STUDIES ON AEROBIC MATERIALS AND ENERGY BALANCES DURING GROWTH AND ENDOGENOUS METABOLISM PHASES OF NATURAL MICROBIAL POPULATIONS OF SEWAGE ORIGIN

By

PAUL ALLAN PERKINS

Bachelor of Science

University of California

Davis, California

1971

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1972

STUDIES ON AEROBIC MATERIALS AND ENERGY BALANCES
DURING GROWTH AND ENDOGENOUS METABOLISM PHASES
OF NATURAL MICROBIAL POPULATIONS OF
SEWAGE ORIGIN

Thesis Approved:

Anthony F. Gaudy Jr.
Thesis Adviser

Don F Kincannon

Richard N. DeWitt

D Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to take this opportunity to express his gratitude to Dr. A. F. Gaudy, Jr., for his guidance and encouragement over the past year.

The author also wishes to thank Dr. R. N. DeVries and Dr. D. F. Kincannon for their careful reading of this manuscript.

Special thanks to Carl L. Goldstein for his help and guidance throughout this study.

A sincere thanks is deserved by Mrs. Grayce Wynd for accurately typing this manuscript.

Also, gratitude is extended to my parents for their interest and encouragement throughout my education.

This investigation was made possible through the financial support provided by the Federal Environmental Protection Agency graduate training grant 5T2-WP-238 (03, 04).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Quantitative description of growth of natural or heterogeneous microbial populations is important in order to obtain technological control of the aqueous environment, e.g., water pollution control. The maintenance of high quality of the aqueous environment is facilitated by the treatment and disposal of municipal and industrial wastewaters. The research investigation herein presented is concerned with the growth of heterogeneous microbial populations (e.g., activated sludge) which convert the organic pollutants in waste streams to new cells, carbon dioxide and water. The total oxidation or extended aeration type of activated sludge system is a purification process which utilizes the assimilative and oxidative capabilites of biological cells. Endogenous respiration or aerobic auto-oxidation of the excess bio-mass provides the ultimate sludge disposal process in this system.

Kinetics and mechanisms of biological purification of waters containing organic matter has been an important research area in the water pollution control field for many years. Most kinetic models require the establishment of a relationship between growth rate and substrate concentration. The "Monod" equation (1) has been found to express adequately this relationship for heterogeneous (i.e., natural) populations. The kinetic behavior of growing microbial systems can be mathematically

described by the maximum specific growth rate, μ_{max}, and saturation constant, K_s, for the system. The development of kinetic models for heterogeneous microbial populations during the substrate removal and growth phase is highly dependent on determination of these kinetic "constants" and an adequate quantitative estimate of the cell or sludge yield. It is also essential to have some way to make an overall check on the validity of the expected data. The aerobic material or energy balance offers a way to determine if the substrate disappearing from the system can be recovered or accounted for as synthetic products, i.e., increased bio-mass (sludge) and as inorganic carbon or equivalent O_2 uptake.

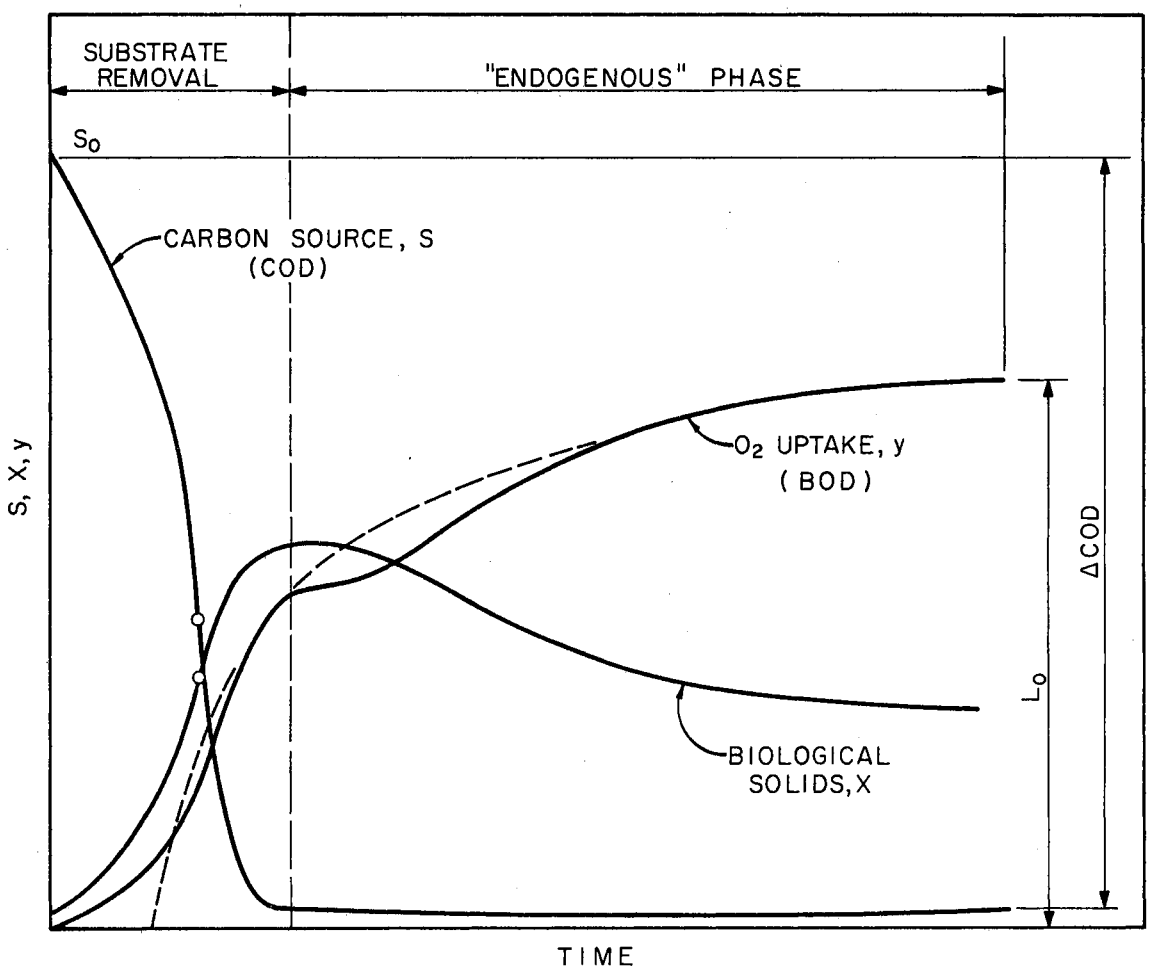
Recently, Gaudy and Gaudy have completed a research project on biological concepts for design and operation of the activated sludge process. Their report brings together various aspects of biological concepts which have emerged and been clarified over the past decade of research in their bioengineering and microbiological laboratories (2).

Figure 1 is reproduced from the above mentioned report (2). This figure represents occurrences in both the purification and endogenous phases. The major occurrences are those which describe the fate of the organic matter in the waste, the growth of the biological solids, and the course of oxygen uptake (i.e., exertion of biochemical oxygen demand).

The purification phase (or phase of removal of exogenous substrate) of biological growth is that phase in which there is an accumulation of biological solids resulting from the microbial metabolism of substrate to cellular compounds and end products. This phase corresponds to a rapid growth of bacterial cells which causes the assimilation of the

Figure 1. Generalized Plot of Substrate Concentration, Biological Solids Concentration, and Oxygen Utilization During Exertion of Biochemical Oxygen Demand.

Circles mark inflection points (from reference 2).


SUBSTRATE REMOVAL
"ENDOGENOUS" PHASE
S_o
CARBON SOURCE, S (COD)
O_2 UPTAKE, y (BOD)
BIOLOGICAL SOLIDS, X
L_o
ΔCOD
S, X, y
TIME

exogenous carbon sources. The available carbon source provides the oxidizable substrate for energy required for microbial metabolic activity and provides the "building blocks" for the synthesis of new cells.

Following the purification phase, it has been shown with reasonable certainty by Gaudy, Bhatla, and Abu-Niaag (3) that there is a "plateau" or discontinuity which occurs in oxygen uptake (see Figure 1). In their investigations, seed and substrate were varied in concentration and type. Known multicomponent carbon sources were also used as substrate. In all, thirty-seven systems were examined for the occurrence of the plateau, and it was found to exist with varying severity and duration, in all of the systems except two. In these two, it was felt that the diphasic nature of the oxygen uptake for the seed served to mask the plateau in net oxygen uptake. Bhatla and Gaudy (4) have proposed four theories to explain the occurrence of this plateau. The proposed theories are as follows:

1. After reaching the maximum population, some of the cells in heterogeneous populations begin to die off and release intracellular components which may become food for other organisms. Since the latter organisms were not the predominating ones, time is required before they are present in sufficient numbers so that their metabolism of the released components is expressed in measurable oxygen uptake, hence, the appearance of a plateau or pause between the first and second stages of oxygen utilization. Alternatively, the cells themselves may become a substrate for predators (such as protozoa).

2. After reaching a maximum population some of the predominating organisms die and release intracellular components. There may be no change in the predominating species, but an acclimation period may be required before the living or intact cells can metabolize the released components.

3. After reaching maximum population the cells may not die off, but may lose their ability to replicate. An acclimation period may be required before the cells can metabolize certain cellular components which were synthesized in the replication process (e.g., excess protein or nucleic acid); that is, an acclimation period may be required before

certain cellular components can be metabolized endogenously.

4. During the rapid metabolism which takes place in the first stage, the cells may release intermediates into the medium. An acclimation period may then be required before these new exogenous compounds can be metabolized. Also, an induction period may be needed to produce catalysts to transport these compounds back into the cell.

It is impossible to eliminate, unequivocally, any of these theories. However, theory 2 and theory 4 may be disregarded for the present studies based on the conclusions reached by Bhatla and Gaudy (4) in their work with heterogeneous populations using glucose as the growth medium. They found that there was a release of soluble organic material into the medium at various times during the second stage, but there was no indication in any of their experiments of such a release at a time which could affect the occurrence of a plateau as postulated in theory 2. Washed cell suspensions used for endogenous blanks were also observed to yield a plateau. Theory 4 may be discounted on the basis of an experiment showing the occurrence of a plateau at a time when there was no exogenous substrate present in the medium.

In a later study, Bhatla and Gaudy (5) determined that theory 3 could also be eliminated as a generally occurring cause for the plateau. The addition of chloramphenicol, which completely stopped bacterial replication and protein synthesis, before the beginning of the plateau did not inhibit oxygen uptake during the second stage or endogenous phase. This provides direct evidence that bacterial synthesis and replication is not the controlling factor in the second stage of oxygen uptake. However, the results of the study provided definite experimental proof of the validity of theory 1. Investigation with pasteurized seed (i.e., protozoa free) showed that the second stage of oxygen uptake

is almost entirely the result of the metabolic activity of protozoa, proving that the plateau in the oxygen uptake curve for a heterogeneous population represents the transition from a system dominated by bacteria to one dominated by protozoa. The length of the plateau is dependent on the time lag between the peaks in bacterial and protozoan populations.

Exertion of biochemical oxygen demand (oxygen uptake) continues after the purification phase is ended and the system is in the endogenous or autodigestive phase. The continuation of oxygen uptake is at a much slower rate during the endogenous phase than during the purification phase, due to the steady decline in biological activity. When the system passes into the endogenous phase, the original exogenous organic matter or carbon source is no longer available since it has already been utilized by the microorganisms in the system. Substrate for continued microbial activity may be present due to the lysing of some biological cells, or may be the cells themselves as they are utilized by predator organisms (protozoa). Since the microorganisms must now utilize their own mass as a substrate source, the biological solids in the system decrease. The reduction of the biological solids all the way down to their initial concentration is termed "total oxidation," and has been a subject of controversy in the field for some time.

Regarding the concept of "total oxidation," Forney and Kountz (6) performed a study using skim milk waste in a continuous flow system and concluded that a long-term biological solids equilibrium was possible and that the concept of "total bio-oxidation" is thus feasible. In their later studies on total oxidation, Symons and McKinney (7) concluded that total sludge recycle was not possible on a long-term basis, and that sludge wastage is essential for the successful operation of an

activated sludge system. Using a daily batch-fed system grown on a sodium acetate substrate and operated for a period of thirty-five days with 100 percent sludge recycle, they found that the volatile biological solids increased throughout the test period for each experiment. They concluded that the accumulated material which was observed to be mostly extracellular polysaccharide was resistant to biological degredation. On this basis they felt they had refuted the concept of total oxidation.

After Symons and McKinney (7) published their paper, Kountz and Forney (8) re-evaluated their stand on the total oxidation concept. From the results of their studies on metabolic energy balances in a multi-unit total oxidation system operated for six months, they concluded that a residual material (polysaccharide material) remained, equivalent to 20 to 25 percent of the new sludge produced. They also concluded that there is an endogenous loss of 2 percent/day and an accumulation of 0.6 percent/day of non-oxidizable sludge in the system. From these conclusions, Kountz and Forney (8) rejected the concept of total oxidation on the basis that it is not possible within a reasonable time and with a reasonable size treatment system.

In later research on the accumulation of volatile biological solids in batch-fed and continuous flow systems with various carbon sources, Washington and Symons (9) concluded that there was a sludge buildup of 10 to 15 percent of the ultimate BOD of the carbohydrate or fatty acid substrate removed. After studying that fraction of the biological solids which is biologically inert and resistant to degradation during the endogenous phase, they concluded that these solids are mainly polysaccharides (47 to 56 percent), with protein (39 to 47

percent) and fats (3 to 8 percent) comprising the remainder.

Gaudy, Ramanathan, Yang, and DeGeare (10) operated a bench-scale pilot plant of the extended aeration activated sludge system for nearly two years, and concluded that such a system can be operated with reasonably good biochemical efficiency without continual solids accumulation and without sludge wasting. Also, there was no buildup of carbohydrate or protein in the sludge composition. During the entire experiment, all of the biological solids were retained in the system. The only solids removed were those taken out for sampling and a small amount for auxiliary experiments (no more than 0.2 percent). They found that there is a fluctuation in the concentration of biological solids due to natural biological internal regulation, which caused the accumulation of biological solids to be periodically reduced. Since there were no solids wasted, either purposely or inadvertently, this reduction of the biological solids that had accumulated must be due to their now being utilized as a carbon source by other members of the population. Investigation of the substrate-consuming capability of the biological solids and their O_2 uptake capacity showed that as the biological solids accumulates, the endogenous O_2 uptake of the sludge drops significantly. However, any possible loss in the unit's purification efficiency can amply be made up by the large number of organisms present to feed on the waste. It was observed at times that the cell concentration became so great that it caused settling problems in the clarification chamber, and an excessive concentration of biological solids in the overflow. Gaudy, Yang, and Obayashi (11) proposed a solution to this problem, and called it the "hydrolytic assist." Their solution is to hydrolyze chemically part of the return sludge and return

it to the aeration chamber along with the regular stream of influent substrate. A pilot plant, in which the hydrolytic assist process was utilized, was operated for one year and it was found that this process is operationally feasible and provides a means to control the concentration of sludge in the total oxidation process (12).

In his work with activated sludge heterogeneous populations during prolonged endogenous aeration conditions, Goldstein (13) observed the "total oxidation" of solids accumulated during the substrate removal phase in many of his batch-activated sludge-extended aeration systems. Further investigation revealed that there was no buildup in carbohydrate content of the sludge during endogenous metabolism, thus providing additional strong evidence that carbohydrate material is not biologically inert. The theoretical feasibility of total oxidation or the extended aeration process is usually contested on the basis that there is an inactive or biologically inert fraction of the bio-mass which cannot be metabolized and therefore builds up in the system, making sludge wastage necessary. Recent investigations by Obayashi and Gaudy (14) were conducted for the express purpose of determining whether extracellular heteropolysaccharides of microorganisms can serve as the carbon source for the growth of other microorganisms. Short-term batch experiments were conducted, using microbial polysaccharide obtained from a variety of microorganisms. The results obtained provide direct evidence that extracellular polysaccharide cannot be classified as biologically inert material. Consequently, their conclusion is that buildup of extracellular polysaccharide cannot be validly cited as evidence against the theory of the total oxidation or extended aeration process.

Again, attention is directed to Figure 1, which represents the

whole spectrum of occurrences during the exertion of biochemical oxygen demand.

The organic matter or exogenous substrate in a waste is partitioned between respiration and synthesis as it is utilized by microorganisms. That portion synthesized shows up as biological solids, and that which is respired shows up as oxygen uptake. The partition of the substrate is often estimated by measuring oxygen utilization and removal of chemical oxygen demand. The amount of COD which is not accounted for as oxygen uptake is assumed to be utilized in sludge synthesis. This partitioning can be checked by means of a materials or energy balance. In an article by Gaudy, Bhatla, and Gaudy (15) four ways to calculate such balances are presented. Each requires independent measurements of substrate removal, oxidation of substrate, and conversion of substrate to cellular material. The primary difference between each of the four methods is the means used to measure the three parameters, or convert them to common units.

1. Materials Balance, Weight Calculation

With synthetic wastes containing single known carbon sources, both COD and oxygen uptake measurements can be converted to equivalent weights of substrate (hexose in the example below). A direct conversion of substrate mass to cell mass is assumed, and the substrate utilized for cell synthesis is therefore measured as increase in dry weight of cells.

$$\text{Recovery} = \frac{(O_2 \text{ uptake})\ (0.94) + \Delta \text{ cells}}{\Delta \text{ COD}\ (0.94)}$$

2. Materials Balance, Carbon Calculation

Assuming that the carbon content of the biological mass remains constant throughout the substrate removal period, a materials balance may be based on an average content of cell mass. The average figure employed was 51 percent. The increase in biological solids can be converted to an equivalent amount of substrate carbon, and a materials balance can be computed by comparing the amount of substrate carbon removed during any time period with the amount of substrate carbon utilized for synthesis plus the amount oxidized. It is assumed that the amount of carbon dioxide can be calculated from the oxygen uptake. This method is applicable only to studies using known carbon sources.

$$\text{Recovery} = \frac{(O_2 \text{ uptake})\ (1.375)\ (0.273) + \Delta \text{ cells}\ (0.51)}{\Delta \text{ COD}\ (0.94)\ (0.4)}$$

3. Energy Balance, Based on Empirical Formula for Composition of Activated Sludge

Based on the sludge formula $C_5H_7NO_2$, the COD value of the cells is calculated as 1.414 times the cell weight. The balance can be calculated by comparing the reduction in COD of the waste at any time with the sum of the measured oxygen uptake and the COD of the cells.

$$\text{Recovery} = \frac{O_2 \text{ uptake} + \Delta \text{ cells}\ (1.414)}{\Delta \text{ COD}}$$

4. Energy Balance, Based on Measurement of Cell COD

The COD removed in any time period is compared with the summation of oxygen uptake and the COD of the cells produced during this time period, thus providing an energy balance.

$$\text{Recovery} = \frac{O_2 \text{ uptake} + \Delta \text{ COD cells}}{\Delta \text{ COD}}$$

Based on their investigation of method 4 and its comparison with the other three methods, Gaudy, et al. concluded that method 4 has a much sounder technical basis than any of the other methods. Therefore, they recommend its use in waste treatment research.

From the foregoing discussion, it can be seen that if total oxidation does or can occur and if the balance idea is valid, then one should be able to account for all of the COD which has been removed as oxygen uptake. The accumulated amount of oxygen uptake should approximate the amount of biological organic matter available to the microorganisms, i.e., the theoretical oxygen demand would be approximately equal to the theoretical COD. Goldstein and Gaudy began special problems research to determine if balances could validly be made all the way along the oxygen uptake curve in cases where total oxidation was observed, or where the material was nearly totally oxidized, in any event. The present work has been conducted to verify and extend the previous studies.

CHAPTER II

MATERIALS AND METHODS

A. Experimental Protocol

From an initial sewage seed obtained from the primary clarifier effluent of the municipal treatment plant at Stillwater, Oklahoma, natural microbial cell populations were grown on glucose as the sole carbon source. The synthetic waste (growth medium) consisted of the following nutrients and inorganic salts (per liter): glucose, 500 mg; 1.0M potassium phosphate buffer (pH 7.0), 10 ml; $(NH_4)_2SO_4$, 500 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; $FeCl_3 \cdot 6H_2O$, 0.5 mg; $MnSO_4 \cdot H_2O$, 10 mg; $CuCl_2$, 7.5 mg; tap water (for trace elements), 100 ml; distilled water to volume.

The cells were acclimated to the growth medium as follows: Five ml of sewage seed were added to 45 ml of growth medium, and this mixed liquor was aerated. Aeration was provided by a shaker apparatus operating at 100 oscillations/minute. Daily, 5 ml of the mixed liquor was transferred to a new flask containing 45 ml of fresh growth medium. The cells were acclimated to the substrate for three days prior to using them for an experiment. All acclimation procedures were conducted at room temperature ($21 \pm 2^{o}C$), and for each experiment performed a different initial sewage seed was used.

Each of the experiments was carried out in the same manner, the procedure of which is as follows: After acclimation, the cells were

inoculated into Warburg flasks containing fresh growth medium. The same amount of inoculum (5 ml) and growth medium (35 ml) was contained in each flask--the assumption then being that conditions in each of the flasks were identical and the experiment as a whole could be treated as a single batch experiment. The pH in all systems was 7.0 $\pm$ 0.15. The flasks were then connected to the Warburg respirometer and allowed to equilibrate for 15 minutes before closing the manometers and initiating the experiment. At various time intervals, individual flasks were removed from the Warburg respirometer and their contents analyzed for the biological solids concentration, the chemical oxygen demand of the mixed liquor and the filtrate, and for ammonia-nitrogen and nitrate-nitrogen. Of the 40 ml of mixed liquor in each flask, 10 ml of the mixed liquor was used for the COD determination, and 30 ml was used for the determination of biological solids. Twenty ml of the filtrate from the biological solids determination was required for a COD analysis, and the remainder was analyzed for ammonia-nitrogen and nitrate-nitrogen. Each experiment was continued until the oxygen uptake was increasing at only a very slow rate, indicating that the biological activity was very slight (approximately 2.5 to three weeks for each experiment).

During initial growth stages of each experiment, optical density determinations of an identical mixed liquor were employed to give an indication of the course of biological growth in the system. This mixed liquor was maintained on a shaker apparatus, and its optical density determined every half-hour. Percent transmittance was measured and converted to optical density using a chart which had been prepared in accordance with the equation $OD = \log_{10} T$. All measurements were

made at a wave length of 540 mm using a distilled water blank. A Bausch and Lomb 120 spectrophotometer was used.

The values for optical density were plotted on semi-logarithmic paper (base 10) versus time. The resulting growth curve gave an indication of conditions in the system connected to the Warburg respirometer. This comparison was continued until the growth curve showed that the peak of the biological solids concentration (measured as optical density) had been passed. This growth curve was utilized as a guide in selecting sampling times during the substrate removal phase.

B. Analyses

1. Biological Solids

Biological solids concentrations were determined by the membrane filter technique as outlined in Standard Methods (16). For the first experiment, tare dishes weighing approximately 1.2 gm were used to hold the 0.45 micron Millipore membrane filters. In subsequent experiments, aluminum dishes weighing approximately 0.2 gm were used.

2. Substrate Removal

The chemical oxygen demand procedure was followed as outlined in Standard Methods (16). Mercuric sulfate (H_gSO_4) was added to prohibit any excess chlorides from interfering with the dichromate reaction.

3. Oxygen Uptake - Warburg Respirometer

Flasks were prepared by placing one milliliter of 20 percent KOH in the center well to absorb CO_2. Forty ml of mixed liquor was added to the sample flasks, and 40 ml of distilled water was used in the two blank (barometer) flasks. The flasks were then sealed to their

individual manometers and placed in the constant temperature water bath at 25 $\pm$ 0.5°C, and operated at 90 oscillations/min. After equilibrium (15 minutes), the manometers were set and closed; readings were taken at various time intervals. After correcting each reading for the barometer flask deflections, the accumulated oxygen uptake was calculated using previously determined flask constants.

4. Nitrate-Nitrogen

Nitrate-nitrogen was determined by the Brucine method as outlined in Standard Methods (16). The standard used was anhydrous potassium nitrate.

5. Ammonia-Nitrogen

Ammonia-nitrogen was determined by a method developed by Niss (1957) and described by Ecker and Lockhard (17). Two reagents were employed. Reagent A contained: 4.7 gm sodium citrate, 1.7 gm citric acid, 9.6 gm phenol, and distilled water to 480 ml. Reagent B contained: 6.0 gm boric acid (H_3BO_3), 8.0 gm sodium hydroxide, 30.0 ml of commercial Chlorox bleach, and distilled water to 200 ml. The cell-free samples were diluted if needed to give between two and 20 mg/l of ammonia-nitrogen per ml. To 1.0 ml samples were added 5.0 ml of reagent A and 2.0 ml of reagent B. The samples were mixed, heated in a boiling water bath for five minutes, and cooled rapidly in ice water. The optical density for the sample was then determined at a wavelength of 615 mm against a distilled water-reagent blank using a Bausch and Lomb 120 spectrophotometer. The optical density readings were compared to a standard curve plotted using known concentrations of $(NH_4)_2SO_4$. This standard curve was prepared immediately prior to the ammonia-nitrogen determinations.

CHAPTER III

RESULTS

A. General Comments

Two long-term batch experiments were conducted. For experiment one, the results are plotted in Figure 2, and O_2 uptake curves for all Warburg flasks used for measurement of O_2 uptake during this experiment are plotted in Figure 3. The results are summarized in Table I, and materials and energy balances are given in Table II. For experiment two, the results are plotted in Figure 4, and the individual O_2 uptake curves are shown in Figure 5. Primary results for this experiment are summarized in Table III, and materials and energy balances are given in Table IV.

In presenting these results, two aspects are emphasized, the recovery of substrate, i.e., the materials and energy balances, and the degree of total oxidation during the period of the experiments.

B. Substrate Recovery

The general course of oxygen uptake, mixed liquor COD, total filtrate COD, and biological solids was similar for both experiments one and two (see Figures 2 and 4). The oxygen uptake curve in these two figures is an average curve in which each plotted point is the result of averaging the oxygen uptake values for each manometer being read at that

Figure 2. Changes in Biochemical Parameters During the Course of BOD Exertion in Experiment 1.

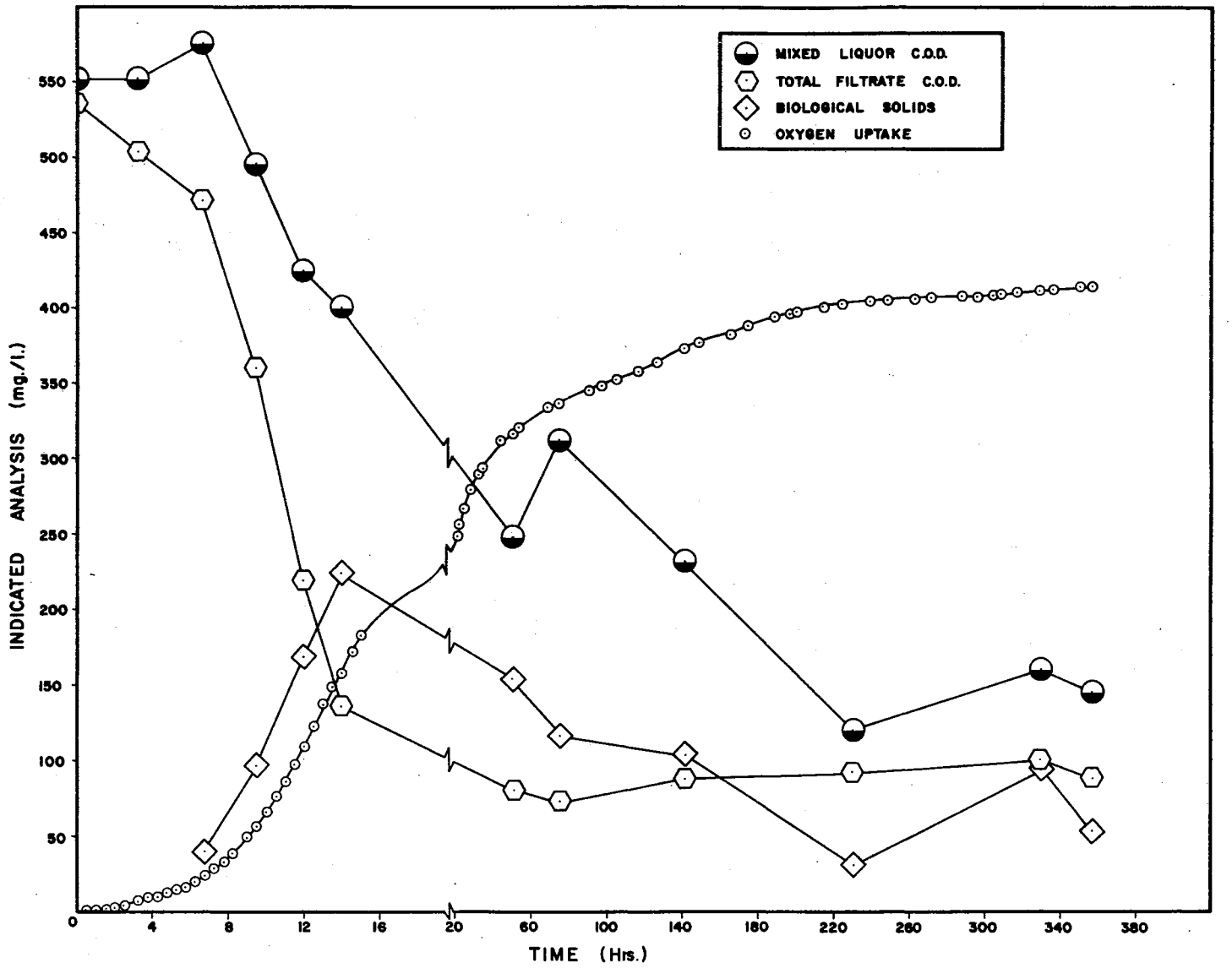

MIXED LIQUOR C.O.D.
TOTAL FILTRATE C.O.D.
BIOLOGICAL SOLIDS
OXYGEN UPTAKE
INDICATED ANALYSIS (mg./l.)
550
500
450
400
350
300
250
200
150
100
50
0
4
8
12
16
20
60
100
140
180
220
260
300
340
380
TIME (Hrs.)

Figure 3. Accumulated Oxygen Uptake During Experiment 1.

Each curve was obtained from individually-treated Warburg flasks.

Triangles represent O_2 uptake calculated as the amount of mixed liquor COD removed using the values shown in Figure 2.

Note the expanded scale for the first 20 hours. The dotted portion of the curves shows O_2 uptake plotted entirely on the smaller scale.

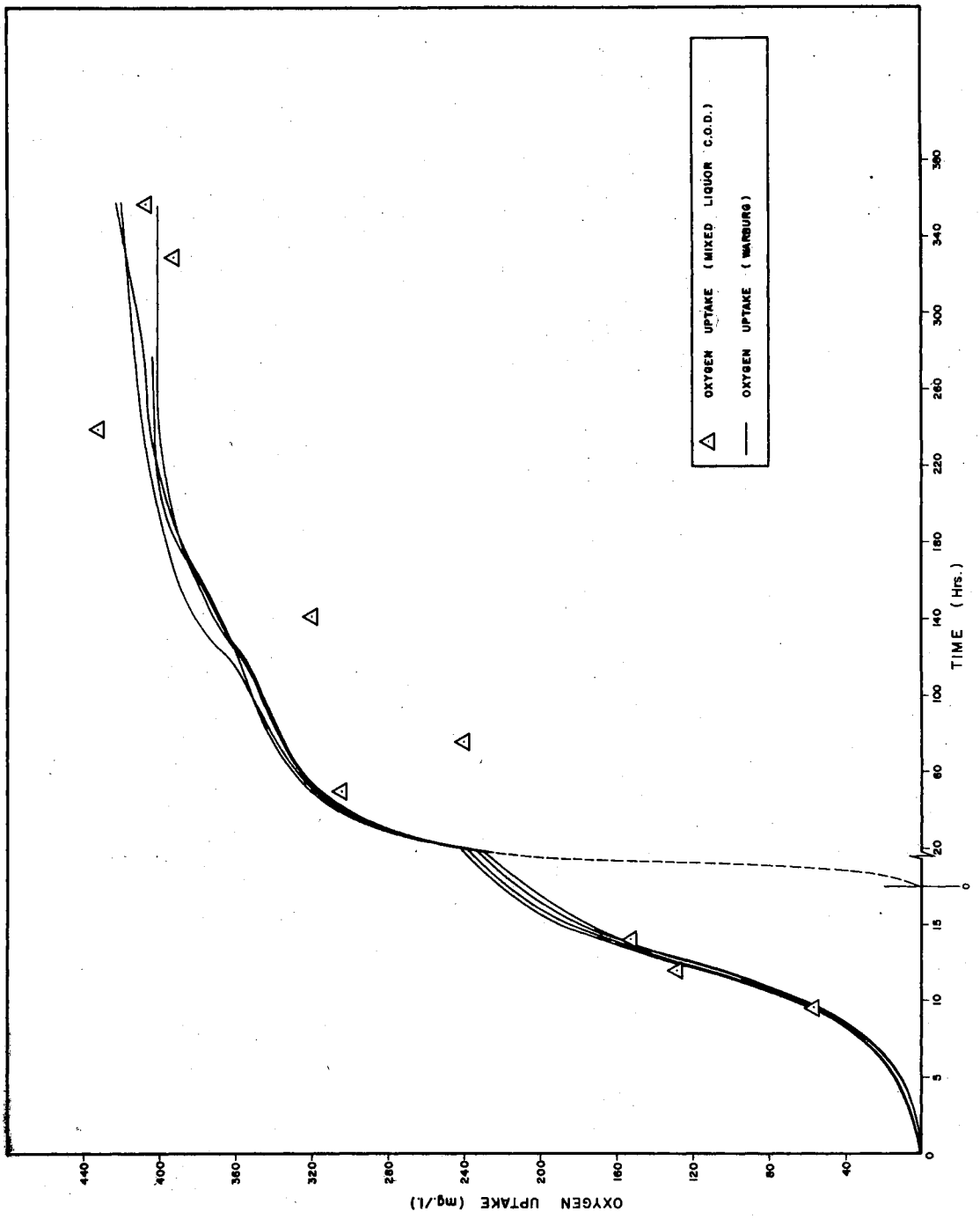

OXYGEN UPTAKE (mg./l)
TIME (Hrs.)
OXYGEN UPTAKE (MIXED LIQUOR C.O.D.)
OXYGEN UPTAKE (WARBURG)

TABLE I

SUMMARY OF BIOCHEMICAL CHARACTERISTICS, EXPERIMENT 1

Time (hrs)	COD Mix. Liq. mg/l	COD Filtrate mg/l	COD Cells mg/l	ΔCOD Mix. Liq. mg/l	ΔCOD mg/l	ΔCOD Cells mg/l	Cells mg/l	ΔCells mg/l	Oxygen Uptake High mg/l	Oxygen Uptake Low mg/l	Oxygen Uptake Avg mg/l	Ammonia Nitrogen mg/l	Nitrate Nitrogen mg/l
0	552	536	16	-	-	-	-	-	-	-	-	62.91	-
3.25	552	504	48	0	32	32	-	-	8.13	5.16	6.18	61.10	-
6.75	576	472	104	-	64	88	40	-	23.44	22.36	22.93	55.45	-
9.50	496	360	136	56	176	120	96.7	56.7	57.72	53.32	56.20	50.53	-
12.00	424	219	205	128	217	189	170	130	112.66	104.58	109.09	57.00	-
14.00	400	136	264	152	400	248	223	183	165.51	154.08	159.79	56.00	-
22.50	-	-	-	-	-	-	-	-	257.66	252.68	255.94	55.00	-
50.50	248	80	168	304	456	152	153	113	319.23	315.02	317.00	61.95	-
75.75	312	72	240	240	464	124	117	77	340.22	335.18	337.67	62.45	-
141.25	232	88	144	320	448	128	103	63	381.81	369.11	373.37	65.43	-
239.25	120	92	28	432	444	12	30	10	408.81	400.05	404.44	64.74	-
329.00	160	100	60	392	436	44	93	53	418.12	398.64	411.34	64.07	-
357.00	145	88	57	407	448	41	45	5	422.09	400.05	414.34	63.40	-

TABLE II

SUBSTRATE RECOVERY EMPLOYING VARIOUS METHODS OF COMPUTATION, EXPERIMENT 1

Time (hrs)	Calculated Substrate Recovery (Percent)														
	I. Materials Balance Weight			II. Materials Balance Carbon			III. Energy Balance Empirical			IV. Energy Balance Cell COD			V. Energy Balance ΔCOD Mix. Liq.		
	High	Low	Avg	High	Low	Avg	High	Low	Avg	High	Low	Avg	High	Low	Avg
3.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.50	67.06	64.56	66.20	76.43	73.94	75.58	78.34	75.84	77.48	100.97	98.47	100.11	103.07	95.21	100.35
12.00	115.64	111.92	114.00	133.08	129.37	131.44	136.62	132.90	134.98	139.01	135.29	137.36	88.01	81.70	85.22
14.00	92.78	89.92	88.61	103.36	100.51	101.91	109.70	106.85	104.63	103.37	100.52	101.94	108.88	101.36	105.12
22.50	Center well on Warburg flask overflowed (see text)														
50.50	96.36	95.44	95.87	103.50	102.58	103.01	105.04	104.12	104.55	103.33	102.41	102.85	105.00	103.62	104.27
75.75	90.97	89.89	90.42	95.71	94.62	95.16	96.78	95.70	96.23	100.04	98.96	99.49	141.75	139.65	140.69
141.25	100.20	97.42	98.30	104.19	101.41	102.27	105.15	102.36	103.22	113.79	110.96	111.91	119.31	115.34	116.67
239.25	94.47	92.49	93.48	94.97	93.00	93.99	95.25	93.28	94.27	94.77	92.80	93.79	94.63	92.60	93.62
329.00	108.90	104.43	107.27	112.32	107.86	110.67	113.18	108.71	111.53	105.99	101.52	104.43	106.66	101.69	104.93
357.00	94.14	89.28	93.67	94.31	89.46	93.84	94.52	89.67	94.06	103.36	98.44	101.63	103.70	98.29	101.80

Figure 4. Changes in Biochemical Parameters During the Course of BOD Exertion in Experiment 2.

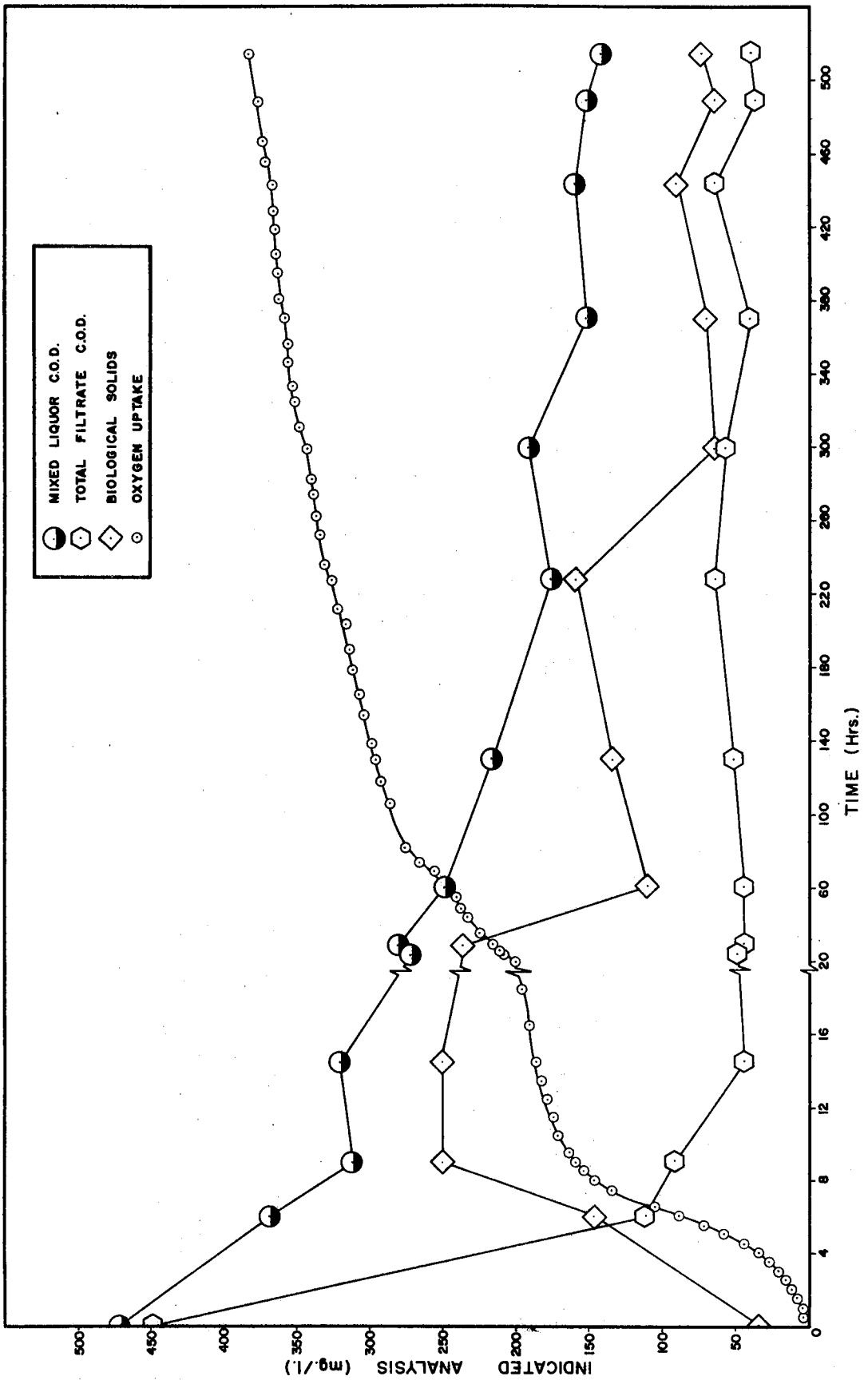

MIXED LIQUOR C.O.D.
TOTAL FILTRATE C.O.D.
BIOLOGICAL SOLIDS
OXYGEN UPTAKE
INDICATED ANALYSIS (mg./l.)
TIME (Hrs.)
0
4
8
12
16
20
60
100
140
180
220
260
300
340
380
420
460
500
50
100
150
200
250
300
350
400
450
500

Figure 5. Accumulated Oxygen Uptake During Experiment 2.

Each curve was obtained from individually-treated Warburg Flasks.

Triangles represent O_2 uptake calculated as the amount of mixed liquor COD removed using the values shown in Figure 4.

Note the expanded scale for the first 20 hours. The dotted portion of the curves shows O_2 uptake plotted entirely on the smaller scale.

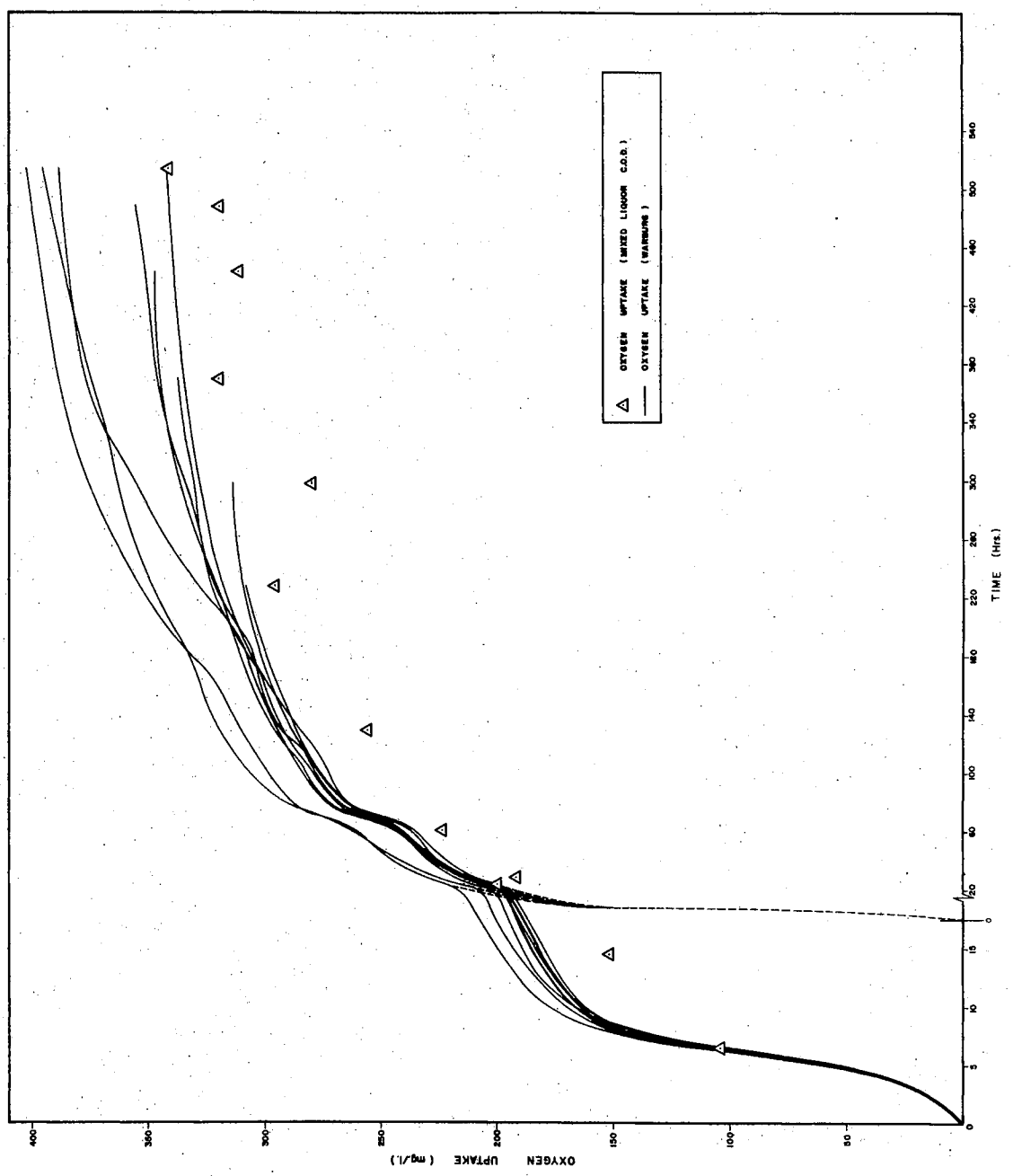

OXYGEN UPTAKE (mg/l.)
TIME (Hrs.)
OXYGEN UPTAKE (MIXED LIQUOR C.O.D.)
OXYGEN UPTAKE (WARBURG)

TABLE III

SUMMARY OF BIOCHEMICAL CHARACTERISTICS, EXPERIMENT 2

Time (hrs)	COD Mix. Liq. mg/l	COD Filtrate mg/l	COD Cells mg/l	ΔCOD Mix. Liq. mg/l	ΔCOD mg/l	ΔCOD Cells mg/l	Cells mg/l	ΔCells mg/l	Oxygen Uptake High mg/l	Oxygen Uptake Low mg/l	Oxygen Uptake Avg mg/l	Ammonia Nitrogen mg/l	Nitrate Nitrogen mg/l
0	472	448	24	-	-	-	34	-	-	-	-	56.84	-
6.0	368	112	256	104	336	232	147	113	94.35	83.76	89.11	37.37	-
9.0	312	91	221	160	357	197	250	216	170.20	154.16	158.92	36.05	-
14.5	320	44	276	152	404	252	250	216	198.51	178.96	185.27	29.74	-
24.0	272	48	224	200	400	200	93	59	223.18	200.86	208.23	32.89	-
29.0	280	44	236	192	404	212	237	203	232.26	209.54	216.86	38.42	-
60.5	248	44	204	224	404	180	110	76	260.54	235.26	245.27	42.37	-
130.0	216	52	164	256	396	140	133	99	319.71	284.95	296.27	48.42	-
228.0	176	64	112	296	384	88	163	129	352.75	309.02	325.94	51.05	-
299.0	192	56	136	280	392	112	63	29	376.70	313.90	342.83	50.00	-
370.0	152	40	112	320	408	88	70	36	389.57	335.00	357.81	50.00	-
443.0	160	64	96	312	384	72	90	56	395.64	339.16	367.35	54.74	-
488.5	152	36	116	320	412	92	63	29	399.57	341.59	375.70	54.21	-
514.0	130	40	90	342	408	66	73	39	403.15	342.98	382.77	53.68	-

TABLE IV

SUBSTRATE RECOVERY EMPLOYING VARIOUS METHODS OF COMPUTATION, EXPERIMENT 2

Time (hrs)	Calculated Substrate Recovery (Percent)														
	I. Materials Balance Weight			II. Materials Balance Carbon			III. Energy Balance Empirical			IV. Energy Balance Cell COD			V. Energy Balance ΔCOD Mix. Liq.		
	High	Low	Avg	High	Low	Avg	High	Low	Avg	High	Low	Avg	High	Low	Avg
6.0	63.85	60.70	62.29	73.65	70.50	72.09	75.63	72.48	74.07	97.12	93.97	95.56	90.72	80.53	85.68
9.0	112.04	107.54	108.88	129.66	125.17	126.50	133.22	128.73	130.06	102.85	98.36	99.69	106.37	96.35	99.32
14.5	106.01	101.17	102.73	121.57	116.74	118.30	124.73	119.89	121.45	111.51	106.67	108.23	130.59	117.73	121.88
24.0	71.48	65.90	67.74	75.70	70.13	71.97	76.65	71.07	72.91	105.79	100.21	102.05	111.59	100.43	104.11
29.0	110.94	105.32	107.13	125.54	119.93	121.75	128.54	122.91	124.72	109.96	104.34	106.15	120.96	109.13	112.94
60.5	84.50	78.24	80.72	89.89	83.65	86.12	91.09	84.83	87.31	109.04	102.78	105.26	116.31	105.02	109.49
130.0	107.33	98.55	101.41	114.51	105.74	108.60	116.08	107.30	110.16	116.08	107.31	110.16	124.88	111.30	115.73
228.0	127.60	116.21	120.61	137.27	125.90	130.30	132.33	127.97	132.38	114.77	103.39	107.79	119.17	104.39	110.11
299.0	103.96	87.94	95.32	105.97	89.97	97.34	106.55	90.53	97.91	124.66	108.64	116.02	134.53	112.10	122.43
370.0	104.86	91.49	97.08	107.29	93.93	99.52	107.95	94.58	100.17	117.05	103.67	109.26	121.74	104.68	111.81
443.0	118.54	103.83	111.17	122.64	107.95	115.28	123.65	108.94	116.28	121.78	107.07	114.41	126.80	108.70	117.74
488.5	104.47	90.39	98.67	106.36	92.31	100.58	106.93	92.86	101.14	119.31	105.24	113.51	124.86	106.74	117.40
514.0	108.98	94.23	103.98	111.61	96.88	106.62	112.32	97.57	107.33	114.98	100.24	109.99	117.88	100.28	111.92

time. In experiment one, four manometers were read throughout the study. For experiment two, nine manometers were read initially, but as flasks were removed for other analyses, the number gradually decreased until four remained at the termination of the experiment. The individual O_2 uptake curves from which the average curve in Figures 2 and 4 were constructed are given in Figures 3 and 5, respectively. In Figures 2 through 5, two time scales are employed. The first twenty hours in general encompassed the purification or substrate removal phase and it was desirable to plot data obtained during this period on an expanded scale. The experiments were of such long duration that this scale could not be employed throughout the experimental period. The break in the scale causes an apparent plateau in O_2 uptake, which is really an artifact. The shape of the O_2 uptake curves during the first twenty hours as related by the total or overall course of O_2 uptake is seen by noting the zero time marker on the contracted scale and observing the dotted line curve which connects with the solid curves (see Figures 3 and 5).

In experiment two (see Figures 4 and 5), the substrate removal and "endogenous" phases are in general demarcated by a plateau or discontinuity in O_2 uptake, whereas in experiment one there is no distinct plateau near the end of the substrate removal phase. The existence of the plateau or discontinuity in O_2 uptake, as well as the fact that it is not always manifested, is in accord with findings of Gaudy, Bhatla, and Abu-Niaaj (3). In Figure 5, and to some degree in Figure 3, there is evidence for plateaus other than that which sometimes separates exogenous and "endogenous" metabolism. These other discontinuities in O_2 uptake are not as pronounced as the first, but there can be little

doubt as to their presence.

In experiments one and two, the conditions in each Warburg flask were as identical as was experimentally possible, thus allowing the system to be treated as one batch study. As can be seen from the oxygen uptake curves of Figures 3 and 5, there is a variation in the oxygen uptake of the different Warburg flasks. During the purification phase, this variation is hardly noticeable, but the longer each experiment proceeds, the more variation there appears to be. In experiment one, the maximum variation is 22 mg/l, and in experiment two, the maximum variation is 60 mg/l. These variations represent a range of $\pm$ 2.7 percent, and $\pm$ 8.1 percent from the median of experiments one and two, respectively. These ranges in oxygen uptake are taken into consideration in the determination of the calculated substrate recovery.

Tables I and III are a summary of the analytical parameters for experiments one and two, respectively. The values presented in each table are those used in the determination of the calculated substrate recovery for each system. Also included in these tables are the results of the ammonia-nitrogen and nitrate-nitrogen determinations. It is readily apparent that the concentrations of ammonia-nitrogen stay relatively constant throughout each experiment, with a slight initial decrease (due to uptake during biological solids accumulation) followed by a return to the initial concentration. There was no nitrate-nitrogen in either system at any of the sampling times; thus, the O_2 uptake represented exertion of carbonaceous BOD.

Table II represents the calculated substrate recovery for experiment one. The substrate recovery values were not available for 3.25 hrs or 6.75 hrs due to an error in the solids determination for the

first two samples. Also, the analyses at time 22.5 hours were deemed to be erroneous, since some KOH from the center well had apparently spilled over into the reaction liquor, thus inhibiting metabolism. Table IV represents the calculated substrate recovery for experiment two.

Tables II and IV both show that the calculated substrate recovery was fairly close to 100 percent. However, it should be noted that the early values in each table are somewhat low (9.5-hour sampling in experiment one, Table II, and six-hour sampling in experiment two, Table IV). This is in agreement with previous findings by Gaudy and Englebrecht (18) in their work with acclimated heterogeneous populations grown on a glucose medium. Except for these early balances and those of methods I, II, and III, at twenty-four hours in experiment two (Table IV), there are no gross discrepancies apparent in the calculated substrate recovery as each experiment progresses to completion. The balance at twenty-four hours in experiment two was low for methods I, II, and III, because of the abnormally low biological solids recorded at this sampling time. For each method considered, three separate values for substrate recovery were calculated, corresponding to the high, low, and average oxygen uptake values presented in Tables I and III. A closer look at the average calculated substrate recovery of both experiments reveals a general trend in the order of the balances. While there is no general order noted for the individual calculations for any specific sampling period, overall average percent recoveries for the two experiments indicate a decreasing magnitude, in order, from method V to I. In order, according to decreasing percentage, they are: method V, 108.78; method IV, 106.89; method III, 104.88; method II, 103.28; method I, 95.81. How close the calculated substrate recovery remains to the 100 percent

value varies between the different methods for each sampling period. However, a determination of the percentage of instances the substrate recovery calculated by each method was nearest the 100 percent value reveals a considerable variation between the calculated recoveries. Their arrangement according to percentage is: method IV, 35.3 percent; method I, 20.7 percent; method V, 20.5 percent; method II, 14.7 percent; method III, 8.8 percent.

Methods I through IV have been discussed previously herein. Method V is an energy balance dependent on the measurement of mixed liquor COD. It represents a direct comparison of O_2 uptake with mixed liquor COD removed at any sampling point along the BOD exertion curve. Substrate recovery is calculated as the ratio of accumulated O_2 uptake and Δ COD of the mixed liquor:

$$\text{Substrate Recovery} = \frac{O_2 \text{ uptake}}{\Delta \text{ COD mixed liquor}}$$

In Figures 3 and 5, the plotted points marked with triangles represent the oxygen uptake as determined by subtracting the mixed liquor COD from the initial mixed liquor COD, i.e., Δ COD. These two methods of measuring oxygen uptake do not yield drastically different results and, as can be seen in Tables II and IV, this method of determining substrate recovery is comparable to the other methods utilized herein.

C. Total Oxidation

As can be seen in Figures 2 and 4, the substrate in each system had not been totally oxidized at the termination of the experiments. For experiment one, the range of oxidation achieved was from 74.65 percent to 78.75 percent, with an average of 77.30 percent, as calculated using

the various oxygen uptake curves presented in Figure 3. Experiment two had a range of oxidation from 76.56 percent to 90.00 percent, with an average of 85.54 percent. The limits of this range are represented by the high and low oxygen uptake curves of Figure 5. It should be noted that experiment two, with the larger percentage of oxidation, was of a longer duration than experiment one. Experiment two was allowed to continue for twenty-one days (comparable to a 21-day BOD), while experiment one was terminated at fifteen days. Experiment one was terminated at this time due to the very slight amount of O_2 uptake taking place.

CHAPTER IV

DISCUSSION

The experiments reported herein exhibit characteristic oxygen uptake curves which have been accepted in the field as describing the exertion of biochemical oxygen demand, i.e., in one there was evidence for a plateau in O_2 uptake near the end of the substrate removal phase, and in the other, this plateau was more manifested. However, these systems have an additional feature in the form of a second plateau or discontinuity and a third stage of oxygen uptake which occurred well into the autodigestive phase. This type of discontinuity was found in previous work by Goldstein (19). In one experiment, he found this stage to be quite a bit more prominent than those observed herein, and in another, less prominent. The phenomenon did not occur to the same degree in each experiment, even though all were run in an identical fashion except that the samples of raw sewage used as the initial seed were necessarily different in each experiment. The second plateau and the following increase in oxygen uptake cannot be explained on the basis of a change in the system from carbonaceous oxygen demand to nitrification. If this were the case, nitrate-nitrogen would be present in the system. Analyses for nitrate-nitrogen have shown that it was not present in these systems.

Since it has previously been determined by Bhatla and Gaudy (5)

that the first plateau and second stage oxygen uptake is the result of a change in the system from domination by bacteria to domination by protozoa, a second plateau and third stage oxygen uptake may be the result of still another change in the dominating species of heterotrophic organisms. Just as the bacteria became a food source for the protozoa, they, in turn, may have become the food source for the next step in the food chain, e.g., the crustaceans. The system observed by Goldstein in which there was no or only slight third stage oxygen uptake may be the result of an absence of crustaceans in that particular sewage inoculum. Samples taken during this period were not examined under a microscope, so the foregoing can be considered only a hypothesis with no experimental verification.

Previous work by Gaudy, Bhatla, and Gaudy (15) has dealt with materials and energy balances calculated for short-term experiments. The work presented herein is concerned with balance calculations for long-term experiments. With the experimental conditions used for this work, which ensures a completely closed system, a calculated substrate recovery of 100 percent should be found throughout the length of the experiments. Within acceptable variation, this was found to be the case, and the recovery of the carbon and energy source was observed throughout each experiment, i.e., the substrate removal and "endogenous" phases. The balances determined previously by Goldstein (19) substantiate these findings. An analysis of the average balances obtained by the various methods for these two experiments reveals that while there is an overall trend in the percent recoveries, going down in order from method V to method I, this is not necessarily indicative of the relative accuracy of the various methods. A closer look reveals that

method IV yields recoveries closer to 100 percent a greater percentage of the time than any of the other methods. On this basis, it would appear that method IV is to be preferred. This is in agreement with previous findings by Gaudy, Bhatla, and Gaudy (15). A possible explanation for the somewhat low substrate recovery values calculated in the early stages of the experiments is the presence of slightly volatile materials in the system at this stage of growth. If such were the case, some soluble organic substrate could be lost from the system during vacuum filtration; thus, the recorded filtrate COD may be somewhat low. The loss of these materials could account for the low substrate recovery calculated for this stage of growth. In these experiments, the calculated substrate recovery determined by methods IV and V do not reflect the low values obtained by methods I to III for these initial calculation periods. This could be due to the analyses required by these two methods. Method V does not utilize any analysis requiring filtration, so it would not be affected by the loss of COD due to slightly volatile substances. With method IV, the increase in Δ COD of the filtrate resulting from the lost volatiles could be offset by the corresponding increase in Δ COD of the cells.

If volatiles are indeed present in the system, their loss could be prevented by changing the method of determining "filtrate" COD. Centrifugation will provide a satisfactory means for solids separation without allowing any volatiles to escape (20).

Neither of these experiments resulted in total oxidation, i.e., O_2 uptake equal to the entire amount of substrate initially recorded. On the other hand, not all biological activity had ceased in either system at the termination of the experiments, so if allowed to continue, total

oxidation may have been achieved. Under identical conditions, Goldstein (19) observed 93 percent oxidation in one system, and 97 percent oxidation in another. In both cases, biological activity was still taking place at the termination of the experiments. Thus, while total oxidation in a totally closed system such as the Warburg flask may require a very long time, its occurrence surely appears to be an expectable one. Its occurrence undoubtedly depends upon the ecological balance in a system. It is interesting to note that the O_2 uptake curves for the Warburg flasks did not diverge much during the substrate removal phase but did to a larger degree during the second (and third) phase of O_2 uptake. These latter phases are the ones one would expect to be most dependent upon the ecology of the system, i.e., the proper predator-prey relationship. Even though each flask was seeded from the same sample of acclimated population, slight differences in ecology could ensue and be reflected in the ultimate degree and kinetic course of oxidation. It is interesting to note that greater divergencies in O_2 uptake curves after the plateau than during the substrate removal phase have also been recently observed by Kelly Peil in other studies in our bioengineering laboratory.

CHAPTER V

CONCLUSIONS

Based upon the results of this study, the following general conclusions are drawn:

1. The fundamental concept of aerobic materials and energy balances was determined to be valid throughout the course of these long-term growth studies. The amount of carbon source removed from the system could be accounted for as the sum of cell material and oxygen uptake. In the endogenous or autodigestive phase, the decrease in solids concentration was accompanied by a balancing increase in O_2 uptake.

2. All of the BOD exerted (O_2 uptake) in these studies resulted from the original organic carbon source; there was no evidence that any O_2 uptake was due to nitrification.

CHAPTER VI

SUGGESTIONS FOR FUTURE WORK

1. Further investigation of the causation for the low substrate recovery at the beginning of the substrate removal phase could be useful.

2. Further investigation of the second plateau and third stage in the oxygen uptake curve is needed to determine the cause of this phenomenon. A knowledge of the possible causes should aid in predicting its occurrence.

SELECTED BIBLIOGRAPHY

1. Monod, J., "The Growth of Bacterial Cultures." Annual Review of Microbiology, 3, 371-394 (1949).

2. Gaudy, Anthony F, Jr., and Gaudy, Elizabeth T., "Biological Concepts for Design and Operation of the Activated Sludge Process." Project Report for the Office of Research and Monitoring, Environmental Protection Agency, September (1971).

3. Gaudy, A. F. Jr., Bhatla, M. N., and Abu-Niaaj, F., "Studies on the Occurrence of the Plateau in BOD Exertion." Proceedings, 18th Industrial Waste Conference, Purdue University, Lafayette, Indiana, 183-193 (1963).

4. Bhatla, M. N., and Gaudy, A. F. Jr., "Studies on the Causation of Phasic Oxygen Uptake in High Energy Systems." Journal Water Pollution Control Federation, 38, 1441-1451 (1966).

5. Bhatla, M. N., and Gaudy, A. F. Jr., "Role of Protozoa in the Diphasic Exertion of BOD." Jour. San. Engr. Div. Proc. ASCE, SA3, 63-87 (1965).

6. Forney, C., and Kountz, R. R., "Activated Sludge Total Oxidation Mechanism." Proceedings, 13th Industrial Waste Conference, Purdue University, Lafayette, Indiana, Ext. Ser. 96, 313-320 (1958).

7. Symons, J. M., and McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." Sewage and Industrial Wastes, 30, 874-890 (1958).

8. Kountz, R. R., and Forney, C., "Metabolic Energy Balances in a Total Oxidation Activated Sludge System." Sewage and Industrial Wastes, 31, 819-826 (1959).

9. Washington, D. R., and Symons, J. M., "Volatile Sludge Accumulation in Activated Sludge Systems." Journal Water Pollution Control Federation, 34, 767-790 (1962).

10. Gaudy, A. F. Jr., Ramanathan, M., Yang, P. Y., and DeGeare, T. V., "Studies on the Operational Stability of the Extended Aeration Process." Journal Water Pollution Control Federation, 42, 165-179 (1970).

11. Gaudy, A. F. Jr., Yang, P. Y., and Obayashi, A. W., "Studies on the Total Oxidation of Activated Sludge With and Without Hydrolytic Pretreatment." Journal Water Pollution Control Federation, 43, 40-54 (1971).

12. Yang, Ping-Yi, "Studies on Extended Aeration Activated Sludge and a Modification of the Process Employing Chemical Hydrolysis of Portions of the Return Sludge." PhD Thesis, Oklahoma State University, Stillwater, Oklahoma, May, 1972.

13. Goldstein, Carl Louis, "Studies of Acclimated Microbial Populations Grown on Acetic Acid. I. Substrate Removal and Endogenous Phases. II. The Effect of Substrate Concentration on Exponential Growth Rate Constant." Masters Thesis, Oklahoma State University, Stillwater, Oklahoma, May, 1972.

14. Obayashi, Alan W., and Gaudy, Anthony F. Jr., "Aerobic Digestion of Extracellular Microbial Polysaccharides." Proceedings, 27th Industrial Waste Conference, Purdue University, Lafayette, Indiana, May 2-4 (1972).

15. Gaudy, A. F. Jr., Bhatla, M. N., and Gaudy, E. T., "Use of Chemical Oxygen Demand Values of Bacterial Cells in Waste Water Purification." Applied Microbiology, 12, 254-260 (1964).

16. Standard Methods for the Examination of Water and Wastewater (13th ed.), American Public Health Association, New York (1971).

17. Ecker, R. E., and Lockhart, W. R., "Specific Effect of Limiting Nutrient on Physiological Events During Culture Growth." Journal of Bacteriology, 82, 511-516 (1961).

18. Gaudy, A. F. Jr., and Englebrecht, R. S., "Basic Biochemical Considerations During Metabolism in Growing vs. Respiring Systems." In Advances in Biological Waste Treatment, 11, Pergamon Press, New York (1963).

19. Goldstein, Carl L., Unpublished Data. OSU, Stillwater, OK.

20. Gaudy, A. F. Jr., Turner, B. G., and Pusztaszeri, S., "Biological Treatment of Volatile Waste Components." Journal Water Pollution Control Federation, 35, 75-93 (1963).

VITA

Paul Allan Perkins

Candidate for the Degree of

Master of Science

Thesis: STUDIES ON AEROBIC MATERIALS AND ENERGY BALANCES DURING GROWTH AND ENDOGENOUS METABOLISM PHASES OF NATURAL MICROBIAL POPULATIONS OF SEWAGE ORIGIN

Major Field: Bioenvironmental Engineering

Biographical:

Personal Data: Born September 6, 1948, in Los Angeles, California, the son of Mr. and Mrs. Clifford M. Perkins.

Education: Graduated from Downey Senior High School, Downey, California, in June, 1966; received the degree of Bachelor of Science in Renewable Natural Resources and Soil and Water Science from the University of California, Davis, in June, 1971; completed requirements for the Master of Science degree at Oklahoma State University, Stillwater, in July, 1972.

Professional Experience: Bioenvironmental engineer trainee, September, 1971 - July, 1972.