varied concentrations of glucose, a substrate of fundamental composition. Measurements of the sludge oxidative activities were performed using the Warburg respirometer technique. A run period of four days was employed in this test since a significant portion of second stage BOD was exerted in this time period. The respirometer was operated at 20°C with a shaker rate of 60 cycles per minute. All samples were run in duplicate including the control. At the termination of the test period, each sample was analyzed for pH to determine if a change in this environmental parameter could be correlated with oxygen uptake characteristics.

As a result of a laboratory accident involving the laboratory bench activated sludge unit, the activated sludge used in Experiment I was destroyed. New activated sludge was collected from the aeration tank of the Roanoke, Virginia, municipal sewage treatment plant and transferred to the laboratory bench unit. The new sludge was acclimated

to substrates A and B for seven days prior to its use in this experiment. At this time, the sludge had not reached a steady state condition.

Activated Sludge

The activated sludge used in this experiment had the following characteristics: total suspended solids, 1760 mg/l; volatile suspended solids, 1130 mg/l; per cent volatile solids in suspended solids, 64; sludge volume index, 63; color, very dark brown; and large numbers of free swimming ciliates and colorless euglenids.

Substrate

Two standard grease emulsions were prepared as outlined in the chapter "Materials and Methods" having concentrations of 200 and 1200 mg/l grease. A 2000 mg/l stock solution of glucose was also prepared. Table IV shows the flask additions of activated sludge, grease emulsion, glucose, and distilled water, and the resulting flask concentration. Concentrations of 25, 50, 100 and 600 mg/l grease and 100, 500, and 1000 mg/l glucose were used in this experiment.

BOD Values

Gross and net cumulative BOD values for glucose are shown graphically in Figure 7 and Figures 8 through 10

respectively. Gross and net cumulative BOD values for grease are shown graphically in Figure 11, and Figures 12 through 15 respectively. Data points for both gross and net cumulative BOD curves are an average of the duplicate values for each concentration.

K_{10} and L

The values for K_{10} and L are shown in Table V.

pH

Each sample was analyzed for pH at the termination of the test period. The results of the pH tests are listed in Table VI.

Results

The pH change in any sample (maximum -1 pH unit) was not deemed sufficient to suppress biological activity. The net cumulative BOD curves for glucose clearly show a plateau in the curve for 100 mg/l glucose. Plateaus are also present in the 500 and 1000 mg/l glucose net cumulative BOD curves but are less pronounced. The net cumulative BOD curves for grease also exhibit plateaus. These plateaus increased in magnitude with increasing concentration of grease to a maximum at 100 mg/l grease. As the concentration of grease was increased beyond 100 mg/l grease the magnitude of the plateau diminished until only

slightly discernible at 600 mg/l grease. With the exception of the 25 mg/l grease curve, all other net cumulative BOD curves for grease and all net cumulative BOD curves for glucose exhibited slight "lag" periods. This could have resulted from an initial high food to microorganism (F/M) ratio.

TABLE IV

FLASK ADDITIONS AND RESULTING CONCENTRATIONS
EXPERIMENT II

Flask	Activated Sludge		Grease		Glucose		ml
	ml Added	Concentration mg/l	ml Added	Concentration mg/l	ml Added	Concentration mg/l	Distilled Water
1	10	2000	None	0	None	0	10.0
2	10	2000	None	0	None	0	10.0
3	10	2000	None	0	1.0	100	9.0
4	10	2000	None	0	1.0	100	9.0
5	10	2000	None	0	5.0	500	5.0
6	10	2000	None	0	5.0	500	5.0
8	10	2000	None	0	10.0	1000	None
9	10	2000	None	0	10.0	1000	None
11	10	2000	2.5	25	None	0	7.5
12	10	2000	2.5	25	None	0	7.5
13	10	2000	5.0	50	None	0	5.0
14	10	2000	5.0	50	None	0	5.0
15	10	2000	10.0	100	None	0	None
16	10	2000	10.0	100	None	0	None
17	10	2000	10.0	600	None	0	None
18	10	2000	10.0	600	None	0	None

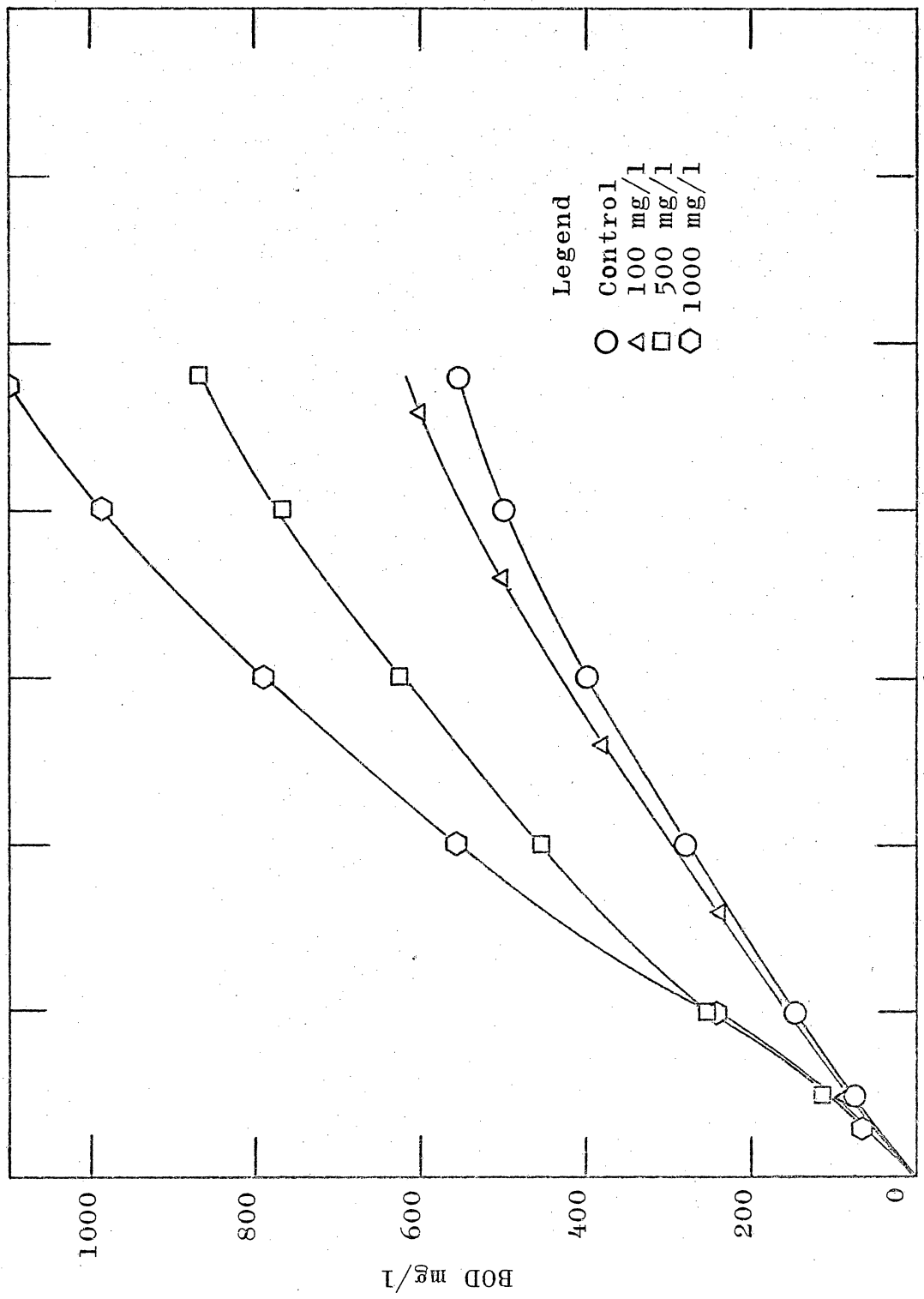

Figure 7. Gross BOD - Glucose - Experiment II.

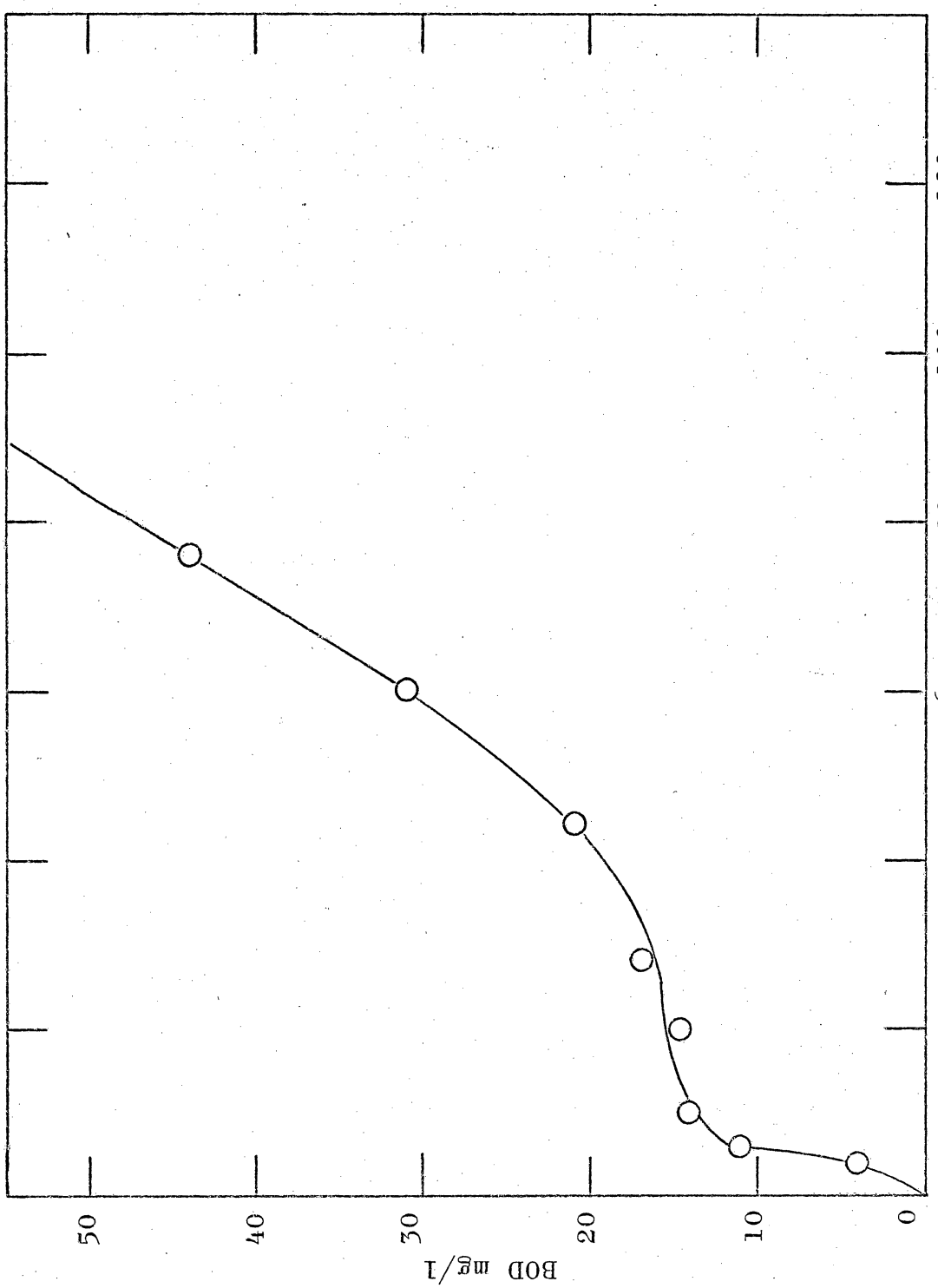

Figure 8. Net BOD - 100 mg/l Glucose - Experiment II.

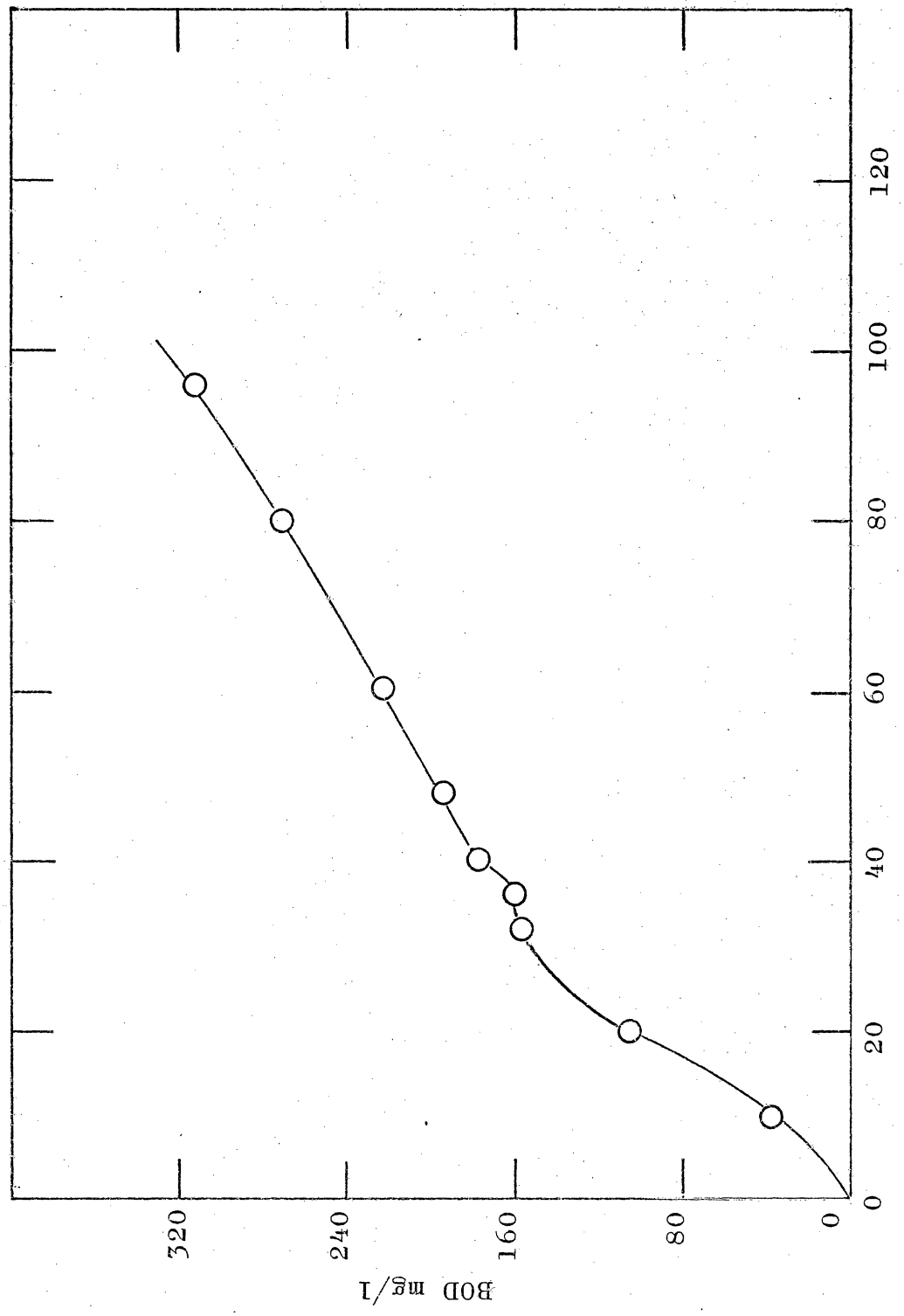

Figure 9. Net BOD - 500 mg/l Glucose - Experiment II.

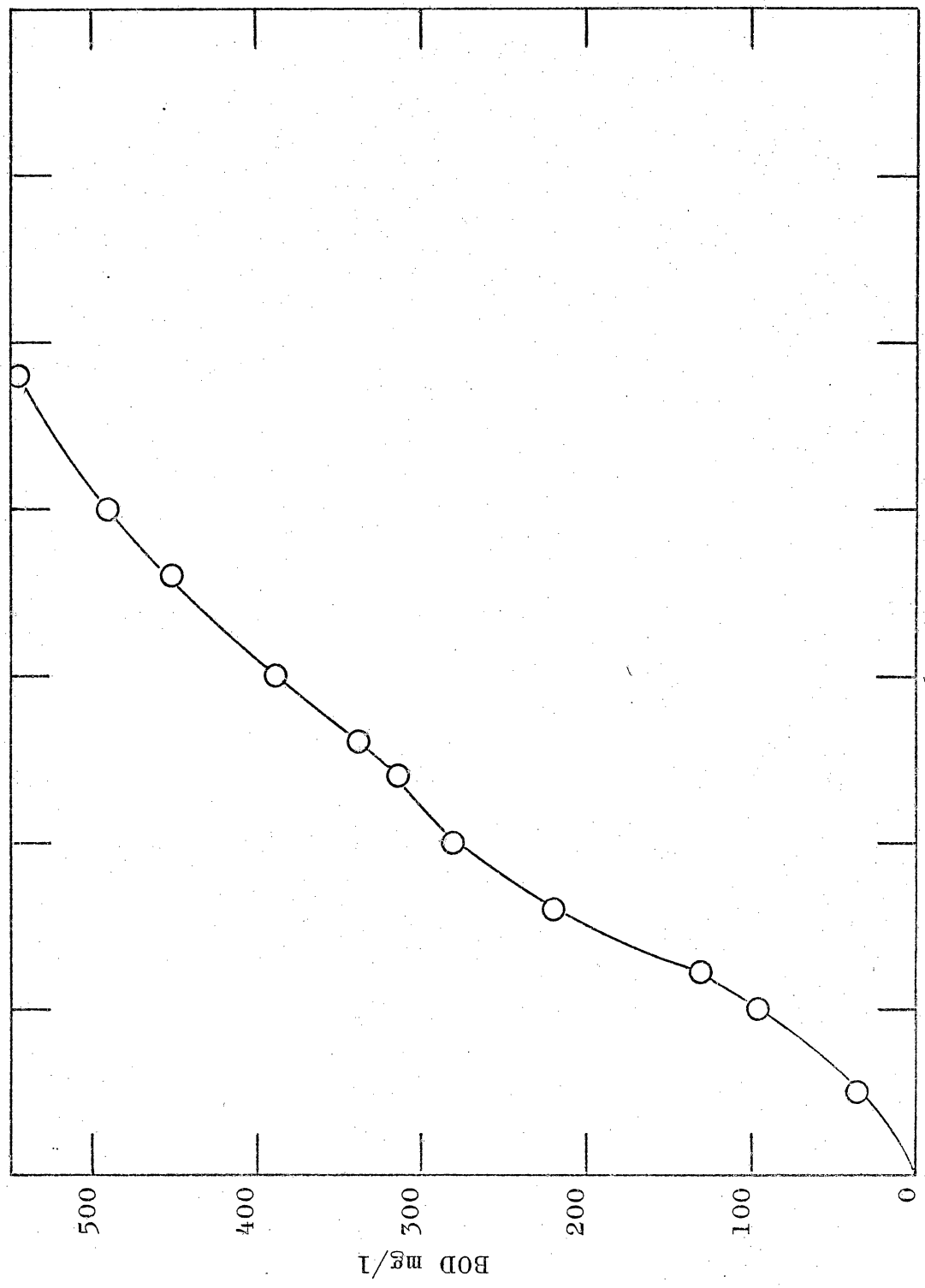

Figure 10. Net BOD - 1000 mg/l Glucose - Experiment II.

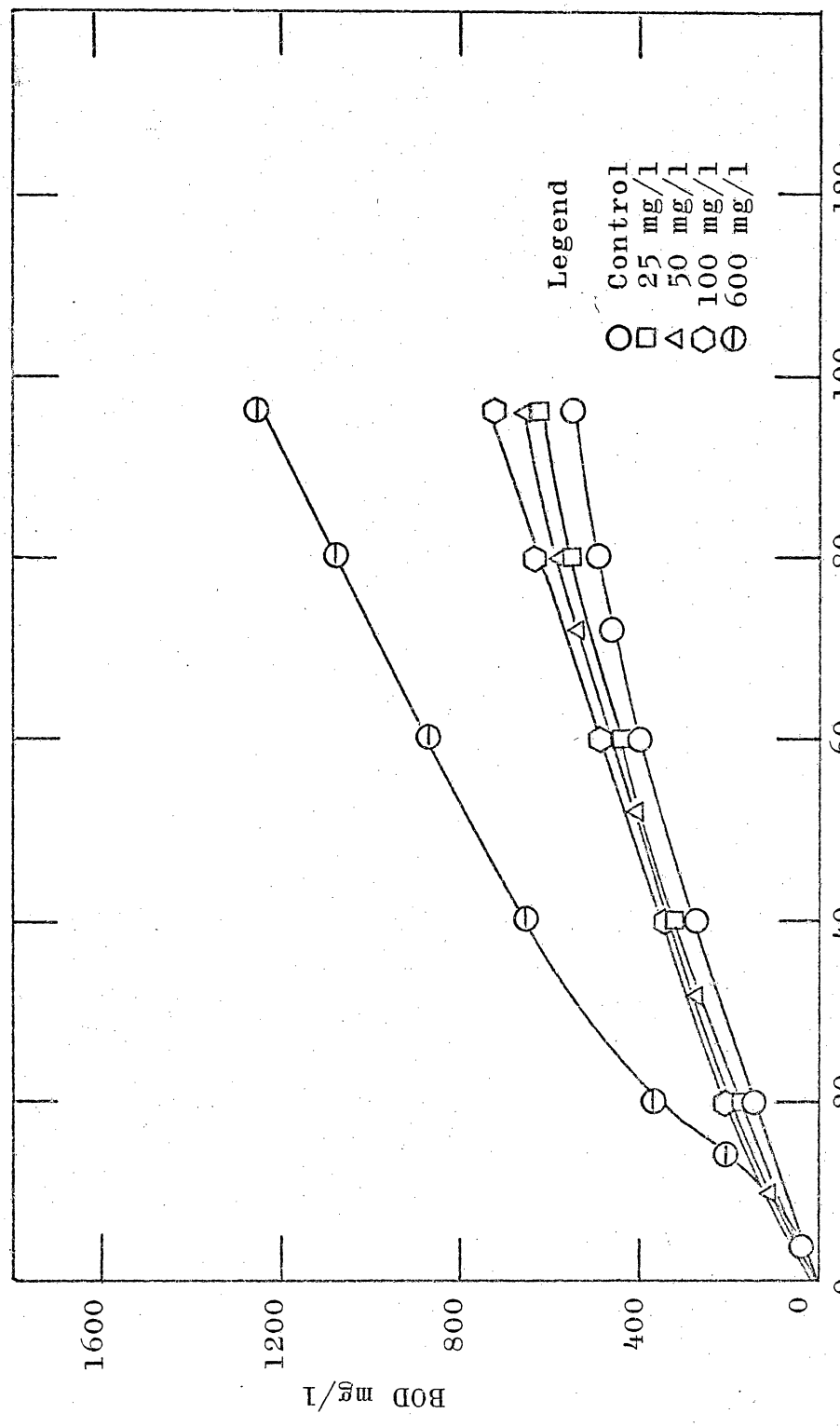

Figure 11. Gross BOD - Grease - Experiment II.

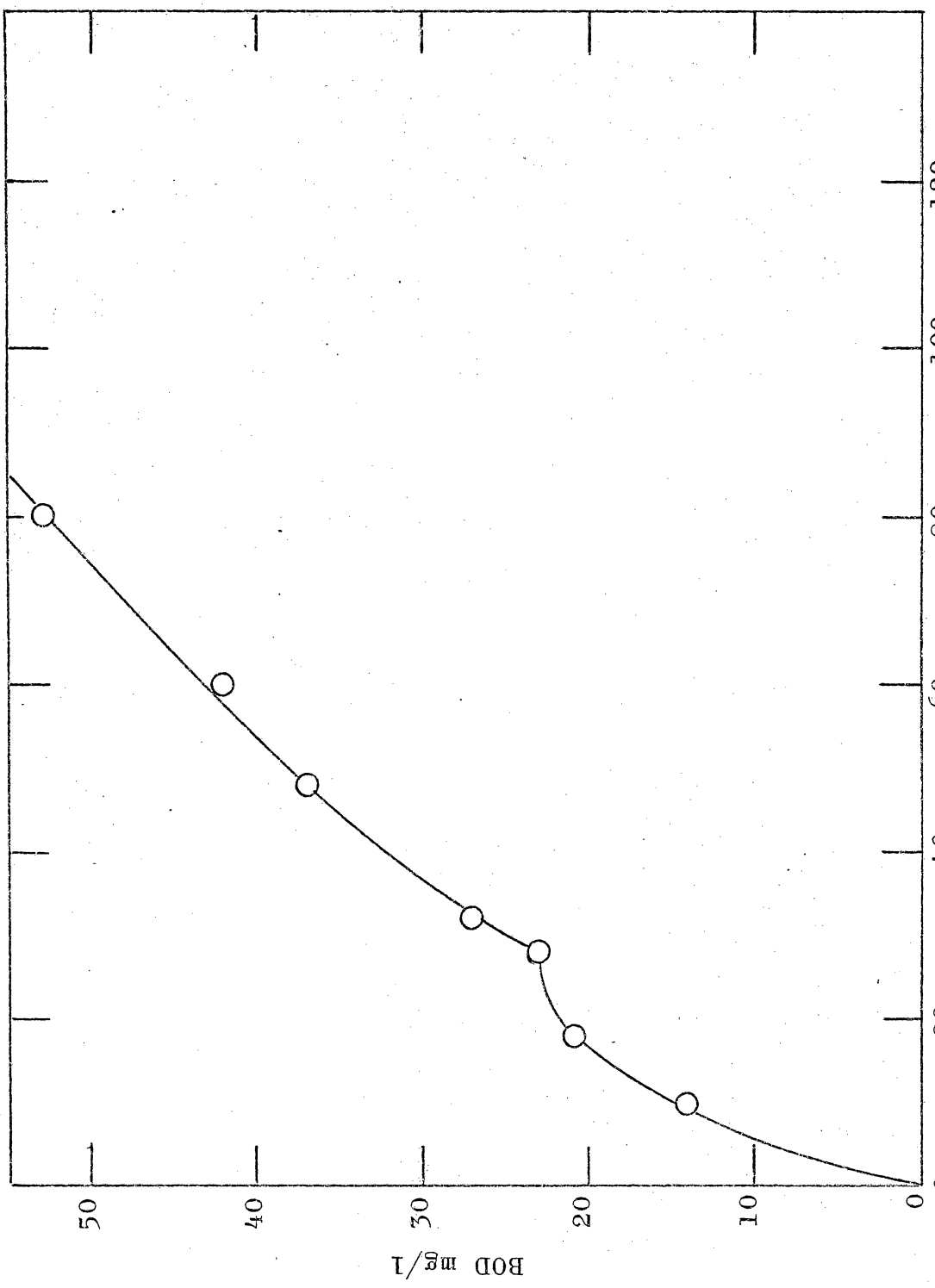

Figure 12. Net BOD - 25 mg/l Grease - Experiment II.

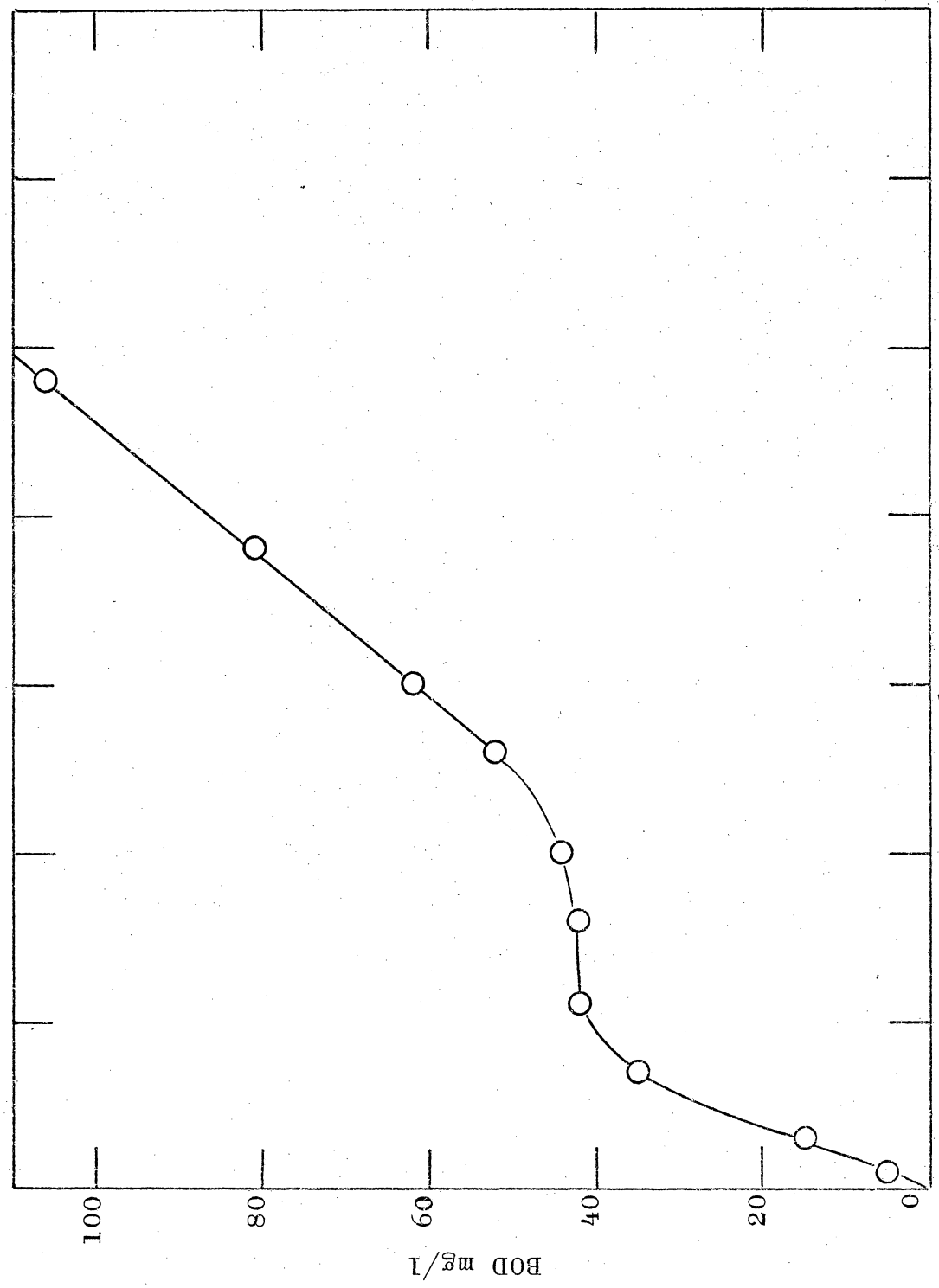

Figure 13. Net BOD - 50 mg/l Grease - Experiment II.

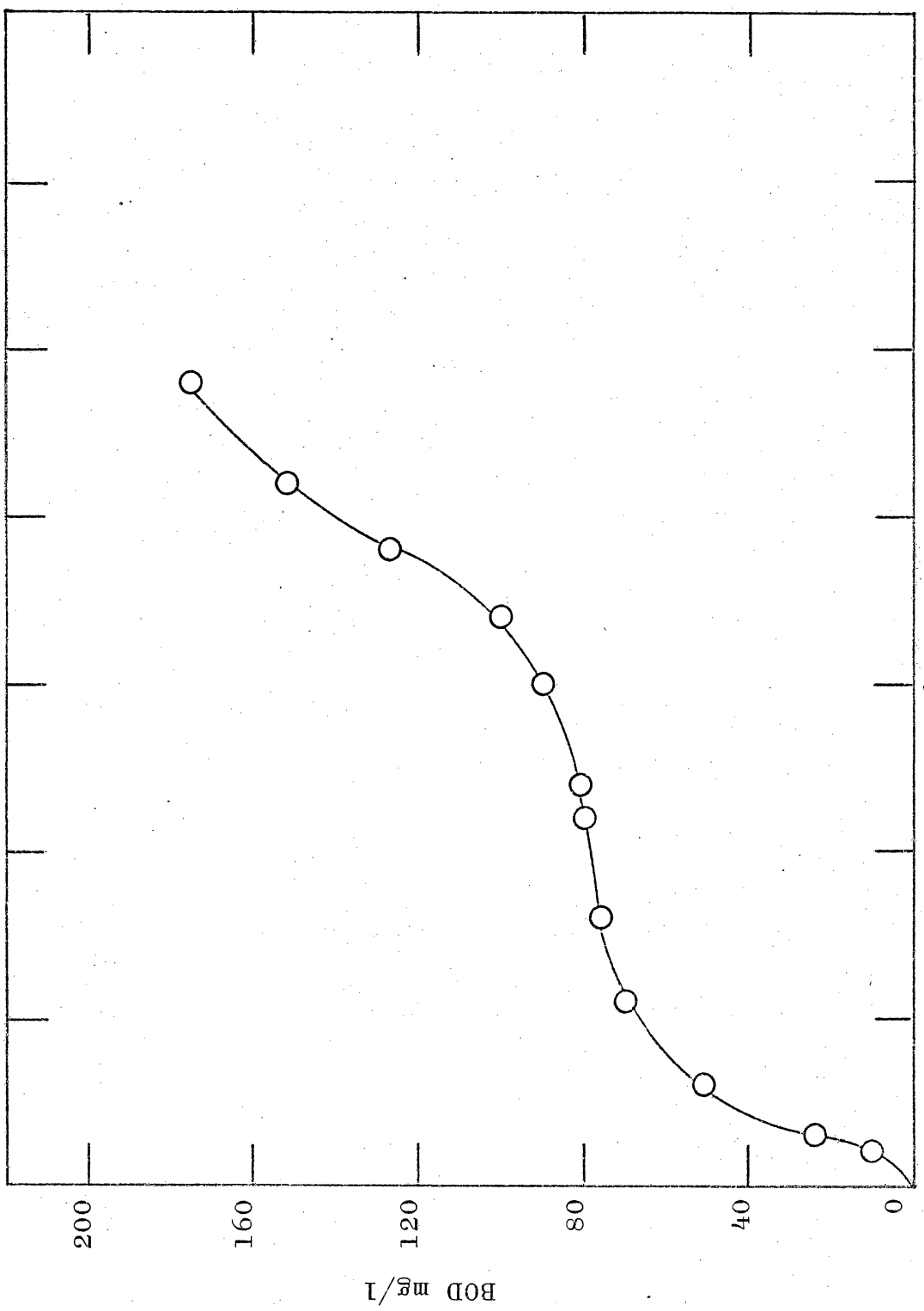

Figure 14. Net BOD - 100 mg/l Grease - Experiment II.

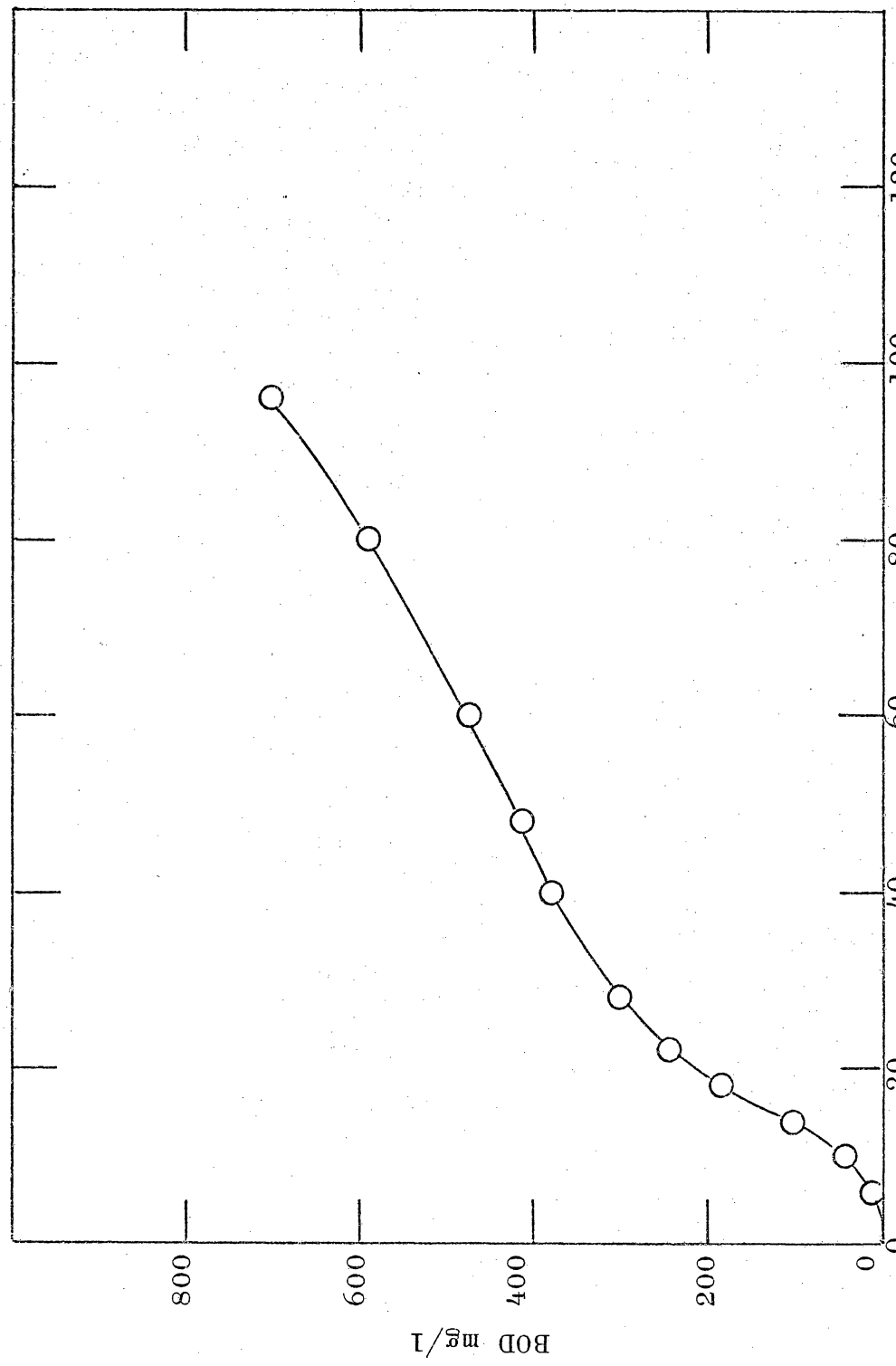

Figure 15. Net BOD - 600 mg/l Grease - Experiment II.

TABLE V

VELOCITY CONSTANT (K_{10}) AND ULTIMATE BOD (L)
EXPERIMENT II

Control	Grease	Glucose	Stage	K_{10}	L
Yes	None	None	1	0.075	1080
Yes	None	None	2	0.151	453
	25	None	1	0.670	31
	25	None	2	0.088	82
	50	None	1	1.789	45
	50	None	2	U.D.	U.D.
	100	None	1	0.766	91
	100	None	2	U.D.	U.D.
	600	None	1	0.862	420
	600	None	2	U.D.	U.D.
	None	100	1	U.D.	U.D.
	None	100	2	U.D.	U.D.
	None	500	1	0.572	195
	None	500	2	U.D.	U.D.
	None	1000	1	0.268	579
	None	1000	2	U.D.	U.D.

Note: U.D. signifies reaction was not first order.

TABLE VI

pH VALUES
EXPERIMENT II

Flask Composition	pH	
	Initial	Final
Control	6.3	5.6
Control	6.3	5.7
100 mg/l glucose	7.0	6.1
100 mg/l glucose	7.0	6.0
500 mg/l glucose	7.0	6.6
500 mg/l glucose	7.0	6.7
1000 mg/l glucose	7.0	7.4
1000 mg/l glucose	7.0	7.4
25 mg/l grease	6.9	5.9
25 mg/l grease	6.9	6.1
50 mg/l grease	6.9	6.1
50 mg/l grease	6.9	6.1
100 mg/l grease	6.9	6.3
100 mg/l grease	6.9	6.3
600 mg/l grease	6.9	7.3
600 mg/l grease	6.9	7.3

Experiment III. Effect of Oxidative Activity of Activated Sludge on Total Grease Content and pH.

The objectives of this experiment were to measure the change in total grease content and pH at given time intervals resulting from the oxidative activities of activated sludge, and to correlate the plateau occurring in the cumulative net BOD curve with the depletion of substrate, and/or sudden change in pH. Measurements of the sludge oxidative activities were made using the Warburg respirometer technique. Grease extraction and pH measurements are outlined in the chapter "Materials and Methods." The test period was established as five days but later prolonged due to a failure of the unit's thermostat. The respirometer was operated at 20°C with a shaker rate of 60 cycles per minute. Analysis for total grease and pH were performed on triplicate samples.

Activated Sludge

The activated sludge used in this experiment had the following characteristics: total suspended solids, 1475 mg/l; volatile suspended solids, 875 mg/l; per cent volatile solids in suspended solids, 59; sludge volume index, 88; color, light chocolate brown; and numerous stalked ciliates. This sludge was five days older than the sludge used in Experiment II and was rapidly approaching a steady state condition.

Substrate

A standard grease emulsion was prepared as outlined in the chapter "Materials and Methods" having a concentration of 200 mg/l grease. Table VII shows the flask additions of activated sludge, grease emulsion and distilled water and the resulting flask concentrations. A concentration of 100 mg/l grease was used in all flasks with the exception of the controls.

BOD Values

Gross and net cumulative BOD curves are shown graphically in Figure 16 and Figure 17 respectively.

K_{10} and L

The velocity constant (K_{10}) and ultimate BOD (L) for first stage BOD were 0.402 and 132 mg/l respectively.

pH

Analysis for pH was made at 24 hour intervals. The results are shown graphically in Figure 18.

Total Grease

Analysis for total grease was made at 24 hour intervals. The results are shown graphically in Figure 19.

Results

The thermostat on the water bath malfunctioned shortly after an elapsed time of 90 hours causing the temperature

to rise to 35°C. The sludge was subjected to that temperature for at least one hour before the malfunction was noticed, at which time adjustments were made to slowly return the water bath temperature to 20°C. At that time the net cumulative BOD curve had reached a plateau. The experiment was continued to an elapsed time of 144 hours to determine whether the activated sludge would exhibit the diphasic properties previously observed. The second stage of net BOD exertion did not occur. It was concluded that the sudden subjection of the activated sludge to a high temperature disrupted its ecological system, resulting in atypical oxidative responses.

Grease analysis indicated that the substrate, grease, was depleted after 24 hours. No direct correlation could be observed between reaching the plateau of BOD exertion and depletion of substrate. Changes in pH during the test period were insignificant, and thus deemed to have no adverse effect on the biological system.

TABLE VII

FLASK ADDITIONS AND RESULTING CONCENTRATIONS
EXPERIMENT III

Flask	Activated Sludge		Grease		Distilled Water
	ml Added	Concentration mg/l	ml Added	Concentration mg/l	ml
1	10	2000	None	0	10.0
2	10	2000	None	0	10.0
3	10	2000	None	0	10.0
4	10	2000	10.0	100	None
5	10	2000	10.0	100	None
6	10	2000	10.0	100	None
9	10	2000	10.0	100	None
11	10	2000	10.0	100	None
12	10	2000	10.0	100	None
13	10	2000	10.0	100	None
14	10	2000	10.0	100	None
16	10	2000	10.0	100	None
17	10	2000	10.0	100	None
18	10	2000	10.0	100	None

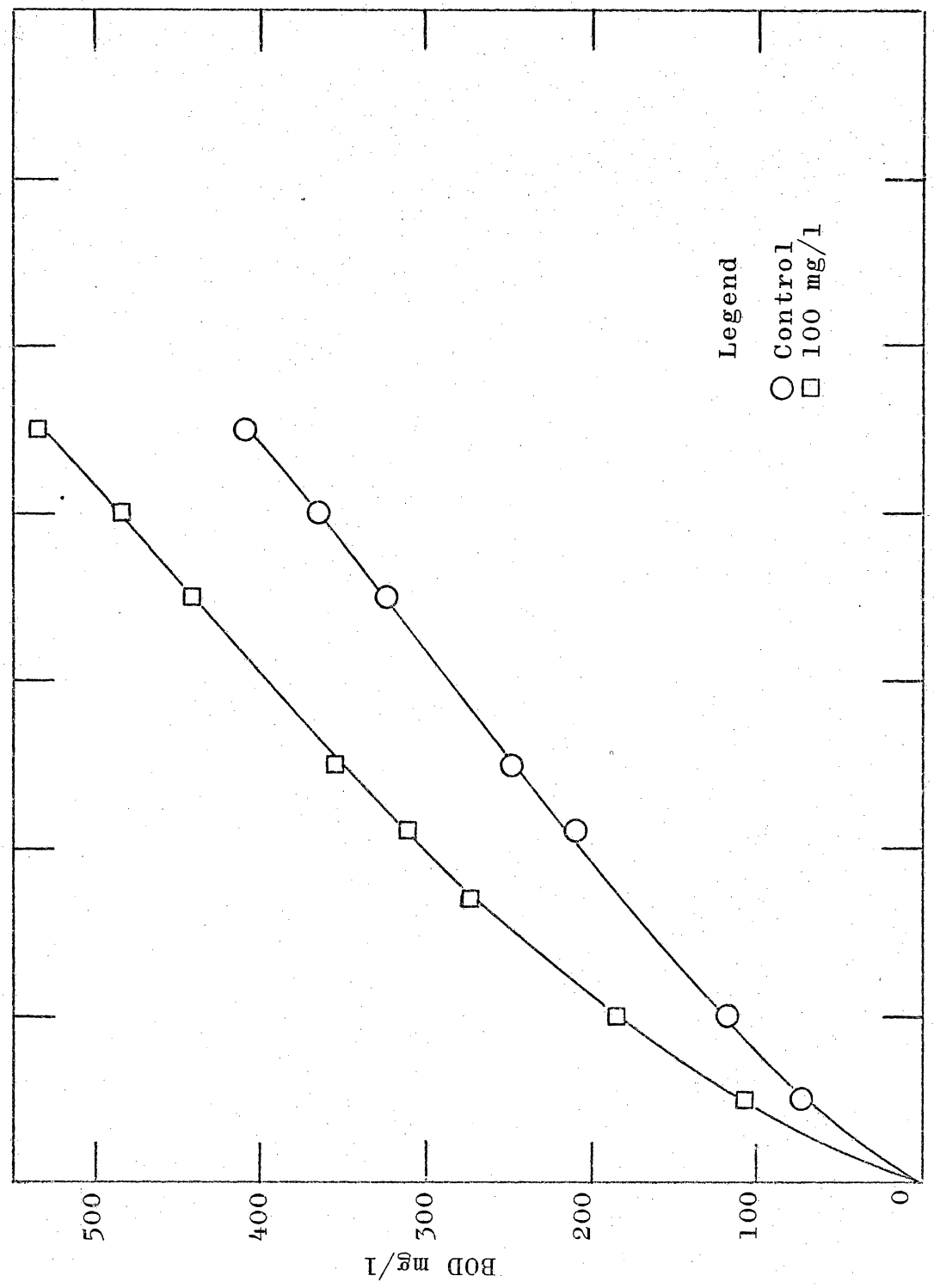

Figure 16. Gross BOD - Grease - Experiment III.

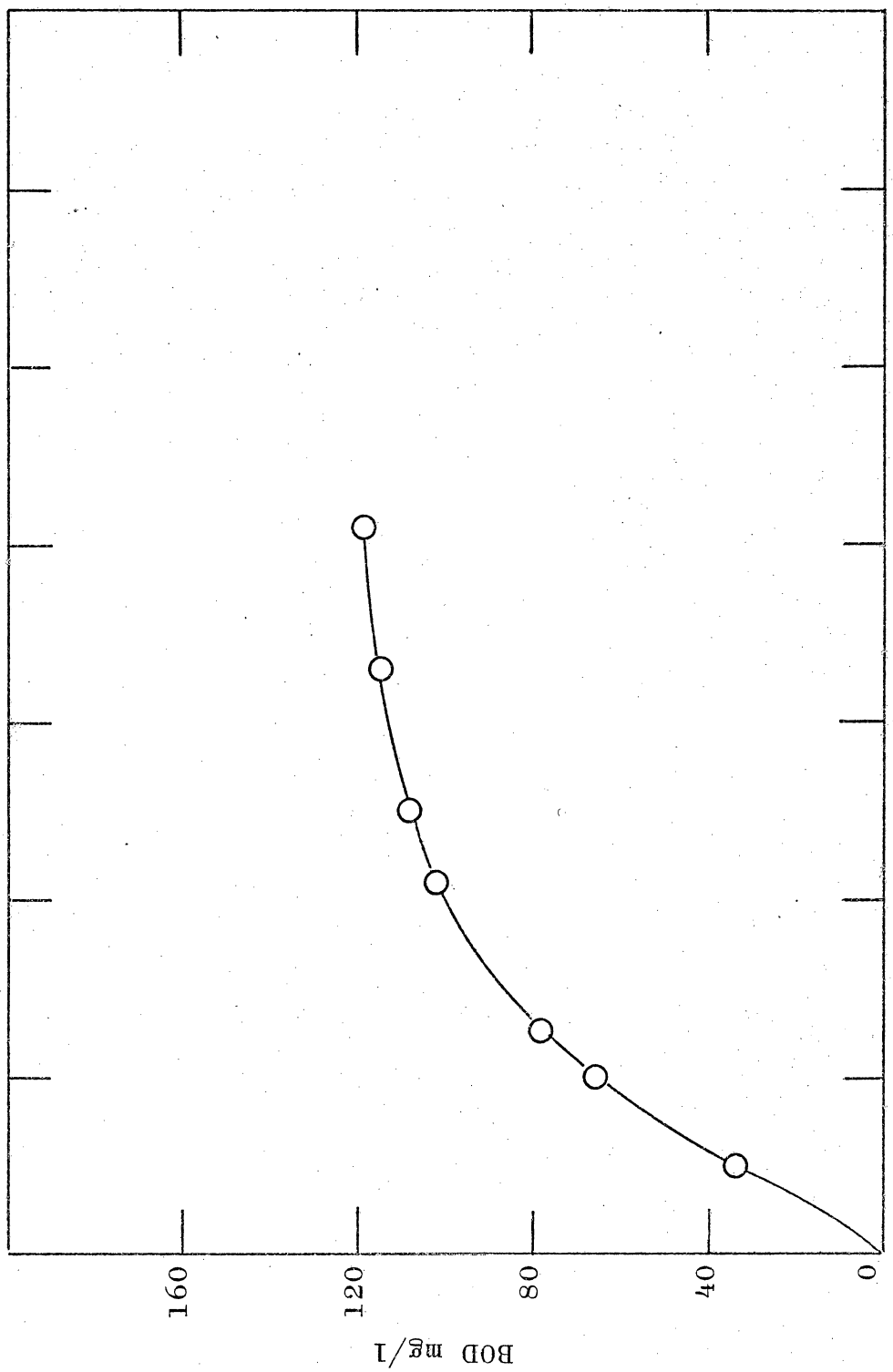

Figure 17. Net BOD - 100 mg/l Grease - Experiment III.

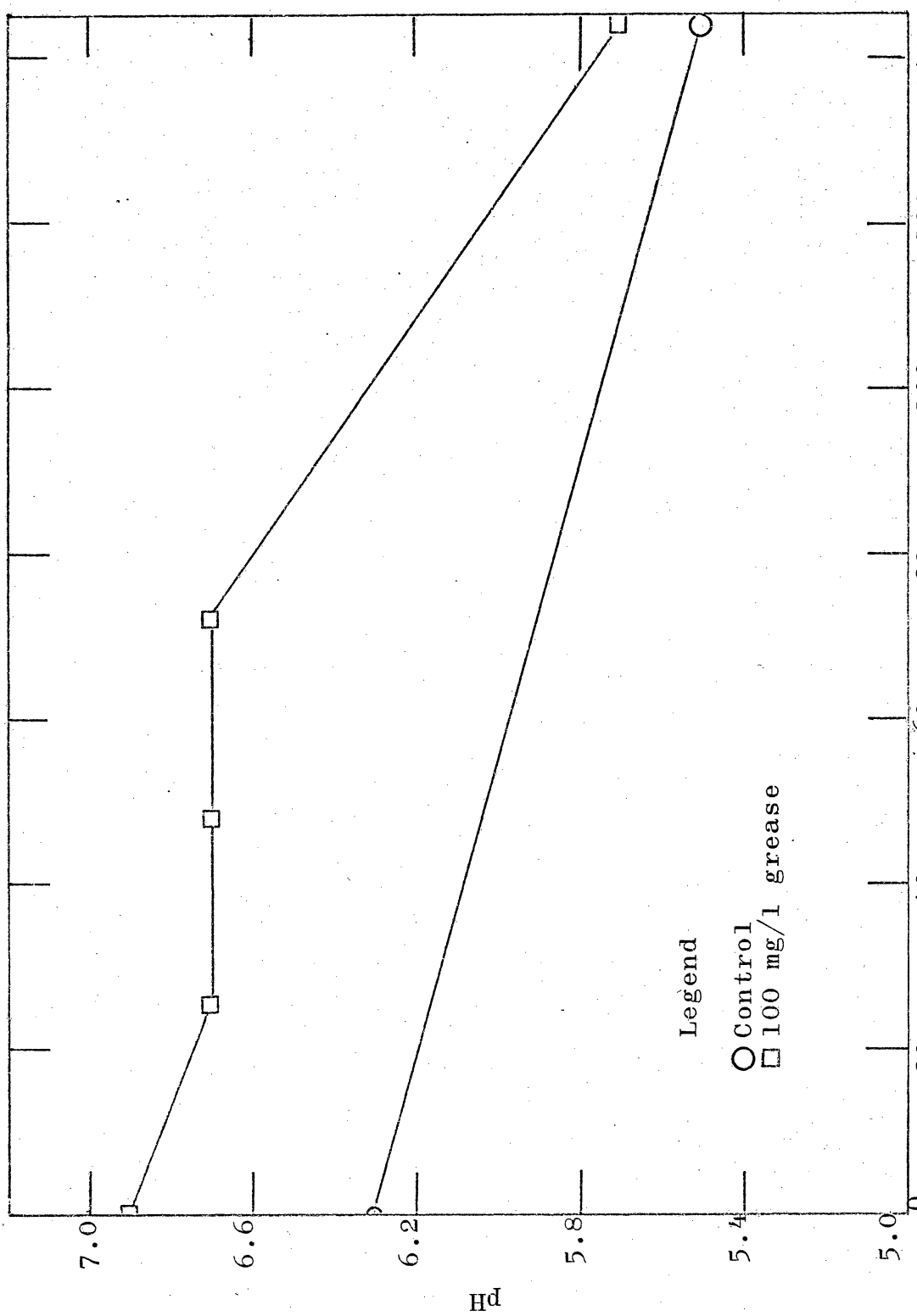

Figure 18. pH versus Time - Experiment III.

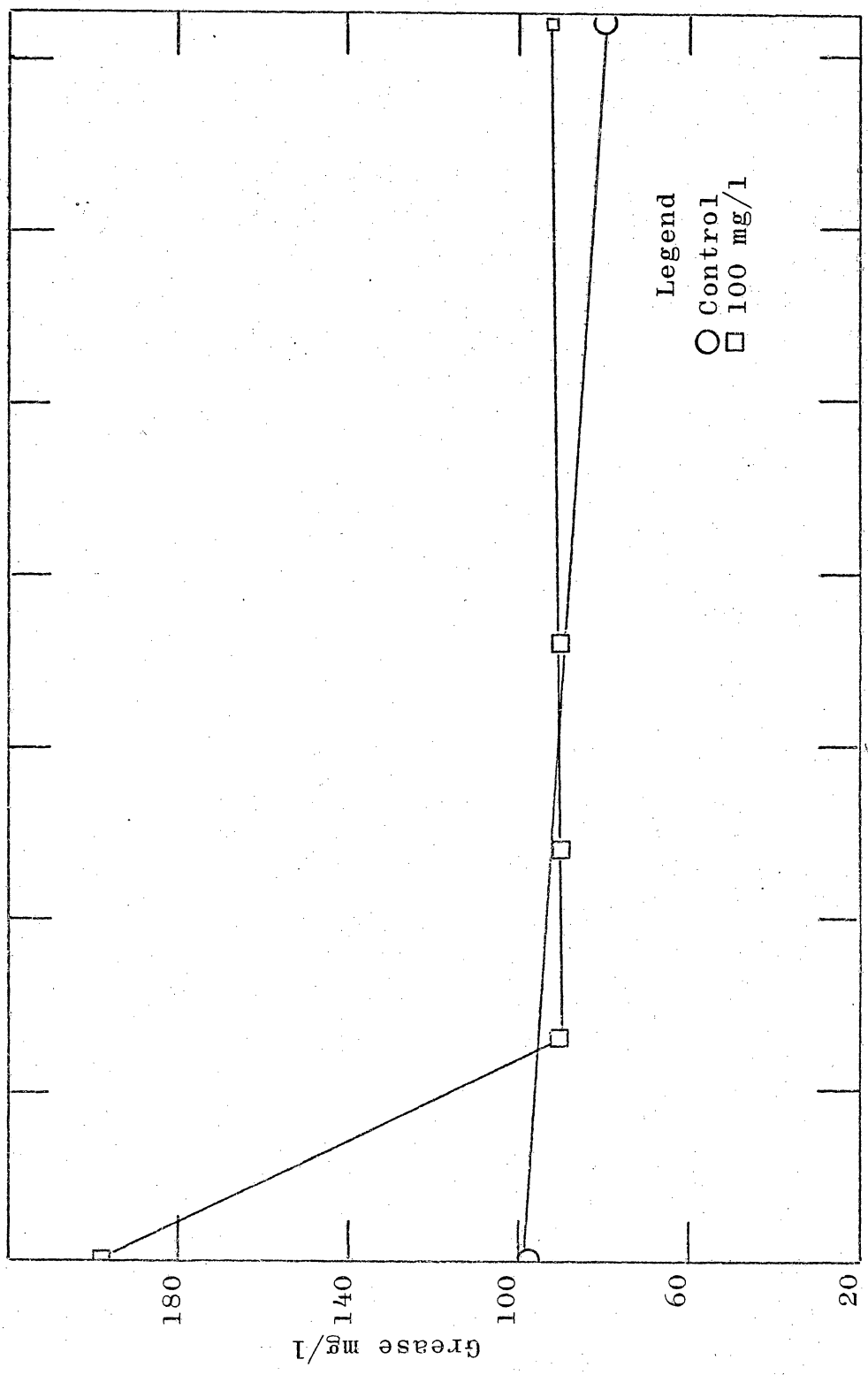

Figure 19. Grease Concentration versus Time - Experiment III.

V. DISCUSSION OF RESULTS

The net cumulative BOD curves clearly show that the grease was readily metabolized by the organisms of activated sludge. Several of the net BOD curves exhibited a slight "lag" period which is characterized by a concave upward curve. Since the BOD reaction is generally accepted as a first order decreasing rate reaction in which substrate concentration is the rate limiting factor, and is characterized by a concave downward curve, the portion of the curve exhibiting an increasing reaction rate must be considered as a "lag". The "lag" could have resulted from a high initial food to microorganism (F/M) ratio which would indicate that a lack of microorganisms was the rate limiting factor. The occurrence of short "lag" periods in themselves is not important to this study since they do not signify an inhibitation in the oxidative activities of the activated sludge, rather they indicate a deficient enzyme supply.

Of significant interest is the mode of BOD exertion exhibited by the activated sludge because it deviates from the classical concept of a single first order decreasing rate reaction governing the metabolism of a substrate(s). (Figure 20) All cumulative net BOD curves for grease exhibit a plateau in BOD exertion with the exception of

Figure 17, which represents the activated sludge that was subjected to high temperature (35°C) due to a malfunction of the water bath thermostat. This phasic or stage BOD exertion has been observed by many investigators and appears to be independent of type or concentration of seed or substrate (5). This is illustrated by the fact that glucose exhibited a plateau in BOD exertion in Experiment II. Gaudy et al (4), using glucose and related organic compounds, reported that increasing substrate concentrations exhibited a compressing effect on the plateau. This phenomenon was also observed in these experiments. Gaudy et al (5) also reported that the occurrence of the plateau did not necessarily coincide with the depletion of initial substrate. This was also observed in Experiment III where the grease substrate was depleted within an elapsed time of 24 hours, while the plateau was not reached until an elapsed time of 70 hours. It was first believed by Gaudy and other researchers that the plateau is caused by a change from metabolism of the original exogenous carbon source(s) to the metabolism of a new carbon source(s) produced in the first stage of oxygen uptake. Thus, the plateau originates due to the time lapse associated with a period of enzyme induction before the new carbon source(s) can be metabolized, or because of the time required for establishment of a secondary population of microorganisms

to a density capable of exhibiting an oxygen uptake (4). This theory may hold true for heterogenous cultures but does not fully explain the existence of plateaus in the BOD studies of Wilson and Harrison (18) using pure cultures.

The data resulting from this study indicates that the first stage of BOD exertion results from metabolism of the initial exogenous carbon sources since the plateau follows the depletion of the grease substrate (see Figure 21). The plateau is the result of an enzyme induction period prerequisite to metabolism of cellular storage products. Thus, the second stage of BOD exertion results from endogenous metabolism of storage products.

For diphasic BOD exertion it is not possible to define the entire curve generated by the carbonaceous demand with the classical equation

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

where y = BOD at any time t

L = ultimate BOD in mg/l

K = velocity constant

t = time in days

since this equation describes a single first order decreasing rate reaction. Each stage of BOD exertion exhibits its own unique K_{10} and L; therefore each stage must be

described by a separate equation. The entire curve can be described by a summation of individual equations as suggested by Streeter (11). A modification of this equation is illustrated below.

$$y = L_A(1 - 10^{-K_A t_A}) + L_B(1 - 10^{-K_B t_B}) + \ldots$$

where y = BOD at any time t

K_A = velocity constant for the first stage of BOD exertion

t_A = time in days from t_1 to $t_2 = t$

K_B = velocity constant for the second stage of BOD exertion

t_B = time in days from t_2 to $= t - t_2$

L_A = ultimate first stage BOD in mg/l

L_B = ultimate second stage BOD in mg/l

t_1 = time zero for first stage BOD exertion

t_2 = time zero for second stage BOD exertion.

Several of the curves generated by the activated sludge did not follow a first order decreasing rate reaction and therefore could not be evaluated for velocity constant (K_{10}) and ultimate BOD(L). Theoretically, the velocity constant K_{10} for a given substrate should be independent of concentration of that substrate. This was substantiated by the results of Experiments I and III where the velocity constant

(K_{10}) varied from a low of 0.402 to a high of 0.487. First stage ultimate BOD plotted against grease concentration in mg/l results in a straight line as shown in Figure 22. This indicates that the magnitude of first stage BOD is a direct function of initial grease concentration. Unfortunately, the relationship does not exist for first stage BOD in Experiment II. This could result from the unsteady state condition of the sludge used in that experiment. Sludge stability appears to be an important factor in any investigation of this nature since unstable sludge does not give reproducible results. Velocity constants (K_{10}) for second stage BOD exertion were not of the same magnitude and ultimate BOD's were not proportional to concentration of substrate. Most second stage curves exhibited long "lag" periods, thus limiting the number of usable data points for calculating K_{10} and L. Thus, the calculated values are not truly representative. Long term Warburg tests utilizing suppression of nitrification are needed to accurately determine the second stage parameters K_{10} and L for the substrate tested.

Sludge characteristics such as total suspended solids, volatile suspended solids, per cent volatile solids in suspended solids, and sludge volume index are commonly used parameters for comparison of the active masses of

microorganisms of one sludge to another. The test data indicate that these parameters are not reliable. The per cent of volatile suspended solids in the total suspend solids were 95 and 59 for Experiments I and III respectively. This would indicate that the sludge used in Experiment I had a greater active mass of microorganisms than the sludge used in Experiment III, and therefore, the sludge used in Experiment I would have a greater capacity for metabolizing the grease substrate. The K_{10} and L values for the first stage of carbonaceous BOD demand using a 100 mg/l grease substrate for Experiment I are 0.485 and 132 mg/l respectively and for Experiment III 0.402 and 132 mg/l respectively. As evidenced by a comparison of K_{10} and L values, the two sludges reacted approximately the same to the 100 mg/l grease substrate. The only observed difference was a two hour "lag" period in the BOD exertion by the sludge used in Experiment III.

The velocity constant K_{10} is the rate at which the ultimate BOD is reached. The magnitude of K_{10} is dependent upon two major factors: 1) the nature of the organic matter and 2) the ability of the organisms present to utilize the organic matter. Soluble organic matter is readily available to the microorganisms while colloidal and suspended matter must await hydrolitic action before it can diffuse into the microbial cells where oxidation

can occur. Domestic waste, which is made up of simple as well as complex soluble, colloidal and suspended organics, has an average K of 0.17. (9) The low K_{10} value of domestic waste is attributed to the complex colloidal and suspended matter. (9) Of further significance in this study is the magnitude of the K values for the first stage of carbonaceous BOD. The sewage grease used in this study was added in the form of a colloid, and grease by its very nature is made up of many complex substances, yet the average K_{10} was 0.452 which is over two and one-half times the average K_{10} for domestic sewage.

The chemical composition of a bacterial cell is classically represented as a carbohydrate having the formula $C_5H_7O_2N$. (8) It is interesting to note that the activated sludge used in Experiment III initially contained 98 mg/l grease (See Figure 22), which is 4.9 per cent by weight based on a suspended solids concentration of 2000 mg/l. It should also be noted that the grease content of the control decreased from 98 mg/l at time zero to 80 mg/l at time 144 hours. This would indicate that grease-like materials (lipids) are stored within the bacterial cells for use during endogenous respiration.

Heukelekian et al (6) reported that the major portion of grease found in sewage is particulate and in the colloidal form. This investigation indicates that the organisms

of activated sludge will readily metabolize grease introduced in the colloidal form. Therefore, it can be concluded that the conventional activated sludge process could prove to be an effective method for treating wastes containing high grease concentrations in the colloidal form.

The major portion of the BOD of the grease would be exerted within the treatment plant since the experimental results indicate that the grease is retained within the cells of the microorganisms of activated sludge to be used during endogenous respiration.

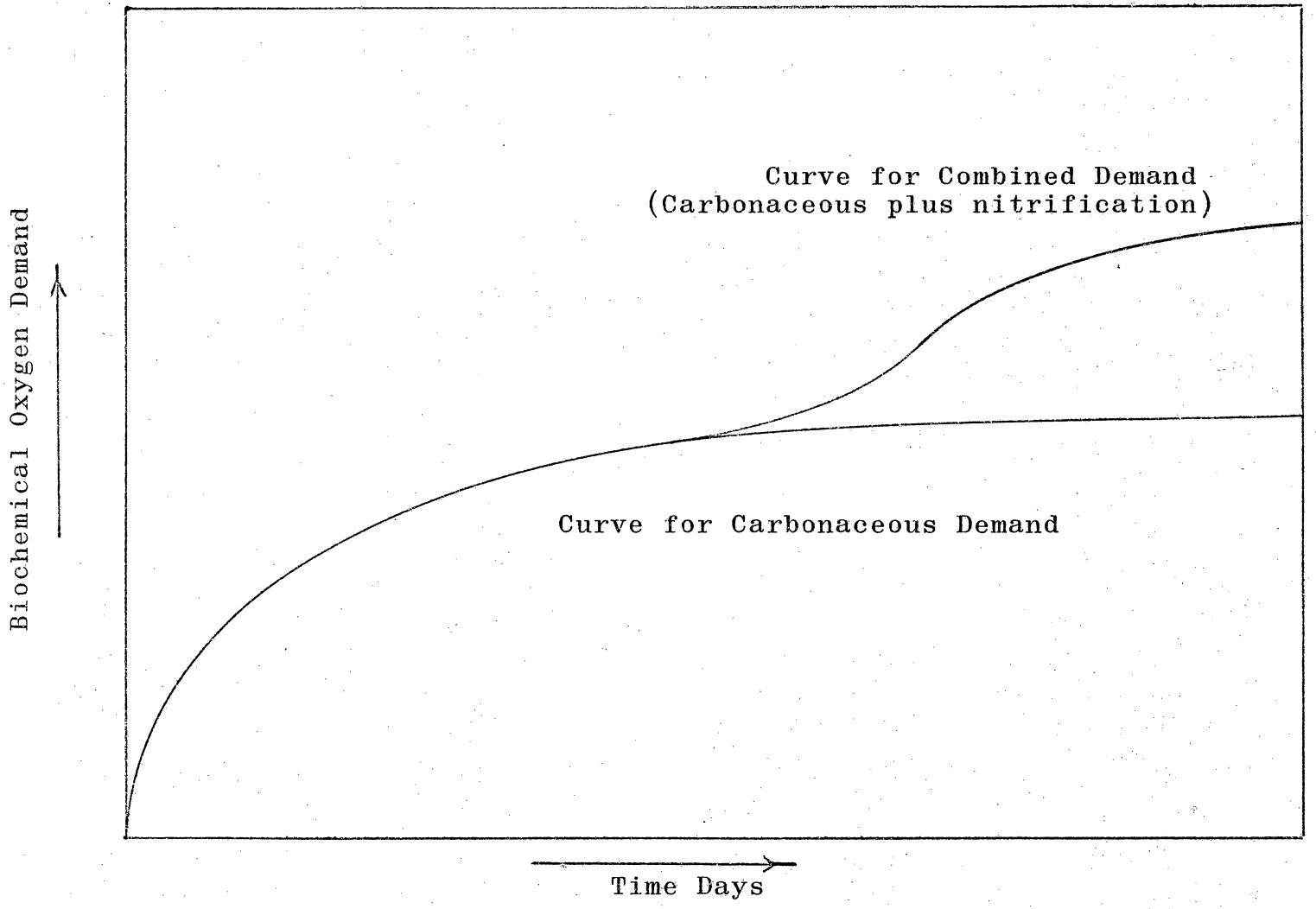

Figure 20. Classical BOD Curve.

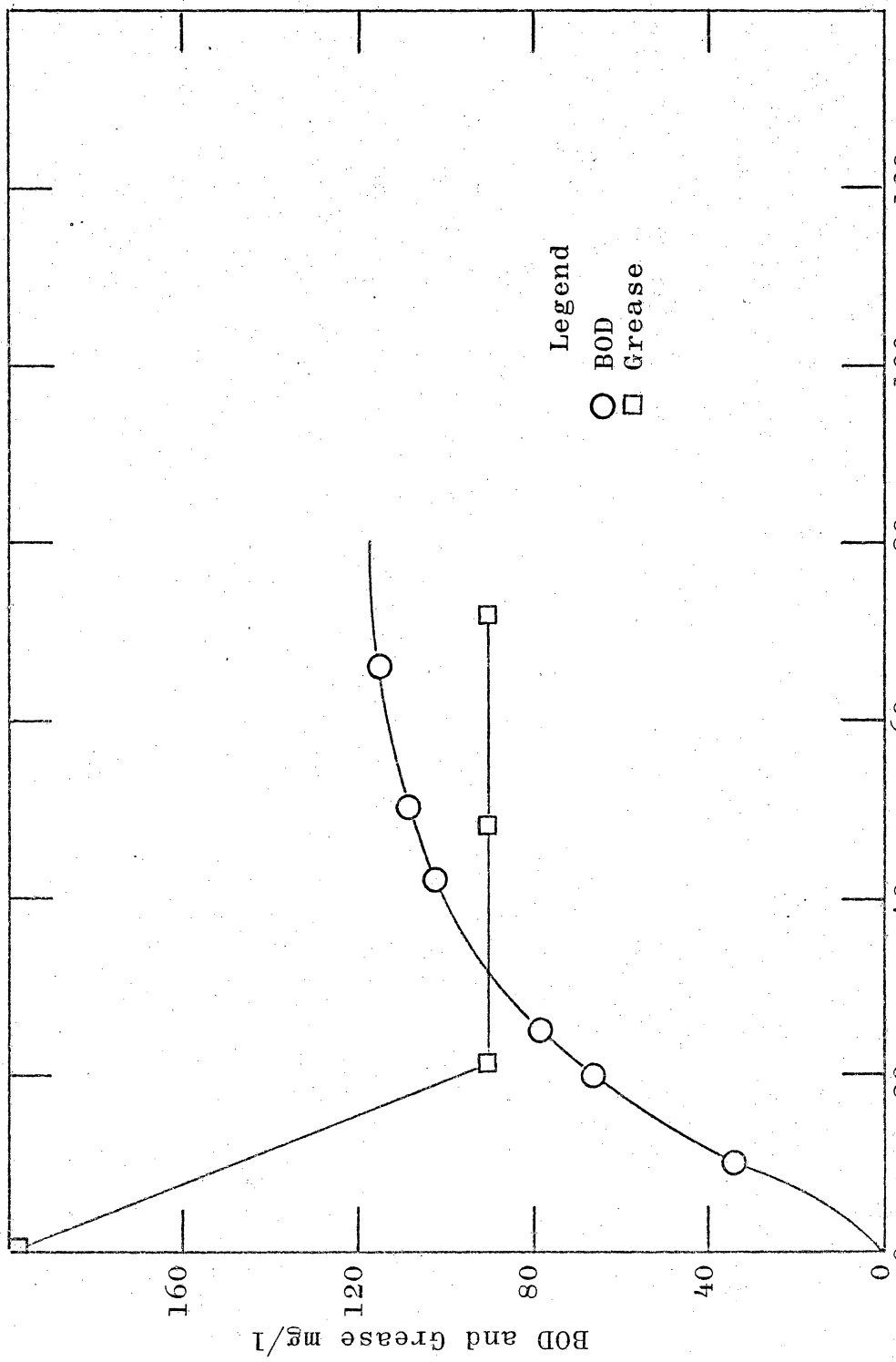

Figure 21. Comparison of Grease Depletion and BOD.

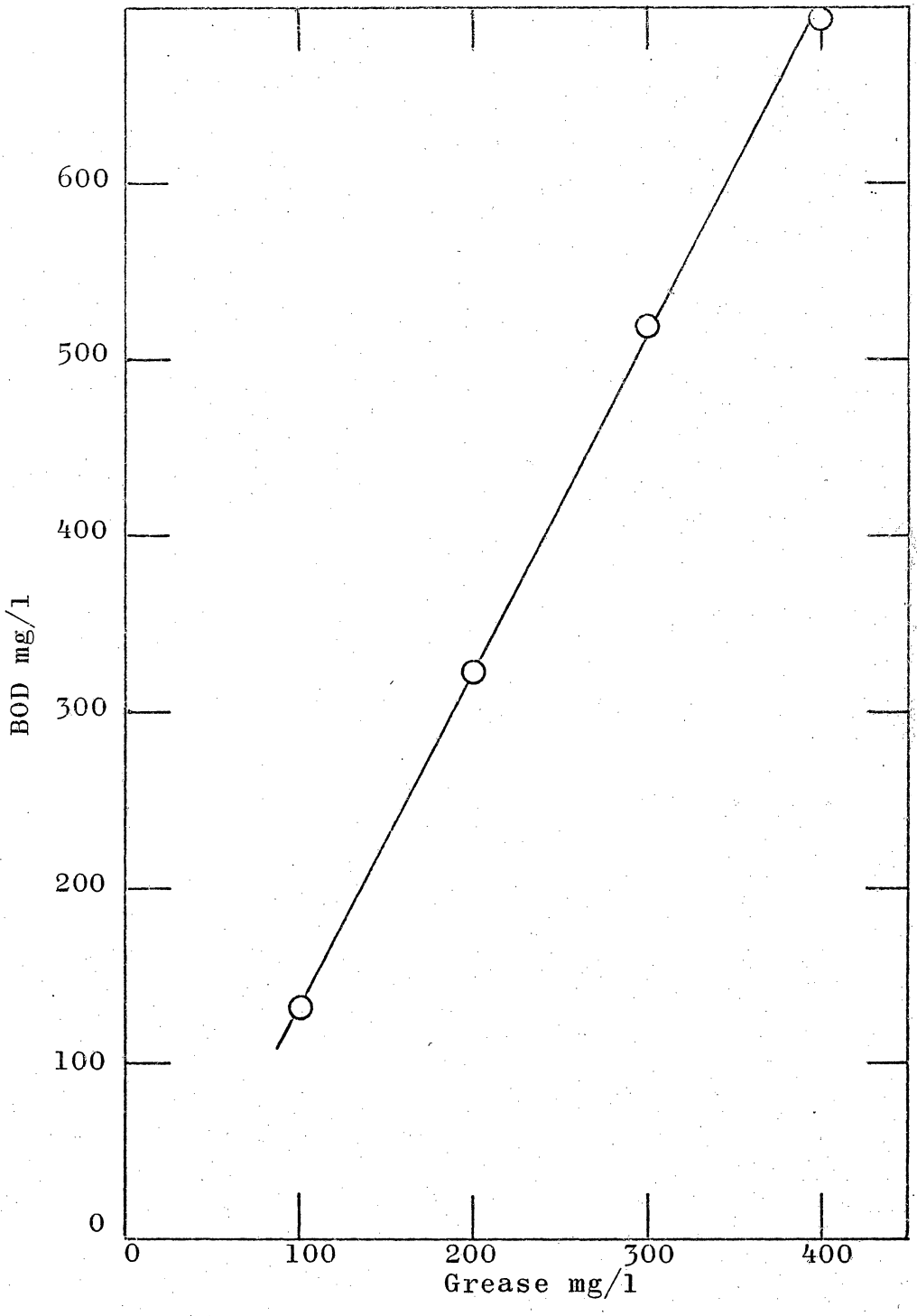

Figure 22. First Stage BOD versus Applied Grease Concentration.

VI. CONCLUSIONS

1. The sewage grease under study was readily metabolized by the organisms of activated sludge. The initial grease substrate was depleted within 24 hours.

2. There were no substantial pH changes resulting from the oxidative activities of microorganisms.

3. Diphasic BOD exertion is typical for the activated sludge used in these experiments but it cannot be concluded that a grease substrate will cause this phenomenon.

4. The velocity constant (K_{10}) and ultimate BOD (L) are not strictly a function of substrate composition or concentration but are also a function of the heterogenous microbial population by which it is metabolized. The longer a heterogenous microbial population is "acclimated" to a given substrate(s) the more readily is the substrate(s) metabolized.

5. The velocity constant (K_{10}) for the first stage of BOD exertion ranged from 0.402 to 0.487 with an arithmetic mean of 0.448.

6. The first stage ultimate BOD (L) is proportional to the concentration of grease.

7. Long term Warburg studies are needed to further define the oxidative activities of activated sludge when exposed to varied concentrations of grease.

8. On the basis of this study activated sludge shows promise as an effective method for the treatment of wastes high in grease content.

VII. SUMMARY

Experimental data obtained in the study of the oxidative activities of activated sludge on a natural sewage grease indicated that the grease was readily metabolized by the microorganisms.

The effect of varied concentrations of grease on the oxygen demand exerted by activated sludge was measured using the Warburg respirometer technique. All tests were performed at 20°C with a shaker rate of 60 cycles per minute.

Diphasic BOD exertion was noted early in the investigation. Glucose was used as a positive control to determine whether the diphasic nature of the BOD curve was caused by the substrate (grease) or by the nature of the microorganisms of the activated sludge. The oxygen demand curves generated by the activated sludge exposed to glucose also showed diphasic BOD exertion. It was concluded that the diphasic nature of the BOD curves was characteristic of the microbial population of the activated sludge.

Tests were also performed to determine the rapidity by which the grease was metabolized by the activated sludge. Test results indicated the grease was assimulated within 24 hours. Samples were analyzed for pH throughout the

test period to determine if a sudden change in pH was a factor affecting the plateau in BOD exertion. There were no adverse pH changes.

Experimental evidence indicates that the activated sludge treatment process could be used to effectively treat grease in a conventional aeration tank.

VIII. ACKNOWLEDGMENTS

This thesis and the graduate program undertaken by the author were supported by an Educational Assistance Grant from the Virginia State Department of Health. The author is indeed grateful for this grant which made his graduate study possible.

The author expresses his appreciation to his thesis advisor, Professor John J. Cibulka, for his suggestion of the topic, guidance, and constructive criticism during the research and preparation of this thesis; to Dr. P. H. King and Dr. C. W. Randall for their advice and counsel; to for his technical assistance; and to his understanding wife, for her interest, encouragement, and devotion throughout the graduate program and the preparation of the thesis.

IX. BIBLIOGRAPHY

1. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, New York, American Public Health Association, 12th Edition, (1965).

2. Bowerman, F. R., Dryden, F. D., "Garbage, Detergents, and Sewers." Journal Water Pollution Control Federation, Vol. 34, p. 475, (May 1962).

3. Fullen, W. J., Hill, H. V., "The Economics of Poor Housekeeping in the Meat Packing Industry." Journal Water Pollution Control Federation, Vol. 39, p. 695, (April 1967).

4. Gaudy, A. F., Jr., Bhatla, M. N., Follett, R. H., Abu-Niaaj, F., "Factors Affecting the Existance of the Plateau During the Exertion of BOD." Journal Water Pollution Control Federation, Vol. 37, p. 444, (April 1965).

5. Gaudy, A. F., Jr., Bhatla, M. N., "Studies on the Causation of Phasic Oxygen Uptake in High-Energy Systems." Journal Water Pollution Control Federation, Vol. 38, p. 1441 (September 1966).

6. Heukelekian, H., Balmat, J. L., "Chemical Composition of the Particulate Fractions of Domestic Sewage." Sewage and Industrial Wastes, Vol. 31, No. 4, p. 413, (April 1959).

7. Mahlie, W. S., "Oil and Grease in Sewage." Sewage Works Journal, Vol. 12, No. 3, p. 527, (May 1940).

8. McKinney, R. E., Microbiology for Sanitary Engineers, New York, McGraw-Hill, (1962).

9. Sawyer, C. N., McCarty, P. L., Chemistry for Sanitary Engineers, New York, McGraw-Hill, 2nd Edition, (1967).

10. Steel, E. W., Water Supply and Sewerage, New York, McGraw-Hill, 4th Edition, (1960).

11. Streeter, H. W., "Measures of Natural Oxidation in Polluted Streams. I. The Oxygen Demand Factor." Sewage Works Journal, Vol. 7, p. 251, (1935).

12. Thomas, H. A., Jr., "Graphical Determination of BOD Curve Constants." Water Sewage Works. Vol. 97, p. 123, (1950).

13. Umbreit, W. W., Burris, R. H., Stauffer, J. F., Manometric Techniques and Tissue Metabolism, Minneapolis, Burgess Publishing Company, 2nd Edition, (1951).

14. Viswanathan, C. V., Phillai, S. C., "Fatty Matter in Sewage Effluents." Indian Institute Science, Golden Jubilee Research Volume, p. 119, (1959).

15. Viswanathan, C. V., Phillai, S. C., "Rapid Removal of Fatty Constituents of Sewage by Activated Sludge." Naturwissenschaften, Vol. 46, No. 5, p. 324, (May 1959).

16. Viswanathan, C. V., Mura Bai, B., Phillai, S. C., "Fatty Matter in Aerobic and Anaerobic Sewage Sludges." Journal Water Pollution Control Federation, Vol. 34, No. 2, p. 189, (February 1962).

17. Watson, K. S., Farrell, R. P., Anderson, J. S., "The Contribution from the Individual Home to the Sewer System." Journal Water Pollution Control Federation, Vol. 39, No. 12, p. 2039, (December 1967).

18. Wilson, I. S., Harrison, M. E., "The Biological Treatment of Chemical Wastes." Journal and Proceedings Institute Sewage Purification, Part 3, p. 261, (1960).

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APPENDICES

APPENDIX A

Grease Extraction

Wash Soxhlet extractor and flask thoroughly and rinse with hexane. Dry extraction flask in a 103°C hot air oven for 30 minutes. Cool in a dessicator for 30 minutes and obtain a tare weight. Add sample to a clean dry beaker with a volumetric pipette being careful to rinse the pipette thoroughly. Fold the Whatman No. 40 filter paper in the shape of a cone and place into a long stemmed funnel which has been fitted to a filtering flask with a rubber stopper. Apply five inches of mercury suction to the filter, at the same time wetting the filter paper with distilled water to seat the filter paper. Acidify the sample with 1 + 1 hydrochloric acid to pH 1. Usually 1 ml is sufficient. Gently mix the sample by swirling for one minute. Filter sample, being careful to rinse the beaker with distilled water. Do not allow the sample to overflow the filter paper. Remove the filter paper being careful not to touch the inside surface of the paper and fold carefully and insert in an extraction thimble. Place extraction thimble containing the filter paper into a hot air oven and dry for 30 minutes at 103°C. Place 20 ml hexane in tared extraction flask, and extract grease at the rate of 60 cycles per hour for four hours. Time from first cycle. Boil off

excess hexane and place in 103°C oven for 30 minutes. Cool in a dessicator for 30 minutes and weigh. The mg/l grease was calculated using the following formula:

$$\text{mg/l GREASE} = \frac{\text{mg GREASE} \times 1000}{\text{ml SAMPLE}}$$

APPENDIX B

Cleaning of Warburg Reaction Flasks

The reaction flasks were rinsed thoroughly in tap water and dried for 30 minutes in a hot air oven at 103°C. The dried flasks were then soaked in chloroform for five minutes to remove the grease from the center well and joints. The flasks were again dried in a hot air oven for 30 minutes at 103°C. After cooling, each reaction flask was soaked in concentrated chrome rinse for 15 minutes to oxidize any organic material that might be in the flasks. Each flask was rinsed in tap water 10 times and distilled water three times. The flasks were again dried in a hot air oven for 30 minutes at 103°C. After drying, the flasks were allowed to cool in the inverted position. The flasks were then ready for reuse.

APPENDIX C

Calculation of Warburg Flask Constant

A flask constant (K) was calculated from the following data as shown below:

V_1 = 10 ml - volume of distilled water added to flask

I = 150 - index for closed leg of manometer

R_2 = 131.5 - reading of open leg of manometer when right index set at 150 and flask containing 10 ml distilled water

V_2 = 100 ml - volume of distilled water added to flask

R_4 = 68.5 ml - reading of open leg of manometer when right index set at 150 and flask containing a total of 110 ml. distilled water.

$$V_T = \frac{V_2(I - R_4)}{R_2 - R_4} + V_1 = \frac{100(150 - 68.5)}{131.5 - 68.5} = 139 \text{ ml}$$

V_T = 139,000 μl - total volume of flask and manometer

V_f = 21,000 μl - total working fluid volume in flask

$V_g = V_T - V_f$ = 118,000 μl - total volume of gas in flask manometer system when containing 21 ml working fluid

T = 293°A - absolute temperature at which measurements are to be taken

$= 0.031$ - solubility of oxygen under test conditions

$P = 10{,}000$ - ratio of specific gravity of mercury to specific gravity of Brodie's solution

$= 0.00143$ mg/µl - density of oxygen

$V_w = 0.02$ l - volume of waste in flask

Then:

$$K = \frac{\text{mg } O_2}{\text{l - mm}} = \left(\frac{V_g \left(\frac{273}{T}\right) + V_f \left(\quad\right)}{P}\right) \left(\quad\right) \left(\frac{1}{V_w}\right)$$

$$= \left(\frac{118{,}000 \left(\frac{273}{293}\right) + 21{,}000 \left(0.031\right)}{10{,}000}\right) \left(\frac{0.00143}{0.02}\right)$$

$$= 0.791 \frac{\text{mg}O_2}{\text{l - mm}}$$

"K" values were calculated for other flasks in a similar manner.

APPENDIX D

Thomas Graphical Method

The following is an example of the calculations needed for determining the velocity constant (K_{10}) and ultimate BOD ($\dot{L}$) by the Thomas Graphical Method.

Hr.	Days t	BOD y	$\frac{t}{y}$	$(\frac{t}{y})^{1/3}$
10	0.417	42	0.00992	0.2145
20	0.833	70	0.01190	0.2280
30	1.250	89	0.01404	0.2410
40	1.667	102	0.01634	0.2540
50	2.083	109	0.01911	0.2670

The values for y were taken from the net cumulative BOD curve. The values $(t/y)^{1/3}$ are plotted against t in days in Figure 23. The intercept A = 0.2015 and slope B = 0.031 are used in the following formulation to solve for K_{10} and L.

$$K_{10} = \frac{2.61\ B}{A} = \frac{2.61\ (0.031)}{0.2015} = 0.402$$

$$L = \frac{1}{2.3\ K\ A3} = \frac{1}{2.3(0.402)(0.0082)} = 132\ mg/l$$

Calculations of all K_{10}'s and L's were made using the Thomas Graphical Method.

When stage BOD exertion occurs and the beginning and ending of the various stages are not readily apparent from observation of a plot of BOD versus time they can be delineated by plotting $(t/y)^{1/3}$ versus time. The points will result in two or more straight lines. The intersection of lines denotes a change in reaction rate and therefore the beginning of another stage of BOD exertion.

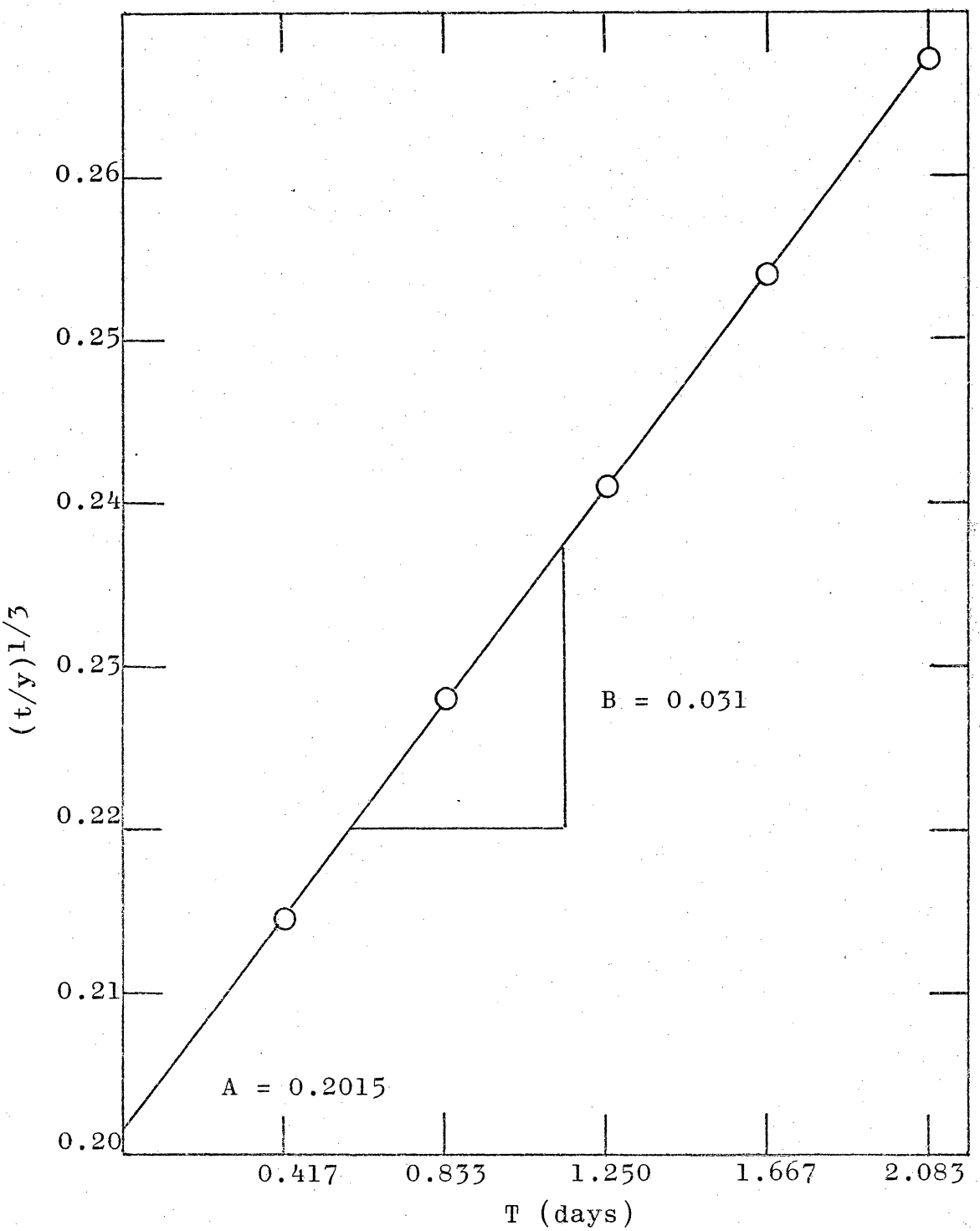

Figure 23. $(t/y)^{1/3}$ versus Time.

WARBURG STUDIES OF THE OXIDATIVE ACTIVITIES OF ACTIVATED SLUDGE SUBJECTED TO VARIED CONCENTRATIONS OF GREASE

by

Eric Herman Bartsch

Abstract

The effects of varied concentrations of a natural sewage grease on the oxidative activities of activated sludge were investigated in an effort to determine the feasibility of using activated sludge as a treatment method for wastes high in grease content.

The method employed to measure the oxidative activities of the activated sludge was the standard Warburg respirometer technique. Cumulative net BOD curves were plotted to determine the magnitude of the oxidation. Total grease analyses were performed throughout the test period to establish a definitive pattern of depletion by oxidation.

The experimental results indicated that the grease was completely assimulated within 24 hours and that there were no adverse pH changes during the course of oxidation.

On the basis of the experimental evidence activated sludge shows great promise as a treatment method for wastes high in grease content.