LIGHT AND DARK MICROBIAL RESPONSE IN SEMI-QUIESCENT WATERS

By

JOHN THOMAS SOLOOK

Bachelor of Science

University of Missouri at Rolla

Rolla, Missouri

1966

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
May, 1968

LIGHT AND DARK MICROBIAL RESPONSE IN SEMI-QUIESCENT WATERS

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. D. F. Kincannon for his invaluable advice and guidance, without which this work would not have been possible.

The author also wishes to express his indebtedness to Dr. A. F. Gaudy, Jr. and Dr. R. A. Mill for their careful reading of the manuscript and valuable suggestions.

The author wishes to express his sincere gratitude to Mrs. Grayce Wynd for her careful and accurate typing of the manuscript.

Lastly, the author wishes to express his most sincere appreciation to his wife, Gail, for her continuing sacrifice and encouragement during this study.

This work was supported by a Research Grant, Project WR-11, "Oxygen Diffusion," sponsored by the Oklahoma Water Resources Research Institution.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The stabilization of organic matter in semi-quiescent bodies of water or oxidation ponds undoubtedly has occurred in nature since the beginning of life on the earth. However, the waste purification potential was not realized until the beginning of the twentieth century. Today, in many parts of the world, these ponds provide a simple and effective means of biological treatment of sewage or waste water containing organic matter.

The oxidation pond is a shallow, earthen basin into which sewage or other liquid waste is discharged and in which natural purification processes take place under proper climatic conditions. The biological principles involved are more complex than those of other conventional treatment processes, since successful operation depends upon heterotrophic (bacteria, protozoa and fungi) and autotrophic (mostly algae) organisms as well as radiant energy. A definite symbiotic relationship exists between these two forms of primitive life. The heterotrophs metabolize the organic matter aerobically releasing various end products such as carbon dioxide, ammonia, water and other inorganic

compounds; the autotrophs utilize these end products for the production of organic material in the form of new cells or excreted organic wastes and liberate oxygen which is then available for continued bacterial oxidation.

Although there has been extensive research dealing with the effects of the different parameters involved in algae growth, such as light, pH, temperature, nutrients, etc., very little data is available concerning organic substrate utilization by algae in the dark.

The purpose of this study was to gain a more complete understanding of algae response to an organic substrate not only in a light-dark cycle, but also in constant darkness. Controlled laboratory experiments were conducted in batch systems with both heterogeneous microbial populations and pure cultures of algae. The relationships among dissolved oxygen concentration, substrate removal, oxidation-reduction potential, oxygen uptake and pH were investigated.

CHAPTER II

LITERATURE REVIEW

The value of sewage purification in ponds was accidentally discovered in the United States at Santa Rosa, California, in 1929, in ponds which resulted from the clogging of a prepared gravel seepage area. In that same year, the California State Bureau of Sanitary Engineering conducted studies on the efficiency of pond purification and as a result recommended the oxidation pond system as an effective means of waste treatment (1). The first operational and design data for oxidation ponds was presented by Texas Agricultural and Mechanical College in 1929 (2).

The merit of oxidation ponds for sewage treatment gained recognition slowly in this country. It was not until the early years of World War II that the construction of oxidation ponds increased ten-fold because of the need to alleviate the overloaded conditions at treatment facilities of the military installations and the surrounding communities. In the years following, construction of oxidation ponds for sewage treatment increased rapidly throughout the United States. In 1959, the results of a survey conducted on the status of oxidation ponds in this country indicated

over 650 cities used this means of treatment (3). However, this survey did not include industry, which was continually adapting the oxidation pond system for treatment of its wastes. A Public Health Service report in 1962 revealed that thirty-one different industries used oxidation ponds as a means of treatment (4).

Although the oxidation pond has gained recognition as being a simple and effective means of biological waste treatment, the effects of the numerous environmental factors such as light, dissolved oxygen concentration and pH, on the biochemical dynamics occurring in an oxidation pond remain to be uncovered.

Light or radiant energy is one of the most important environmental factors affecting the biochemical dynamics in an oxidation pond, since algal growth depends upon photosynthesis. There is now abundant evidence that photosynthesis requires cooperative interaction of two photochemically active systems acting in series (5--9). The two systems include various pigments such as chlorophyll a, chlorophyll b, and the carotenoids, which are present in most photosynthetic algae. The activation of these systems by light results in the evolution of oxygen, and the production of ATP and NADPH, which are necessary for photosynthetic carbon assimilation. Although light is essential for photosynthesis and algal growth, it has been reported that the rate of photosynthesis or oxygen production is dependent upon the light intensity as well as the amount of

algae present (10). Lubbers, et al. investigated mixed algal cultures from oxidation ponds and found that the rate of oxygen production increased as a logarithmic function of light intensity up to approximately 720 ft candles and then rapidly decreased (11). Oswald, et al. also reported similar findings using a pure culture of Euglena gracilis, and attributed the decrease to chlorophyll breakdown. In addition, they found that as the age of the algae increased, the rate of oxygen production decreased (12). In experiments with Chlorella, it was concluded by Kutyurin that the rate of oxygen production was independent of the concentration of oxygen in the water, but that it was dependent upon the light intensity as well as the physiological condition of the algae (13).

The variation of the dissolved oxygen content in an oxidation pond encompasses a broad spectrum, ranging from values far above saturation during the day when photosynthesis is maximum to values near or at zero during the night. This variation or diurnal cycle is affected by climatic conditions as well as the amount of organic material present (14, 15). Wu (16) has shown in a laboratory scale oxidation pond that for organic loadings greater than 400 mg/l there was no evident recovery from anaerobic conditions at the end of a seven-day period. The effect of prolonged anaerobiosis in an oxidation pond will reduce the algal population, because the end products of bacterial fermentation, such as organic acids and ethanol, have been

shown to be toxic to most algae (17). In addition, it has been reported that certain species of Chlorella and Scenedesmus produce similar products from various hexoses under anaerobic conditions (18).

Another factor affecting the biochemical dynamics of an oxidation pond is pH. It has been reported that a pH gradient exists from the influent to the effluent end of an oxidation pond (19, 20). This gradient can be explained by the fact that in the absence of free carbon dioxide the algae present utilize carbon dioxide from soluble bicarbonates which results in an increase of the hydroxyl ion and therefore increases the pH. Rohlich and Fitzgerald (21) reported that it was not uncommon for the pH to reach values as high as 10.0 to 11.0 during the day in an oxidation pond. Nielsen (22) cultured Chlorella pyrenoidosa in a media with pH values in the range of 3.0 to 11.0 with no apparent inhibition of photosynthesis or respiration. In contrast, Oswald (23) found that the light conversion efficiency of algae grown in laboratory cultures on sewage decreases as an approximately linear function of the duration of time that the culture has a pH greater than 8.0. However, he attributed this to the limited amount of carbon dioxide available because bacterial oxidation was inhibited.

Pipes (24) studied the following two methods of pH control in laboratory scale oxidation ponds which received a synthetic sewage medium; 1) adjusting the influent pH of the sewage medium, and 2) loading the ponds only during the

daylight hours. He found that when the pH of the influent waste was reduced to values between 6.0 and 7.5, the pH of the pond was prevented from reaching extremely high values during the daylight. He also noted that there was an increase in the precentage BOD removal in the oxidation ponds having a detention time of five days or less when the pH of the influent waste was between 6.0 and 7.5. However, for oxidation ponds having a detention time greater than twenty days and receiving a waste with pH values ranging from 6.0 to 9.0, he found no significant difference in the percentage BOD removal. In regard to pH control by loading the ponds only during the daylight, he concluded that it did not appear to be a practical method of decreasing the maximum daytime pH of the ponds.

In the preceding paragraphs it has been shown that the pH, the amount of incident light intensity, and the dissolved oxygen content influence the general symbiotic relationship between the biological phases present in an oxidation pond.

The general symbiotic relationship between the two existing biological phases, the bacterial phase and the algal phase, in an oxidation pond has received extensive study and results have shown it to be similar to that presented in Figure 1 (25-30). However, if the biochemical processes outlined in Figure 1 are absolutely true, the algae would not benefit from any of the preformed organic compounds for their metabolism, i.e., heterotrophic growth.

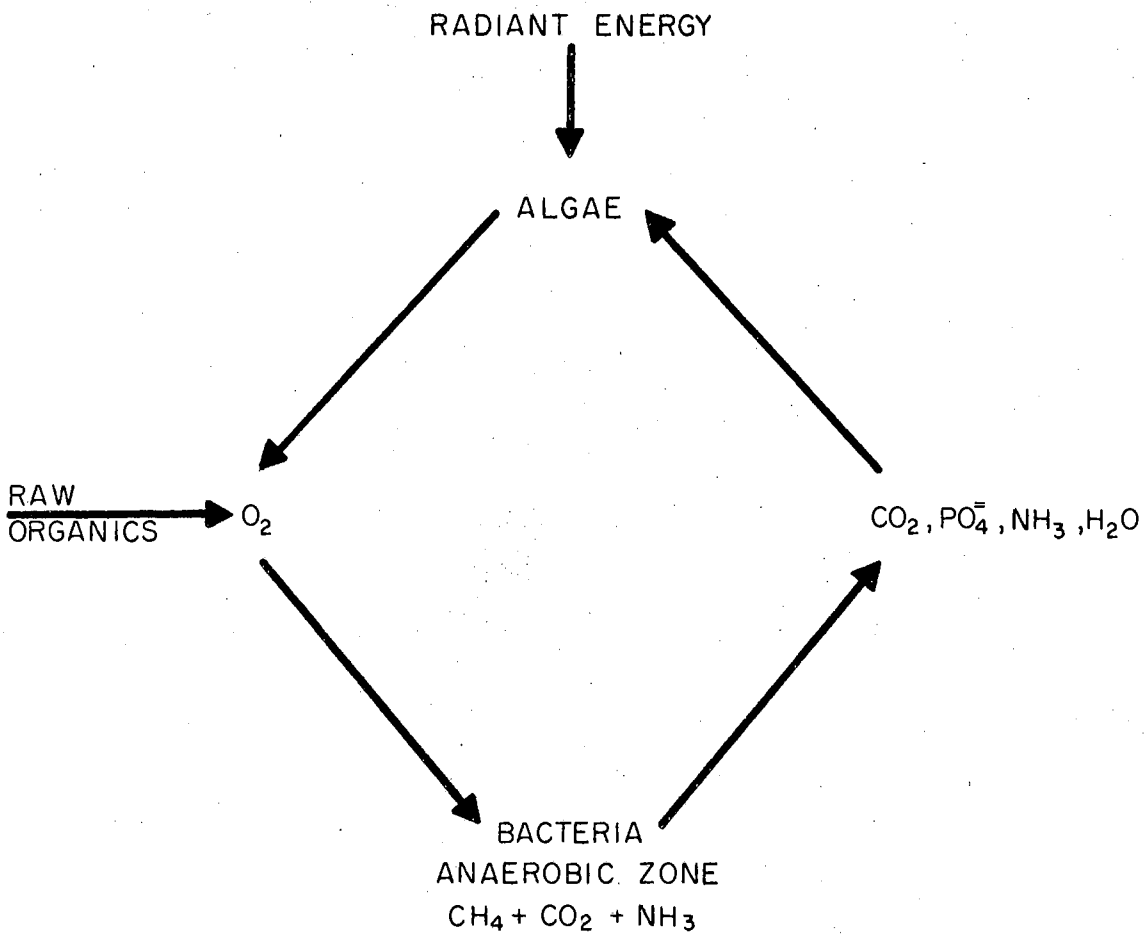

Figure 1. The general symbiotic relationship between the two existing biological phases in an oxidation pond.

It has been reported in the literature that algae, namely Chlorella, Euglena, Chlamydomonas, and Scenedesmus, which often predominate in oxidation ponds, are able to utilize a variety of organic compounds for growth in the presence of light (31-37). Gotaas, et al. (38) found that Chlorella pyrenoidosa was capable of growing on sterile sewage, but when the detention time was greater than three days he found a significant increase in the dissolved volatile solids and in the soluble BOD, therefore indicating secretion of organic substances by the algae. However, Merz, et al. (39) concluded that the effect of excretion of extracellular, soluble organic matter by algae on the efficiency of an oxidation pond is not likely to be significant, because the bacteria present will readily oxidize most of the excreted matter. In contrast, Allen (40) reported that algae were incapable of reducing the organic content of sewage in the light as well as in the dark.

Heterotrophic growth in the dark is one of the most puzzling characteristics concerning the algae. Although many different mechanisms have been suggested, there seem to be just as many mechanisms as algae species investigated, therefore the reasons why and how some algae exhibit this characteristic still remain to be explained.

In studies with forty-four species from eight different genera of Chlorococcales, Parker, et al. (41) found that only twenty-four species were capable of growing on a glucose or acetate salt media in the dark. He noted that there

was clearly no perfect correlation between facultative heterotrophic abilities and taxonomic relationships in this family. The inability of various other algae species to grow heterotrophically in the dark has also been found (42, 43).

In contrast, Pearsall and Bengry (44) found that Chlorella sp. growing in the dark in a glucose medium showed a growth cycle consisting of the following four stages:

1. An exponential phase (1-8 days) when cell number is approximately doubled daily and relative dry weight of the cell falls.

2. A linear increase in growth in number and dry weight for six days.

3. The rate of number-increase falls and from 14 to 40 days there was a period in which the daily rates of number or dry weight increase are constant and the relative weight per cell increases.

4. After 40 days a small increase in cell weight was found.

They noted that only one-fifth of the glucose supplied (500 mg/50 ml) was used by day 14, and after 40 days only one-half of that supplied was absorbed.

Avilov (45) investigated the response of 15 algae strains of the genus Chlorella to various carbohydrates and organic acids in the dark. It was shown that the best growth of most algae was on glucose and galactose, while fructose, mannose and acetate provided good growth only in

some strains. The organic acids such as butyric, lactic, malic and citric, had no nutritional value to any of the strains tested. The most significant increase in cell mass was detected after a fourteen-day lag period.

Myers (32) has shown that Chlorella pyrenoidosa grew well in the dark on added glucose, and that its pigment system remained intact. However, with a culture of Euglena gracilis, he noted a sluggish response to added substrates in the dark and there was notable loss in the chlorophyll content (46).

It has been reported in the literature that the addition of an organic substrate to unstarved algal cells may cause a two or three-fold increase in the rate of respiration, whereas starved cells exhibit at least an eight-fold increase (47). Although the rate of respiration increases after the addition of an organic substrate, the question arises as to whether the endogenous respiratory substrate is continually being used or whether it is somehow suppressed by the exogenous substrate, which then serves as the respiratory substrate. Since evidence has been presented supporting both theories, the effect of an external oxidizable substrate on the rate of respiration still remains unresolved.

In general, there are numerous factors influencing the population mass of an oxidation pond. Although there has been a multitude of reports on the effects of the various factors, much information concerning the basic biological

relationships as well as the engineering design features for an oxidation pond remains to be uncovered. In the present experimental research, an attempt was made to gain a better understanding of the basic biological relationships when the system was subjected to complete darkness.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Apparatus

1. Batch Unit for Light-dark Studies

The experimental pond used throughout these studies was constructed of plate glass with the following geometric specifications:

Width	28.5 cm
Length	48.6 cm
Depth	27.2 cm
Total surface area	1387 cm^2
Total volume	36 liters

Illumination was provided by three gro lux lamps (F15t8-GRO, Sylvania) suspended longitudinally across the pond at a distance of $2\frac{1}{2}$ inches above the water surface to give a constant incident light intensity of 425 candles.

2. Batch Unit for Dark Studies

The reaction vessel used was a 20-liter pyrex carboy which had a sampling port at the 10-liter mark. To ensure homogeneity of the microbial population, a Lightnin mixer was employed. Throughout these experiments the reaction

vessel was in a rectangular closed cabinet to eliminate all light.

B. Microbial Populations

1. Heterogeneous Bacterial Seed

The heterogeneous bacterial seed used in the light-dark studies was obtained from the effluent of the primary clarifier at the municipal waste treatment plant at Stillwater, Oklahoma. However, in the dark studies, the only bacteria present were those occurring as natural contaminants in the system.

2. Algal Seed

The algal seed used throughout these studies was a mixed algal culture with Chlorella as the predominating genus. A stock algal population was maintained to assure a new and healthy algal seed at the beginning of each experiment. The pure culture, Chlorella pyrenoidosa (#7516), used in this study was obtained from the American Type Collection, Washington, D. C. The reasons for using a strain of Chlorella in these experiments were the following: 1) Chlorella has been extensively used in studies of the mass culture of algae on inorganic media, and 2) Chlorella often is the predominating algal group in oxidation ponds.

C. Growth Media

The chemical compositions of the media used in this study are shown in Tables I and II. As noted in Table II,

the glucose concentration varied from experiment to experiment.

TABLE I

SYNTHETIC ALGAL GROWTH MEDIUM (48)

Constituent	Concentration
NH_4Cl	1000 mg/l
$MgSO_4 \cdot 7H_2O$	200 mg/l
$FeSO_4 \cdot 7H_2O$	50 mg/l
$CaCl_2$	20 mg/l
$MnCl_2 \cdot 4H_2O$	2 mg/l
$Na_2MoO_2 \cdot 2H_2O$	1 mg/l
K_2HPO_4	1000 mg/l
$NaHCO_3$	100 mg/l

TABLE II

COMPOSITION OF MINIMAL MEDIUM

Constituent	Concentration
*Glucose	200 mg/l
$(NH_4)_2SO_4$	250 mg/l
$MgSO_4 \cdot 7H_2O$	100 mg/l
$MnSO_4 \cdot H_2O$	10 mg/l
$CaCl_2$	7.5 mg/l
$FeCl_3 \cdot 6H_2O$	0.5 mg/l
Tap Water	100 ml/l
1 M Phosphate Buffer	20 ml/l

*Concentration varied from experiment to experiment

D. Analytical Procedures

Dissolved oxygen concentration was determined by the Alsterberg (Azide) Modification of the Winkler Method, as described in Standard Methods (49).

Biological solids concentration was determined using the membrane filter technique (Millipore Filter Corp., HA, 0.45 mμ) as outlined in Standard Methods (49).

The oxidation-reduction potential (ORP) and pH were monitored throughout all studies using the Beckman Expanded Scale pH Meter (Model 76) in accordance with the procedure outlined in the Beckman Operating and Maintenance Instruction Manual (50).

Filtrate COD was determined in accordance with the procedures given in Standard Methods (49). Filtrate carbohydrate (anthrone) was measured according to the procedure outlined by Gaudy (51).

Oxygen uptake was determined using a Warburg respirometer operating at a shaker rate of 100 oscillations per minute and constant temperature of 25^{o}C. A black polyethylene sheet was placed over the Warburg respirometer during all experiments to ensure that the incident light intensity at the water surface was zero.

In a few experiments the viable bacterial counts were obtained by the spread plate surface counting technique, and this is noted in the protocol for the individual experiments.

The method outlined by Gloyna and Thirumurthi (17) was

employed to check for bacterial contamination during the pure culture experiments, as well as the spread plate surface counting technique.

E. Experimental Protocol

The types of experiments investigated in this study may be placed into the following categories:

1. Batch Studies on the Effects of Various Organic Loadings on the Experimental Oxidation Pond

In these studies the recovery of the dissolved oxygen content in the oxidation pond from various organic loadings was investigated. The medium composition of the pond used throughout these experiments is shown in Table III.

TABLE III

COMPOSITION OF EXPERIMENTAL POND

Sodium Bicarbonate (30 gm/l)	100 ml
Bacterial Seed	100 ml
Algal Seed	800 ml
Algal Growth Medium	550 ml
Glucose Minimal Medium*	3,904 ml
Distilled Water	26,546 ml

*Glucose not included

In all studies an abundant algal growth was allowed to develop before the organic substrate and bacterial seed were added and the experiment begun. During all experiments a

lighting period of twelve hours on and twelve hours off was employed. The light intensity at the water surface was 425 ft candles, and the temperature was maintained at $24^{o}C \pm 1^{o}$. Samples were siphoned from mid-depth of the experimental pond just prior to turning the lights on and off. Dissolved oxygen concentration, biological solids, pH, oxidation-reduction potential, filtrate COD, and carbohydrate content were determined throughout the studies.

2. Response of Heterogeneous Microbial Populations to Organic Loadings in the Dark

In these experiments the following three systems were investigated: 1) open system without mixing, 2) open system with mixing, and 3) closed system with mixing. The synthetic medium used in all cases is given in Table IV.

TABLE IV

COMPOSITION OF BATCH UNIT USED FOR DARK STUDIES

Algal Growth Medium	340 ml
Algal Seed	1,000 ml
Tap Water	2,000 ml
Distilled Water	16,630 ml
Glucose	30 ml

Once an abundant algal growth developed, the organic substrate was added and the reaction vessel was placed in the dark. Samples were taken at various times throughout the experiments, and the following parameters were investigated:

dissolved oxygen concentration, pH, oxidation-reduction potential (ORP), biological solids, oxygen uptake, viable bacteria counts, filtrate COD, and carbohydrate (anthrone) content.

3. Pure Culture Response to Organic Loadings in the Dark Using the Warburg Respirometer

The pure culture, Chlorella pyrenoidosa (#7516) was used in all experiments. The culture was photosynthetically grown in shaker-flasks at ninety strokes per minute on the synthetic growth medium shown in Table I. The experiments were started by aseptically adding the required amount of culture and organic substrate to the reaction vessels (125 ml capacity). The reaction vessels were then placed on the Warburg respirometer, which was operated at 100 oscillations per minute and at a constant temperature of 25°C. Periodically two reaction vessels were removed for analyses of pH, biological solids, and filtrate COD and carbohydrate (anthrone) concentrations.

CHAPTER IV

RESULTS

1. Batch Studies on the Effects of Various Organic Loadings on the Experimental Oxidation Pond in a Light-dark Cycle

For these studies the results of organic loadings of 100-150-200 mg/l glucose on the recovery of the dissolved oxygen concentration in the experimental oxidation pond are shown in Figures 2 through 4. The composition of the experimental pond used throughout was that given in Table III.

Although the systems which received organic loads of 100 mg/l and 150 mg/l glucose, Figures 2 and 3 respectively, exhibited an initial supersaturated DO content; the DO removal rates were very similar and in approximately twenty-three hours the DO content was zero. The similarity of the DO removal rates indicates that the rate was independent of the applied organic loading. In contrast, the system receiving 200 mg/l glucose (Figure 4) exhibited a much slower rate. This decrease in rate may be attributed to the low initial DO concentration of the system, which was attained by aeration with nitrogen prior to the addition of the organic substrate and bacterial seed.

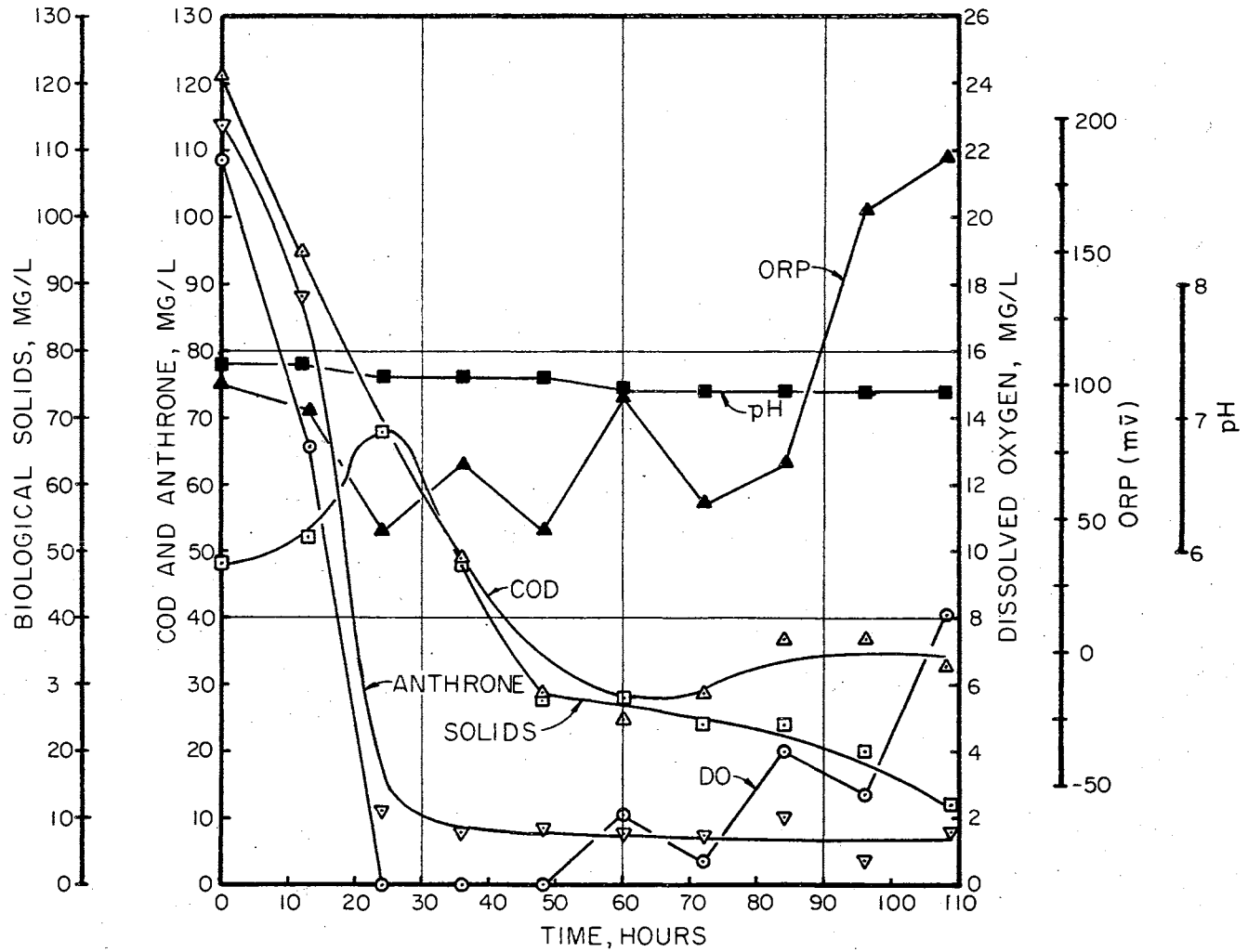

Figure 2. Biological response in semi-quiescent waters to glucose loading of 100 mg/l; lighting conditions, 12 hours on, 12 hours off.

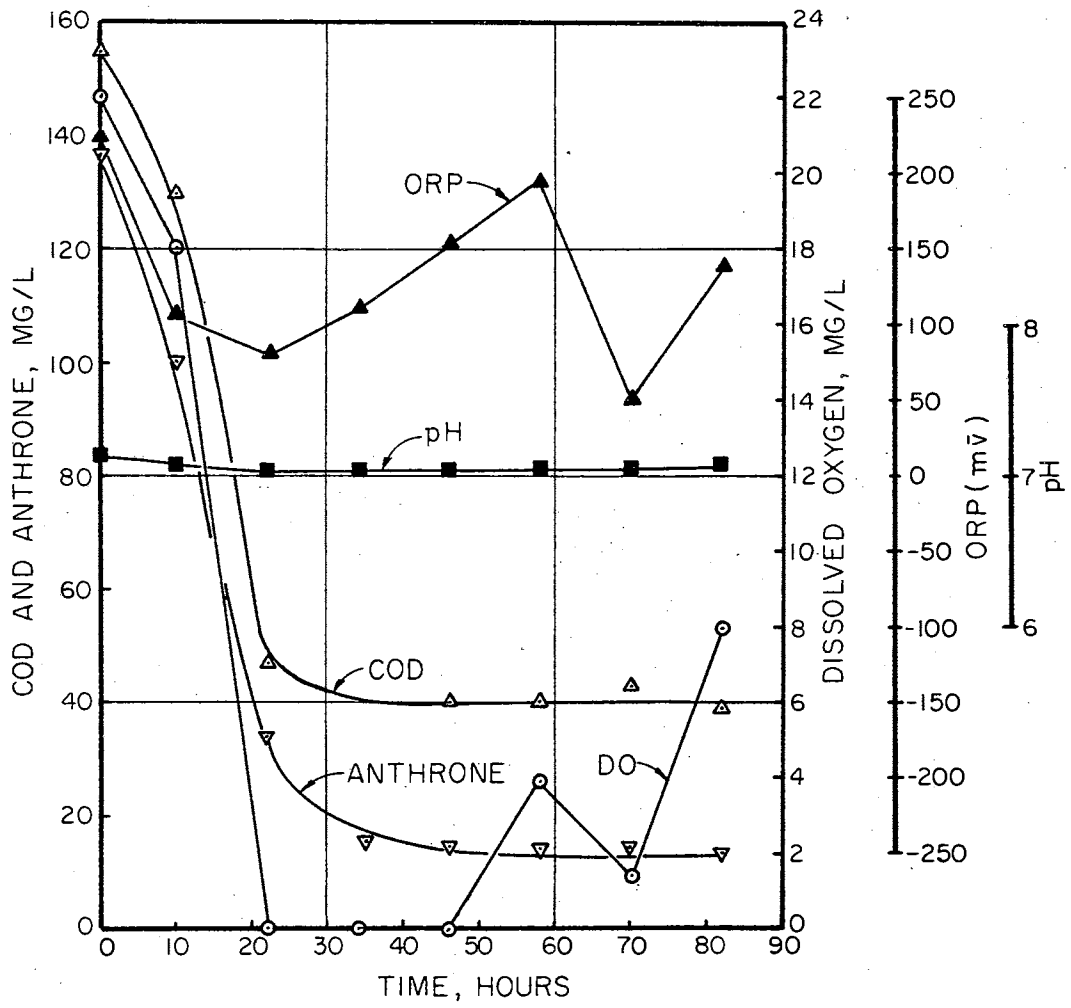

Figure 3. Biological response in semi-quiescent waters to glucose loading of 150 mg/l; lighting conditions, 12 hours on, 12 hours off.

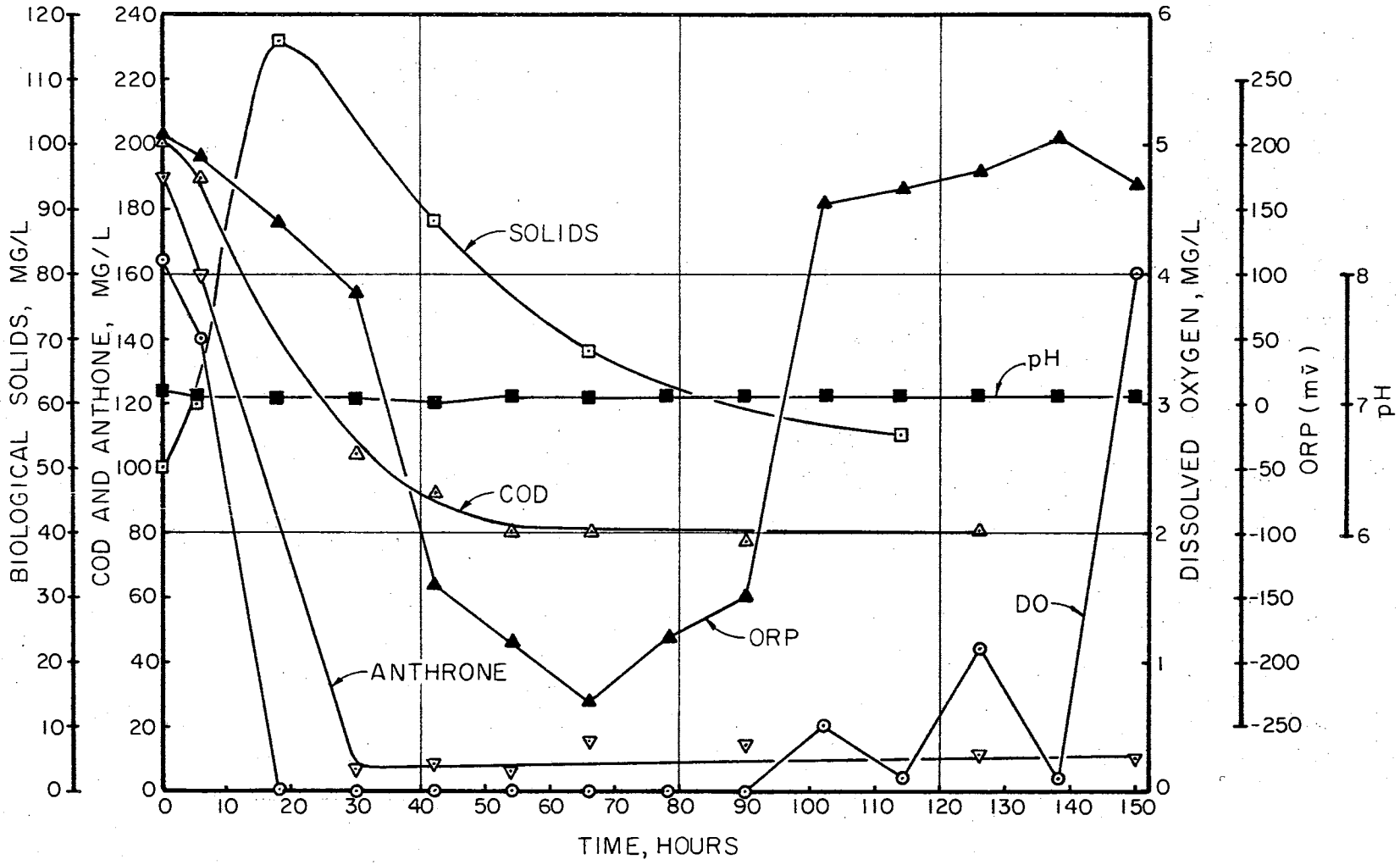

Figure 4. Biological response in semi-quiescent waters to glucose loading of 200 mg/l; lighting conditions, 12 hours on, 12 hours off.

However, it can be seen that the DO recovery rate, which was due to photosynthesis and physical reaeration, decreased with increasing organic loading. In all cases, once the photosynthetic oxygen production was greater than the oxygen consumption of the microbial population, the DO concentration increased in a stepwise fashion corresponding to a maximum gain during daylight and a minimum loss during dark hours.

The rate of glucose and COD removal for all three organic loadings were very similar. However, the amount of metabolic intermediates produced in each system increased as the amount of glucose was increased. As might be expected, the biological solids concentrations in the systems receiving 100 mg/l (Figure 2) and 200 mg/l (Figure 4) glucose were maximum when most or all of the applied loading was removed. It is interesting to note that the oxidation-reduction potentials (ORP) for the 100 mg/l and 150 mg/l systems never attained negative values when the DO content was zero, i.e., under anaerobic conditions. In contrast, the ORP for the 200 mg/l system was very negative under anaerobic conditions. This noted difference may be attributed to the fact that anaerobic conditions persisted longer in the 200 mg/l system than in either the 100 mg/l or 150 mg/l system. As shown in Figures 2 through 4, there was a small decrease in pH during active substrate removal.

2. Response of Heterogeneous Microbial Populations to Organic Loading in the Dark

In this set of experiments, the following types of systems were studied: 1) open system without mixing, 2) open system with mixing, and 3) closed system with mixing. The composition of the medium used throughout was that shown in Table IV.

a. Open System Without Mixing

Figure 5 shows the response of the system to 100 mg/l glucose in the dark. Although the initial DO concentration was extremely high, there was a continual reduction of DO during the experiment. The glucose and COD removal curves indicate an initial lag period followed by complete removal of all organic material. The maximum biological solids concentration occurred approximately fifteen hours after complete substrate removal. Initially, the ORP was continually changing, i.e., dynamic, but as the activity of the system decreased, the ORP attained a fairly stable negative potential. Although this system was not buffered, the initial pH of 8.0 did not seem to affect the system; the pH exhibited a decreasing trend throughout the experiment.

The response of the system in the dark, which was loaded with 200 mg/l glucose, is shown in Figure 6. There are two notable differences between this system and the system previously mentioned. First, the initial DO concentration in the 200 mg/l system was reduced by aeration with nitrogen for five minutes; after shutting the nitrogen off,

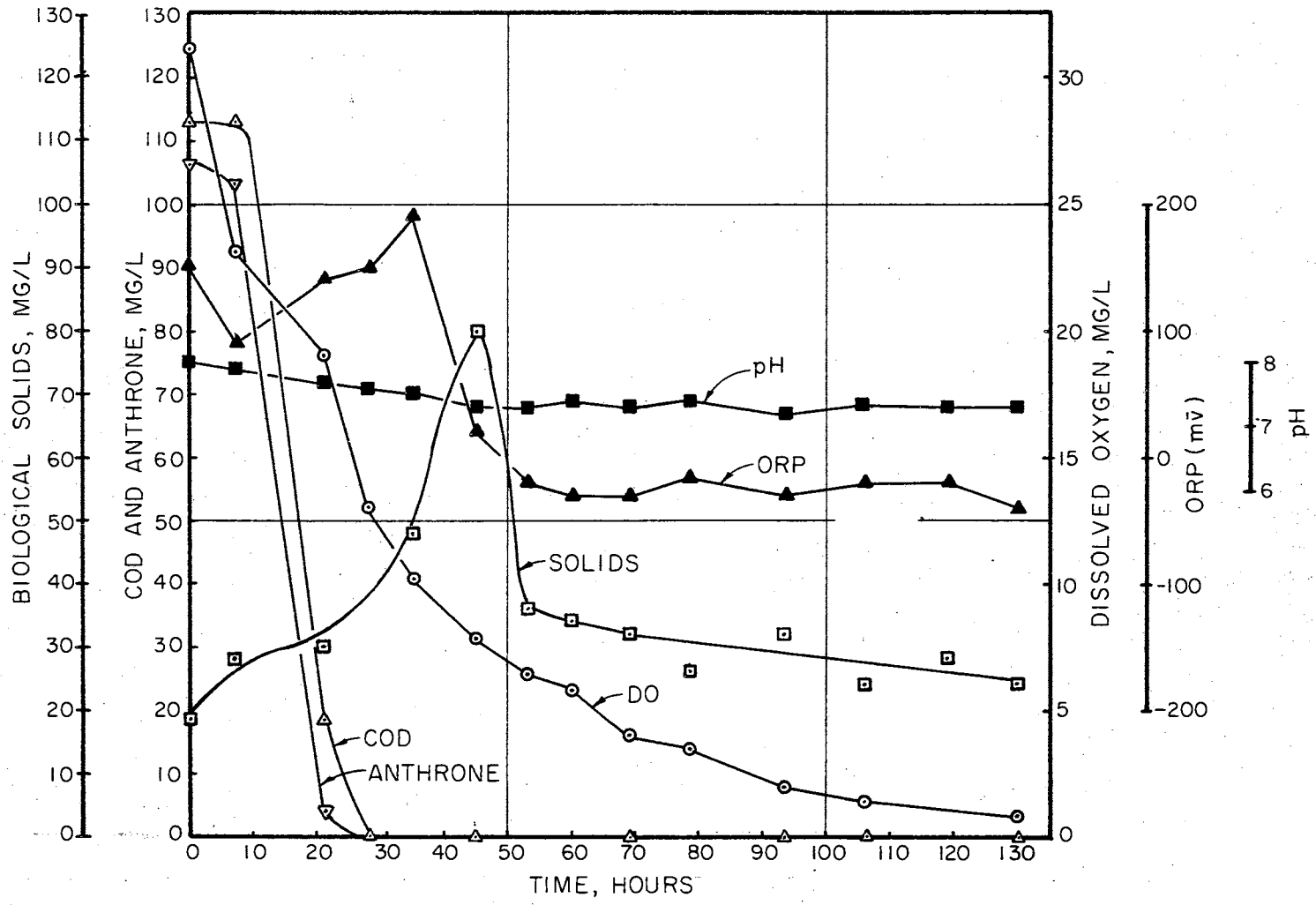

Figure 5. Biological response in semi-quiescent waters to glucose loading of 100 mg/l; lighting conditions, 24 hours darkness.

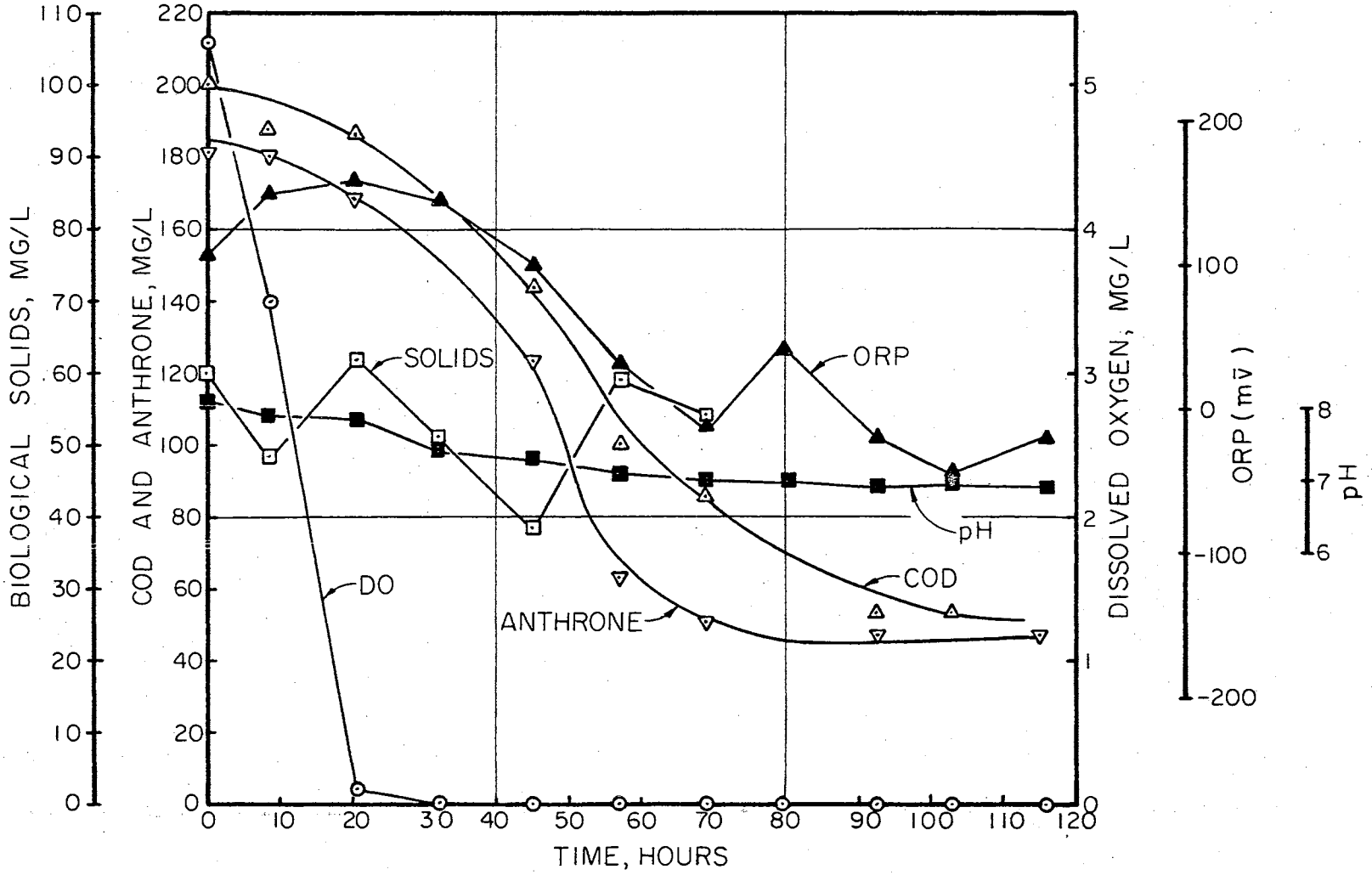

Figure 6. Biological response in semi-quiescent waters to glucose loading of 200 mg/l; lighting conditions, 24 hours darkness.

the organic load was added. The system was aerated with nitrogen to keep it from having an initial supersaturated DO concentration, as shown in the 100 mg/l system (Figure 5). The DO concentration in the 200 mg/l system was reduced to zero within thirty hours, and remained at zero throughout the experiment. Secondly, the initial biological solids concentration in the 200 mg/l system was three times greater than in the 100 mg/l system. The glucose and COD removal curves for the system which received 200 mg/l glucose (Figure 6) were strikingly different than that in the 100 mg/l system (Figure 5). Although both systems exhibited a short lag period, the rate of removal in the 100 mg/l system was approximately three times as fast as in the 200 mg/l system. This significant difference in removal rate may have been due to the fact that the 200 mg/l system was subjected to anaerobic conditions within twenty hours, while the 100 mg/l system remained aerobic throughout the experiment. The changes which occurred in the ORP and pH during the experiment were similar to those previously reported in Figure 5. The biological solids concentration for the 200 mg/l system exhibited continual variation during the experiment, which may have been due to continual sedimentation of the microbial population, namely, the algal population.

b. Open System with Mechanical Mixing

The responses of the system to organic loadings of 100 mg/l and 150 mg/l glucose are shown in Figures 7 through

10. In both systems there was a general decreasing trend in the DO concentration, until the major portion of the glucose was utilized, then an increasing trend in the DO concentration was seen due to reaeration by mixing. The glucose and COD removal curves for both systems again showed an initial lag period followed by a slow removal, similar to that previously reported for the 200 mg/l system (Figure 6).

A comparison between the 100 mg/l (Figure 7) and 150 mg/l (Figure 8) systems shows that the removal rate was faster in the 150 mg/l system than in the 100 mg/l system, hence the 150 mg/l system was biologically more reactive. The biological solids concentrations for both systems (Figures 7 and 8) are maximum at the point when all or most of the glucose was removed. Also, the ORP values for both systems indicate the following: 1) a positive potential throughout, which is indicative of an aerobic system, and 2) a dynamic potential, indicating biological activity. The pH decreased throughout both experiments in spite of the high initial pH of 10.5.

Figures 9 and 10 show the oxygen uptake for these two systems. In both cases the flasks from zero time of the experiment exhibited a lag period, whereas there was no lag period for the flasks added during the experiments, and the oxygen uptake rates for the added flasks correlated very well to flask rates from zero time for the respective systems. The oxygen uptake rates per gram of solids for

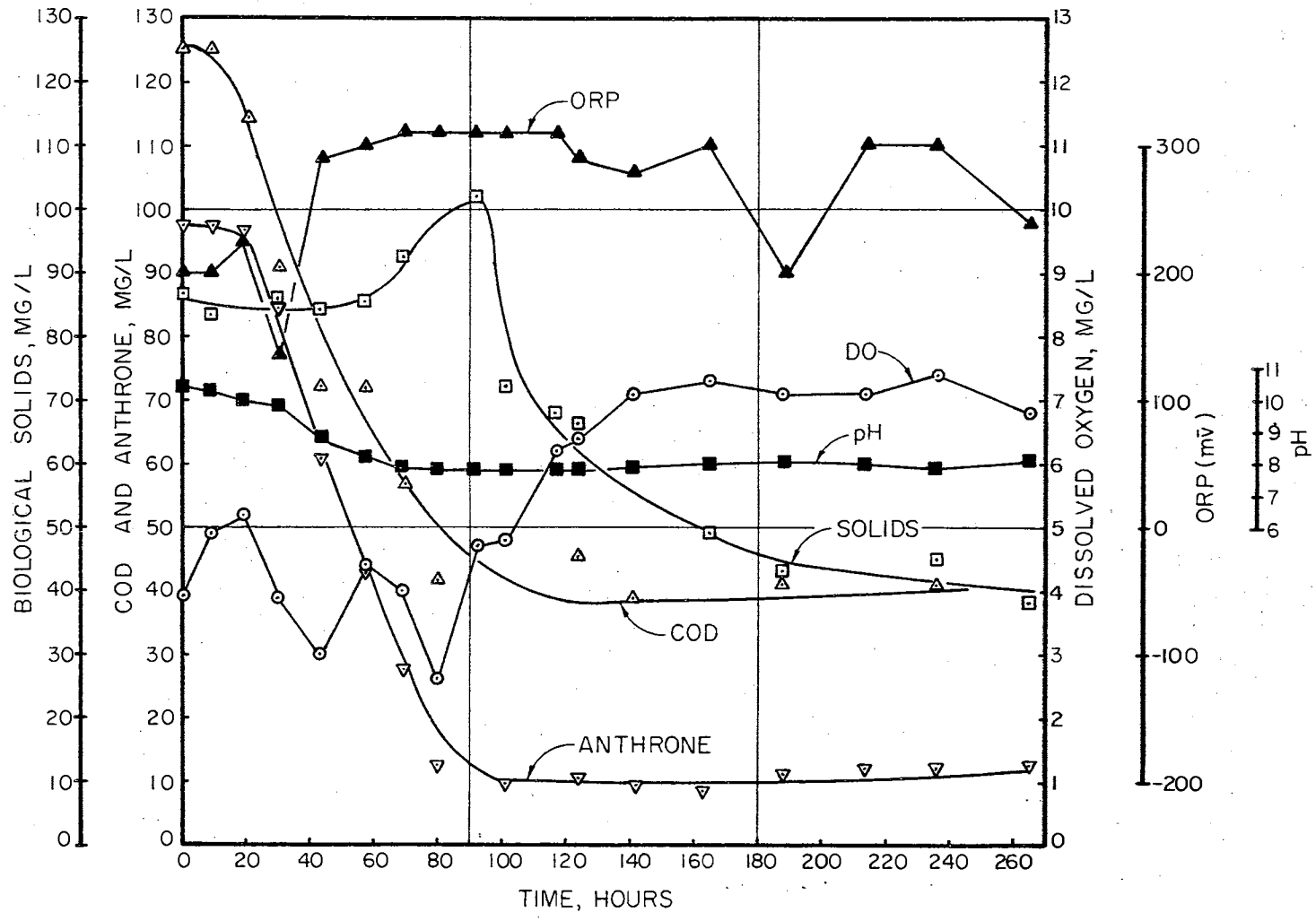

Figure 7. Biological response in semi-quiescent waters to glucose loading of 100 mg/l, with mixing; lighting conditions, 24 hours darkness.

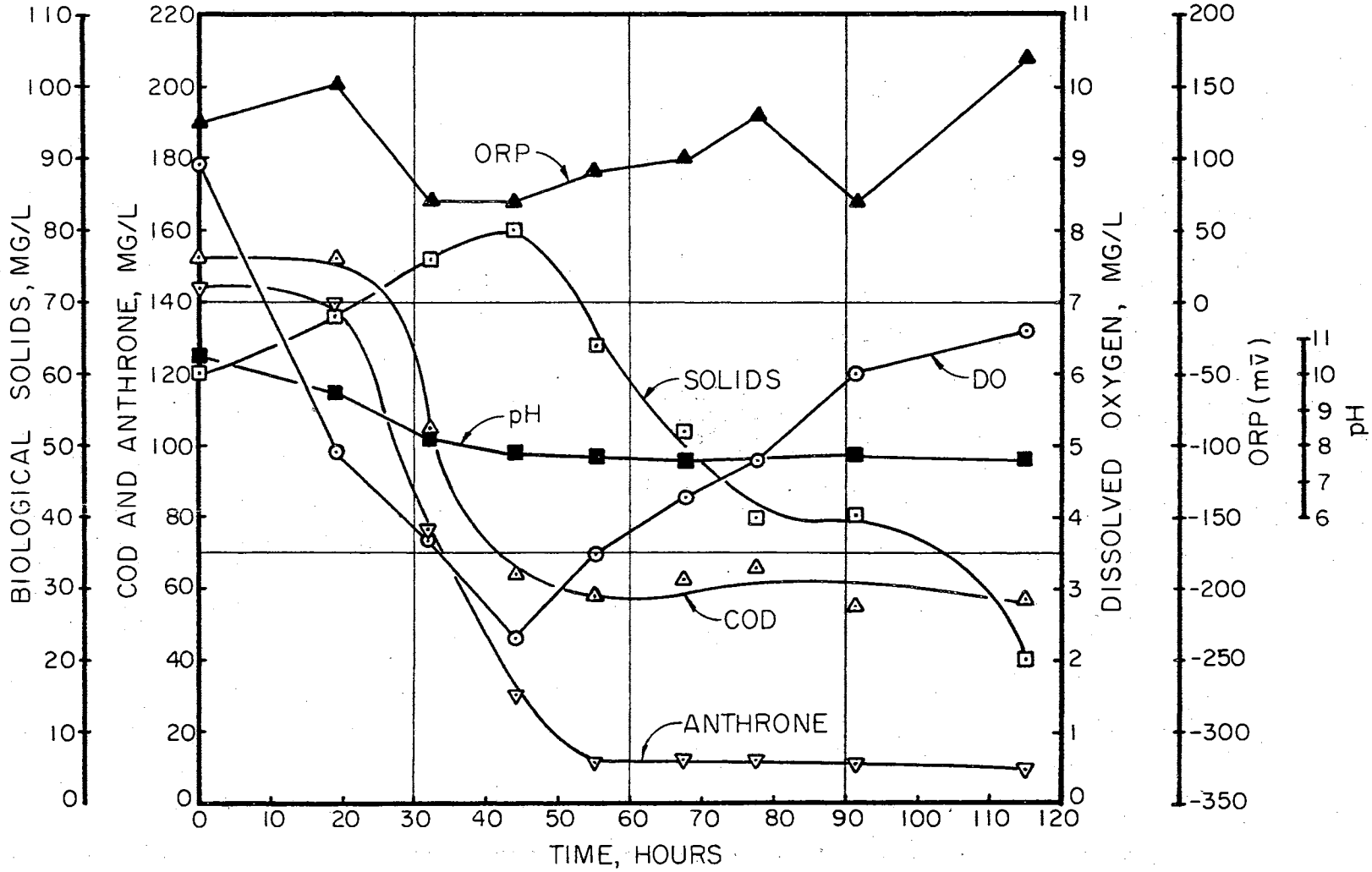

Figure 8. Biological response in semi-quiescent water to glucose loading of 150 mg/l, with mixing; lighting conditions, 24 hours darkness.

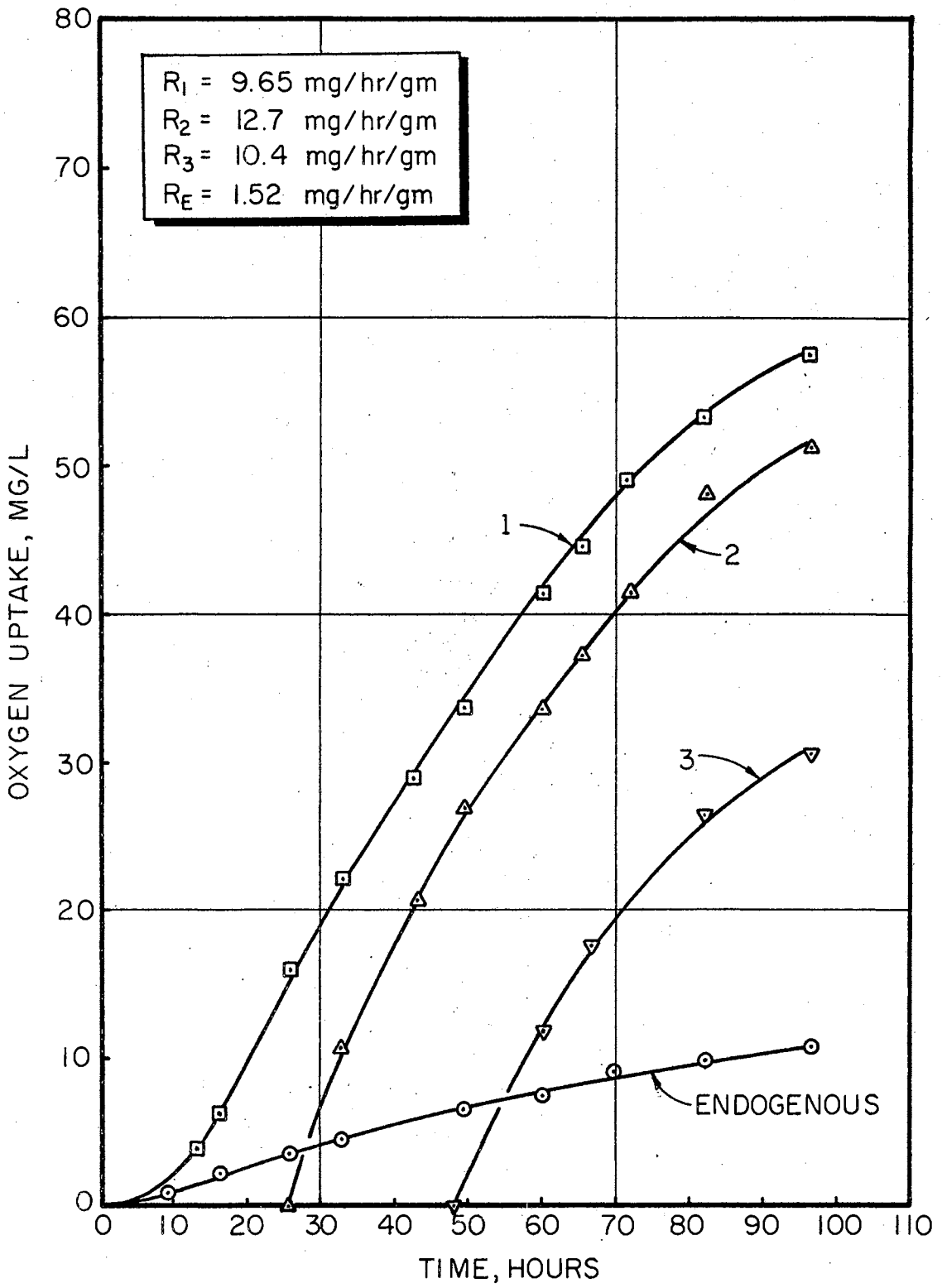

Figure 9. Cumulative oxygen uptake for 100 mg/l system.

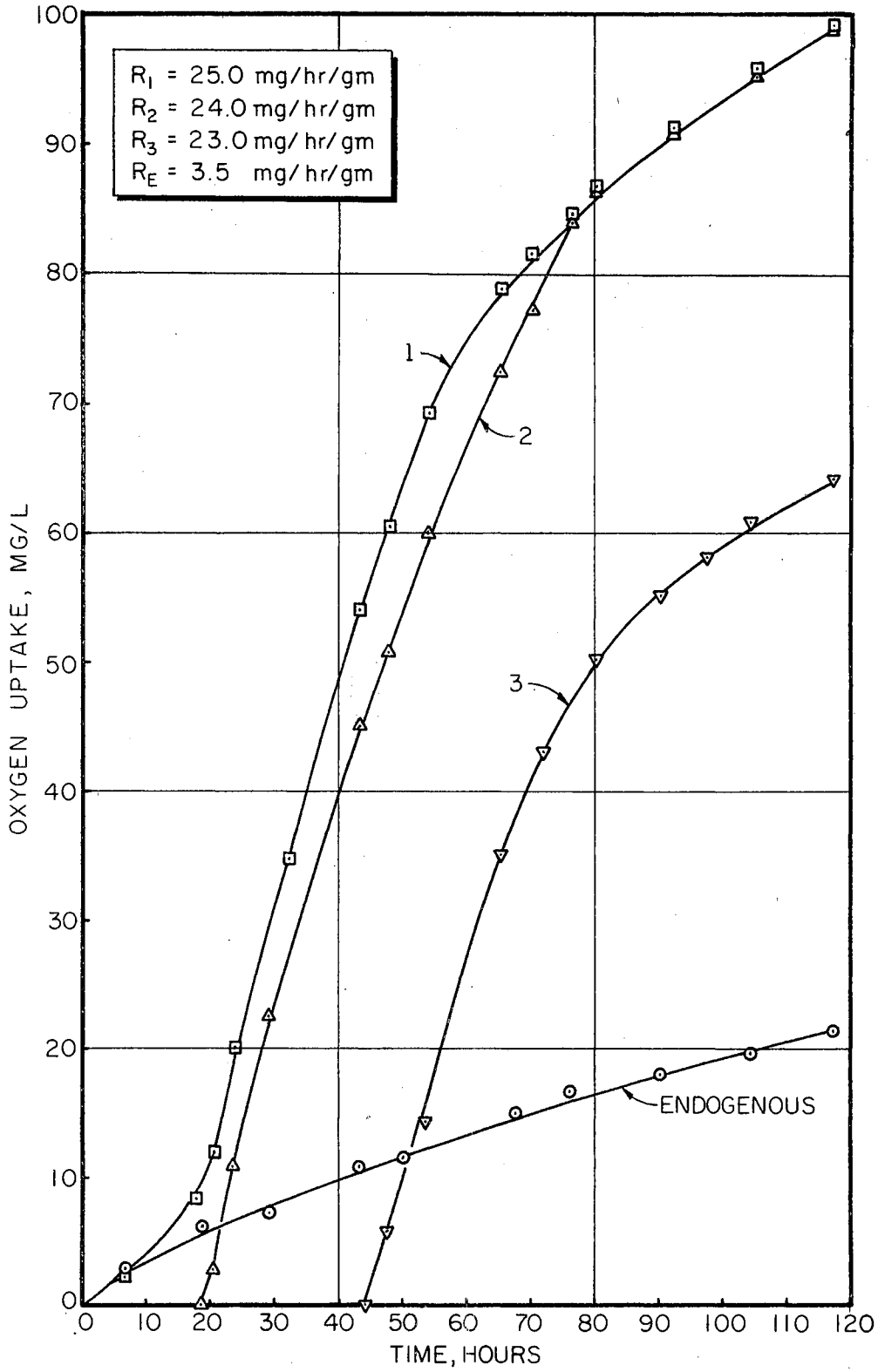

Figure 10. Cumulative oxygen uptake for 150 mg/l system.

both the 100 mg/l (Figure 9) and 150 mg/l (Figure 10) systems were determined in the following manner: the rates for flasks one and two were calculated for the same time interval and using the average solids concentration taken from the batch data during this interval. The rates for flask three were determined for the initial twenty hours using the average solids during this period, and the endogenous rate was based on the initial seventy hours and the initial solids concentration. The 100 mg/l system had an endogenous rate of 1.52 mg/hr/gm solids and an overall average rate of respiration of 10.9 mg/hr/gm solids. In contrast, the 150 mg/l system exhibited an endogenous rate of 3.5 mg/hr/gm solids with an overall average rate of respiration of 24.0 mg/hr/gm solids. A comparison of these data indicates that the 150 mg/l system was biologically more reactive than the 100 mg/l system by approximately 230%.

The relationships between the biological solids and the viable bacteria present in the above-mentioned systems are given in Figures 11 (100 mg/l) and 12 (150 mg/l). A comparison of these figures shows that in both cases the maximum biological solids occurred when the viable bacteria present were at a maximum. It is also interesting to note that both systems exhibited a net negative synthesis. Based on the apparent color change of the systems with time, from an initial dark green to a pale green, it can be said that algal cell loss was primarily the cause for the negative net synthesis.

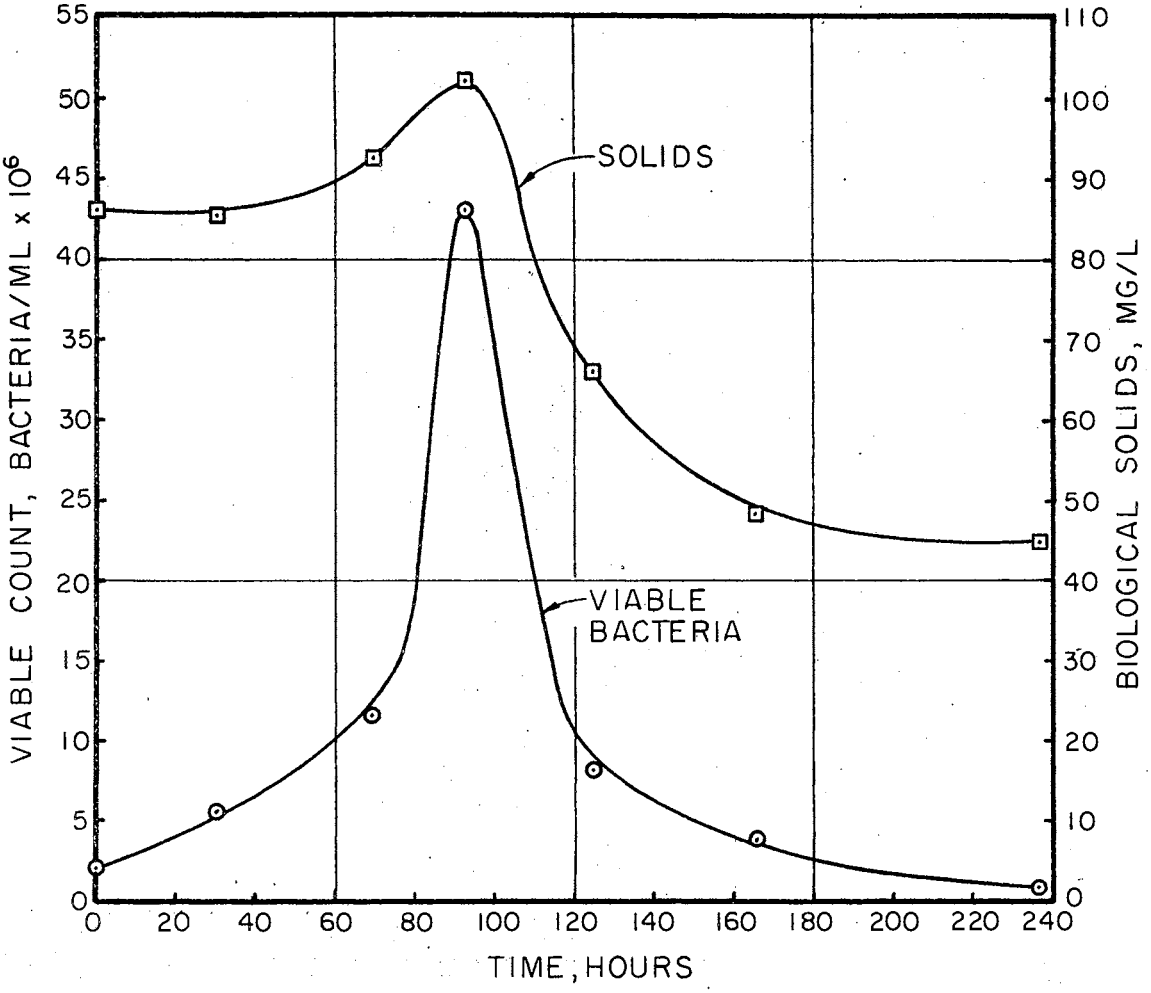

Figure 11. Relationship between biological solids and viable bacteria for glucose loading of 100 mg/l.

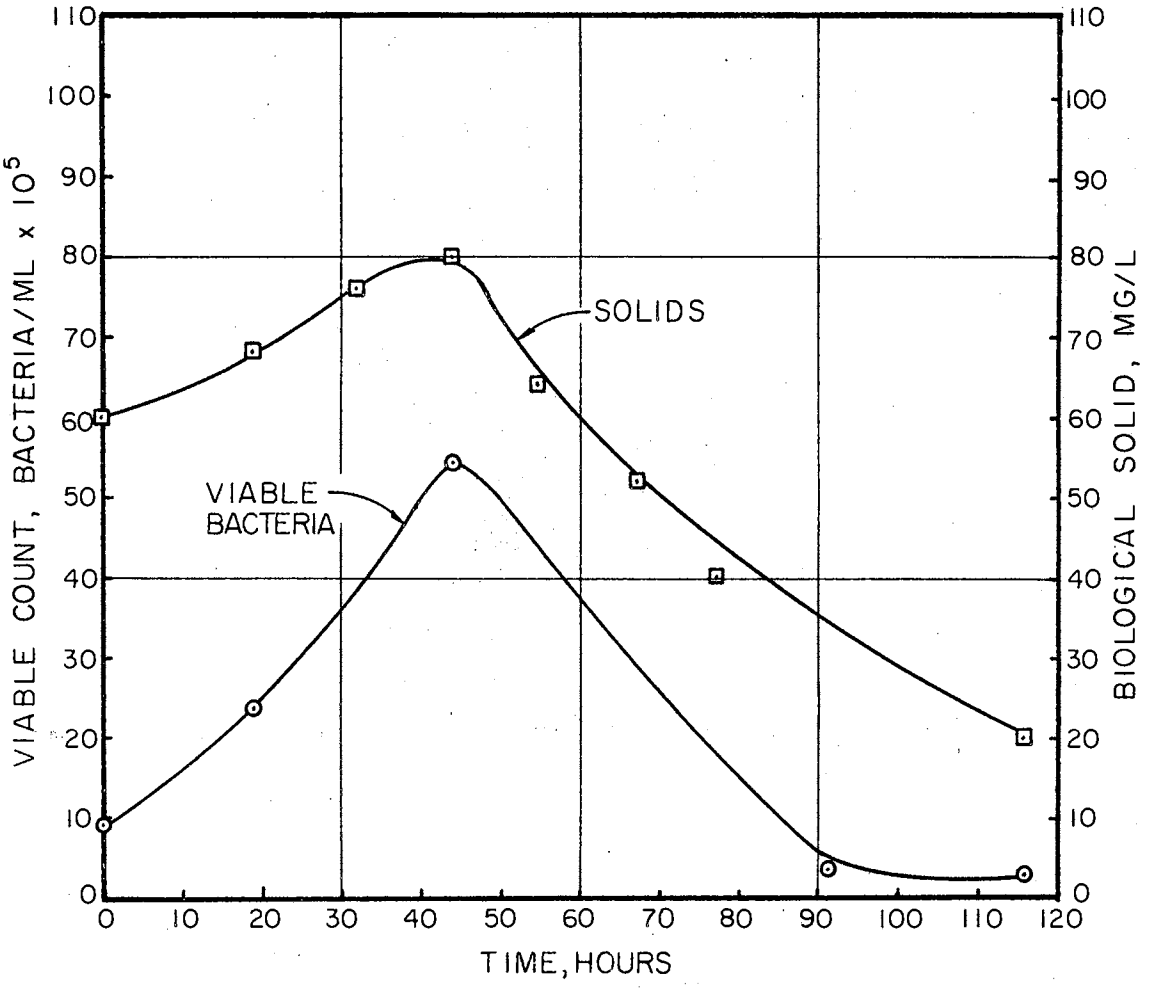

Figure 12. Relationship between biological solids and viable bacteria for glucose loading of 150 mg/l.

c. Closed System with Mixing

In this set of experiments the following two systems were investigated: 1) a non-buffered system, and 2) a buffered system (1 M Phosphate buffer).

Figure 13 shows the response of the unbuffered system to a loading of 150 mg/l glucose. The DO concentration was reduced to zero within thirty hours and then remained at zero throughout the remainder of the experiment. Although there was no lag period in the glucose and COD removal, the system exhibited a much slower removal rate than any of the previously investigated systems. It can be discerned that most or all of the glucose was removed in approximately 120 hours. The growth of this system was somewhat different than any of the previously reported systems. There was a slow continual increasing trend in the biological solids with a maximum occurring after approximately 95 hours, followed by a small decrease to a constant value for the remainder of the experiment.

It is interesting to note that the DO concentration remained at zero during most of the experiment, but the ORP values did not indicate anaerobic conditions as was previously reported in Figures 4 through 6. Although the DO concentration of a system influences the ORP, other factors such as the presence of oxidized metallic ions (Fe^{+++}) and/or the lack of chemical or biological activity also have an effect on the ORP of the system. Therefore these other factors may have caused the ORP to remain at a

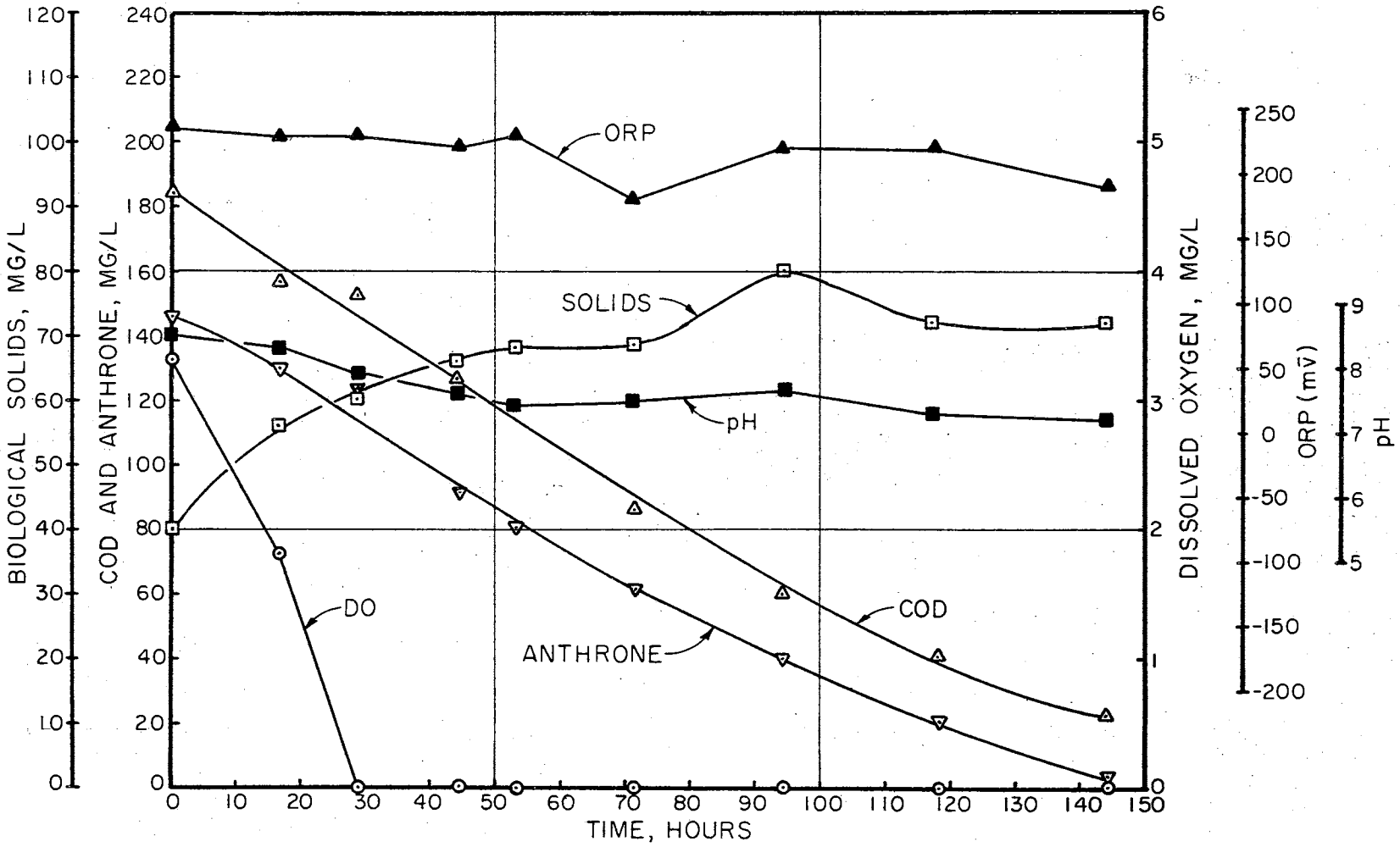

Figure 13. Biological response of closed, non-buffered system to glucose loading of 150 mg/l; lighting conditions, 24 hours darkness.

relatively stable positive potential as indicated in Figure 13 in spite of the absence of dissolved oxygen in the system. There was a slow decrease in the pH throughout the experiment.

The oxygen uptake for the 150 mg/l non-buffered closed system is given in Figure 14. However, no definite correlation can be made to the batch system because of the fact that it was anaerobic after twenty-nine hours, while the oxygen uptake was measured under aerobic conditions. Despite the differences between the two systems, it is interesting to note that the oxygen uptake rate for flask two was 17.4 mg/hr/gm solids, while that of flask one was 14.5 mg/hr/gm solids for the same time interval. In contrast, flask three, which was subjected to anaerobic conditions for approximately forty hours, exhibited an immediate uptake with a rate equal to 21 mg/hr/gm solids. These data do show, however, that the cells possessed a high capability for aerobic metabolism. It can be discerned that the endogenous rate of respiration approximated first order decreasing kinetics.

The response of the closed phosphate buffered system to 150 mg/l glucose is shown in Figure 15. The DO removal rate was greater than in the non-buffered closed system. There was a lag period of approximately seventy hours in the glucose and COD removal. Following this lag period the glucose and COD removal was similar to the dark systems previously shown. The biological solids concentration

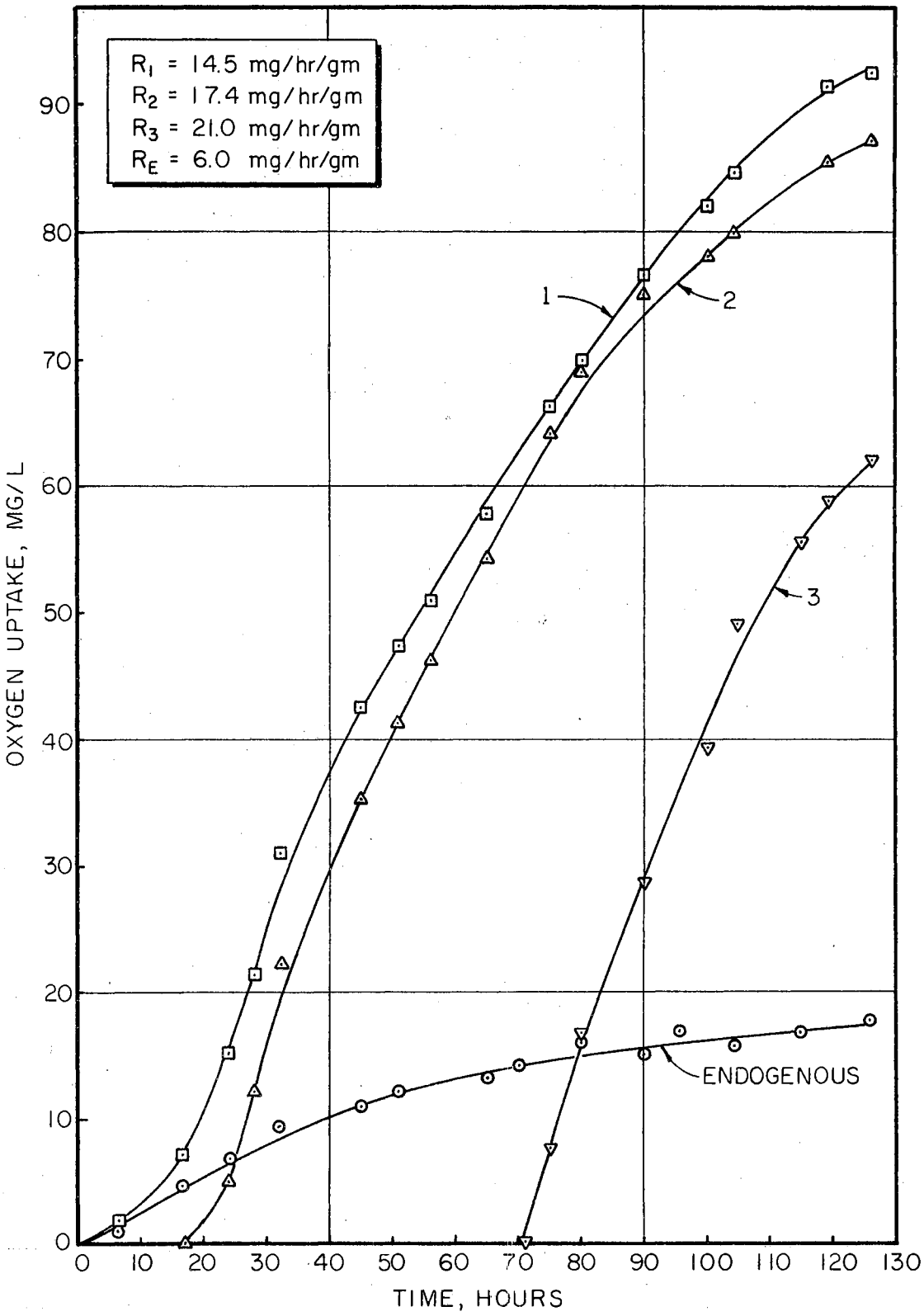

Figure 14. Cumulative oxygen uptake for closed, non-buffered system loaded with 150 mg/l glucose.

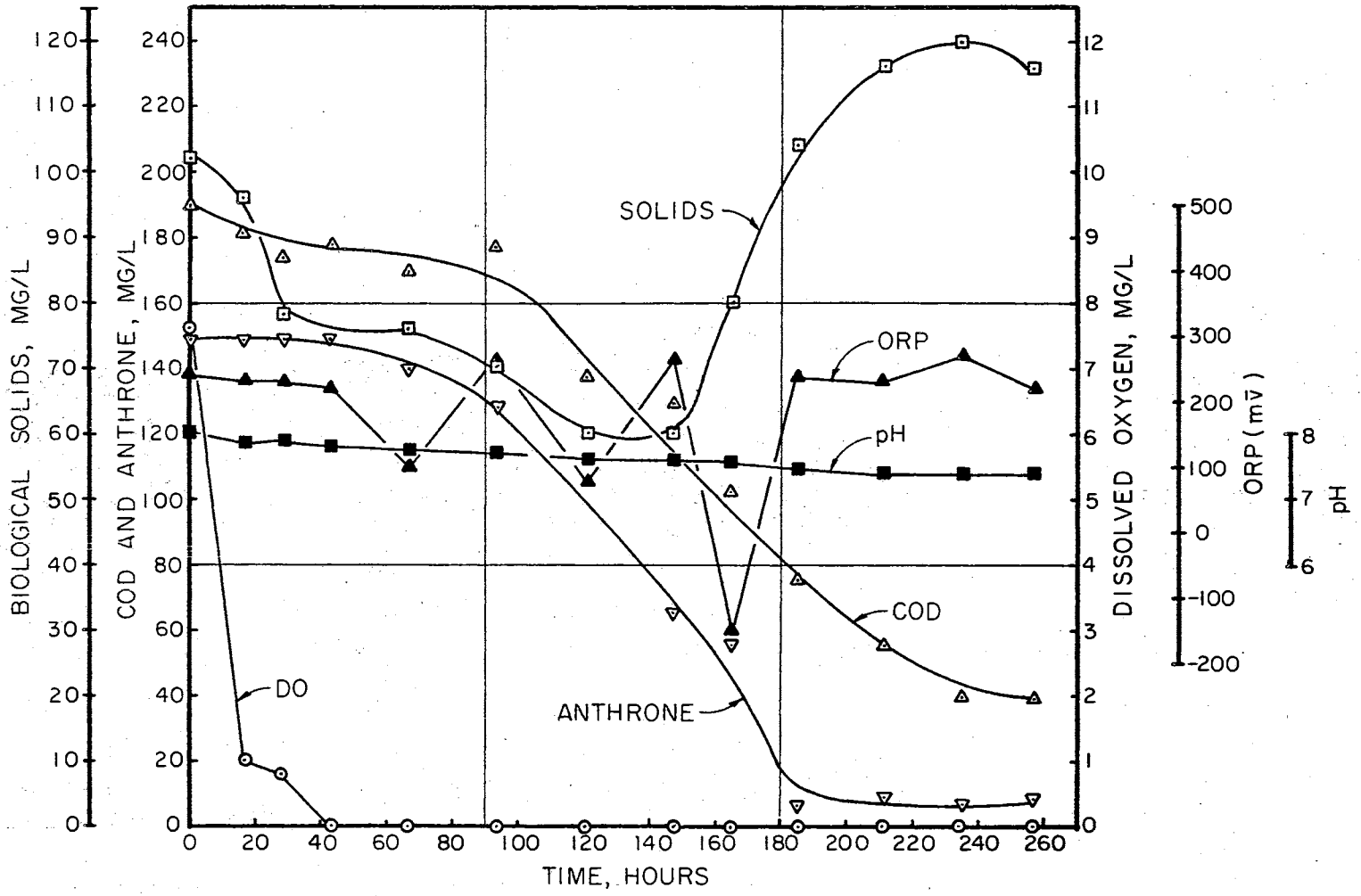

Figure 15. Biological response of closed, buffered system to glucose loading of 150 mg/l; lighting conditions, 24 hours darkness.

exhibited a continual decreasing trend until the system started to utilize the glucose, after which a rapid increase was noted with a maximum occurring when most or all of the applied substrate was removed.

It is interesting to note that during active substrate utilization the ORP values were dynamic, while those during the initial lag period and after complete substrate removal were at a relatively stable positive potential. The ORP values during the lag period indicate the presence of dissolved oxygen as well as the lack of chemical or biological activity, while those after complete substrate removal indicate the lack of biological activity due to the absence of the oxidizable substrate. There was a slight decrease in pH during active substrate removal.

The oxygen uptake of the closed buffered system is shown in Figure 16. A comparison between the endogenous respiration and the zero time sample shows that there was very little difference in the oxygen utilization during the initial twenty hours. The absence of a lag period in the oxygen uptake correlates very well to the observed rapid removal of the dissolved oxygen in the batch system. However, further correlation between the changes in the batch system and the oxygen uptake data again is not possible, because of the differences in the oxygen content of the systems. Upon examination of Figure 16 it can be seen that the rates of oxygen uptake for all flasks never exhibited a constant linear uptake rate. Nevertheless, it is

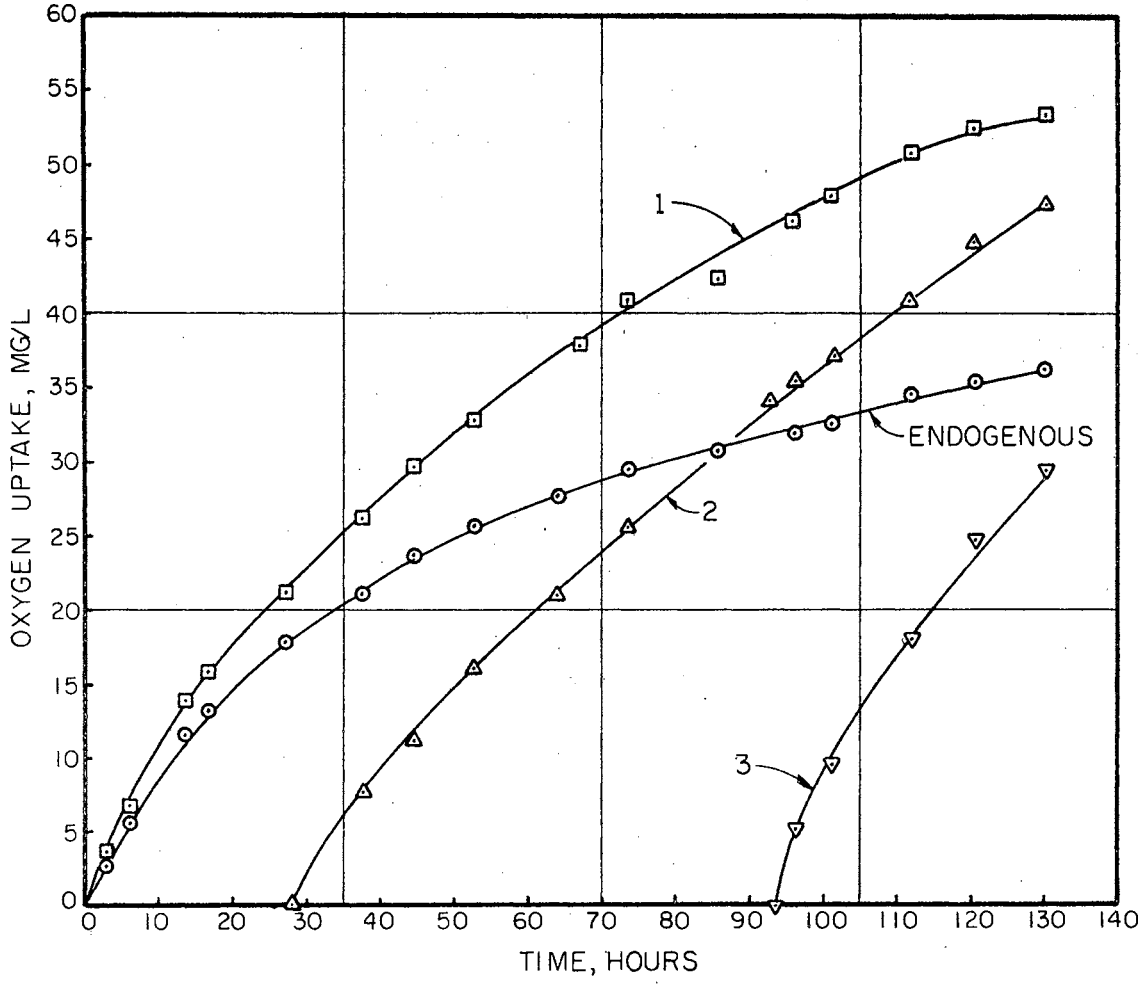

Figure 16. Cumulative oxygen uptake for closed, buffered system loaded with 150 mg/l glucose.

interesting to note that the oxygen uptake data during the initial 100 hours exhibited properties for a system having little biological activity.

The relationships between viable bacteria and biological solids for the non-buffered system (Figure 13) and the buffered system (Figure 15) are shown in Figures 17 and 18, respectively. In Figure 17 it can be seen that as the biological solids increased there was a corresponding increase in the viable bacteria. In contrast, a comparison between the biological solids and the vaiable bacteria in Figure 18 for the buffered system shows a completely different response. As the biological solids exhibited a continual decreasing trend for approximately 150 hours, the viable bacteria slowly increased. Visual and microscopic examination of the samples during this continual decrease in biological solids showed that there was a definite color change from a dark green to a weakening pale green, and an apparent reduction in the algal cell number. Therefore, this decrease may be attributed to the algal population in the system. After 150 hours, there was a rapid increase in biological solids accompanied by a concurrent increase in viable bacteria.

d. Open, Non-buffered System with Negligible Response

The results presented in Figures 19 through 21 are strikingly different than any of the previously shown systems even though the method of developing the system was the same and the initial conditions were quite similar,

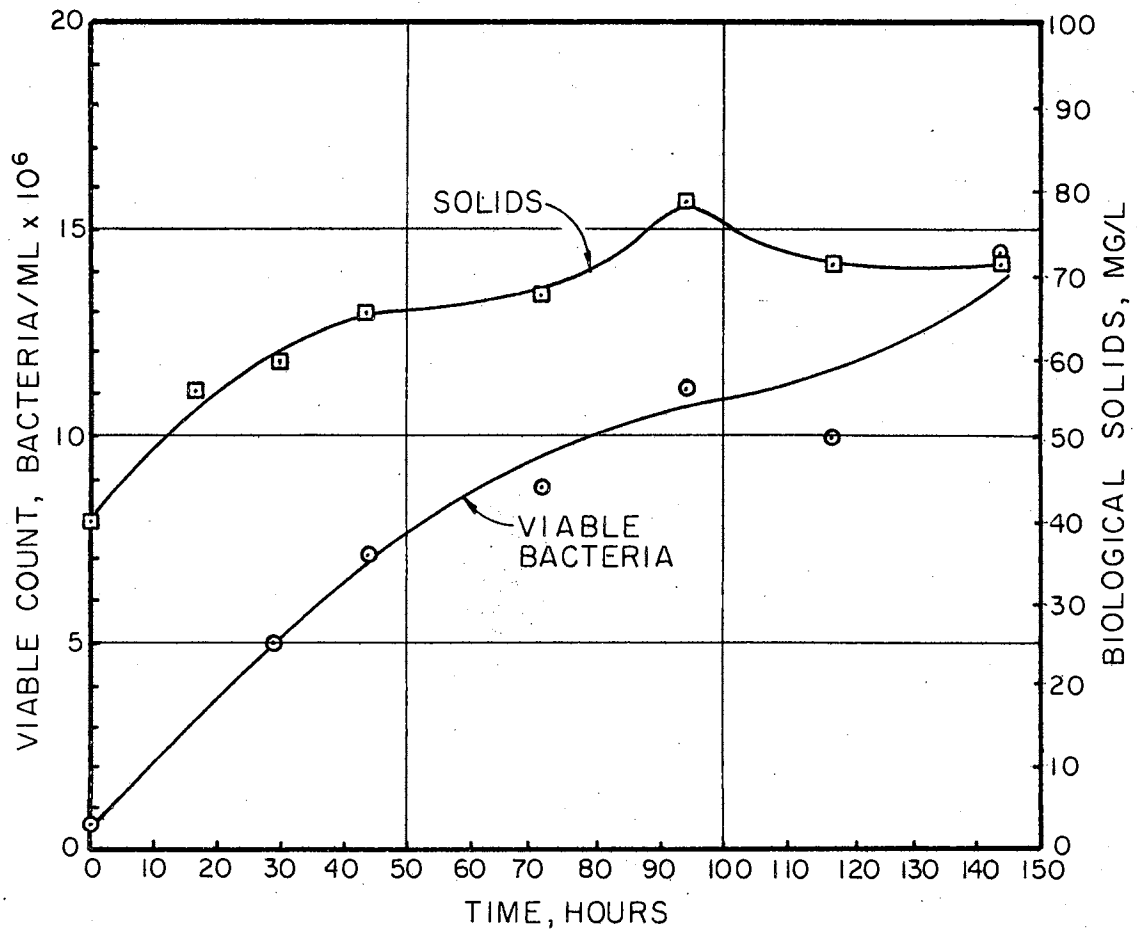

Figure 17. Relationship between biological solids and viable bacteria for the closed, non-buffered system.

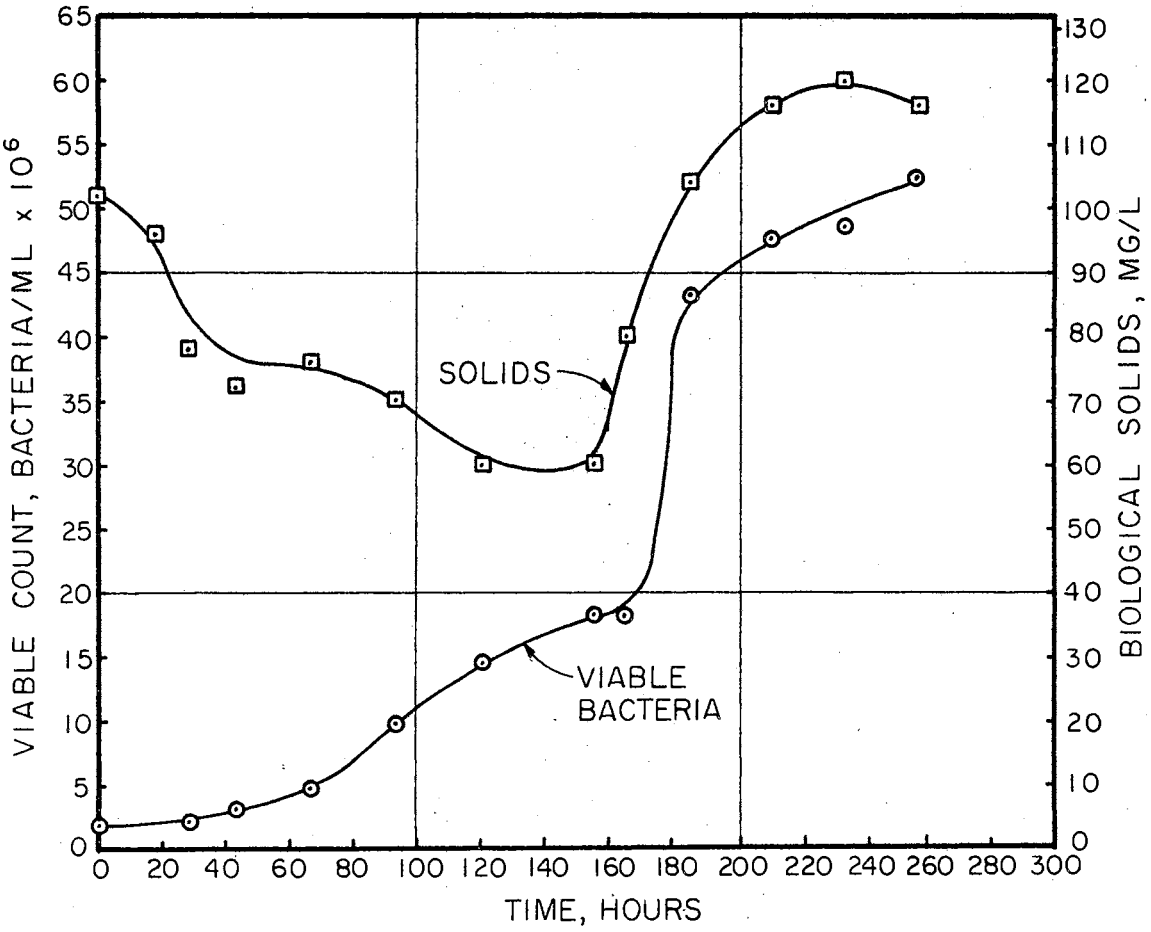

Figure 18. Relationship between biological solids and viable bacteria for closed, buffered system.

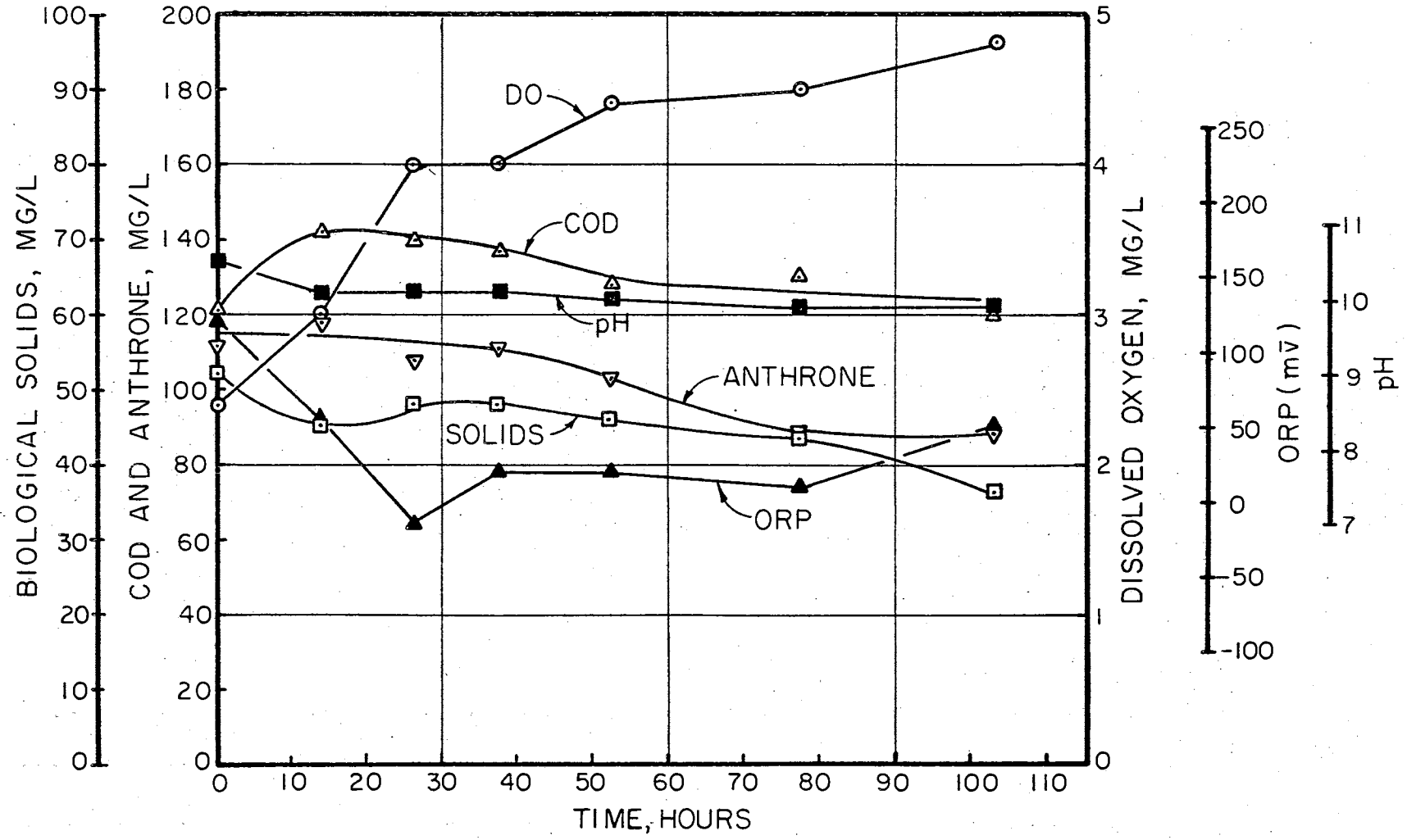

Figure 19. Open, non-buffered system with negligible response to a glucose loading of 100 mg/l; lighting conditions, 24 hours darkness.

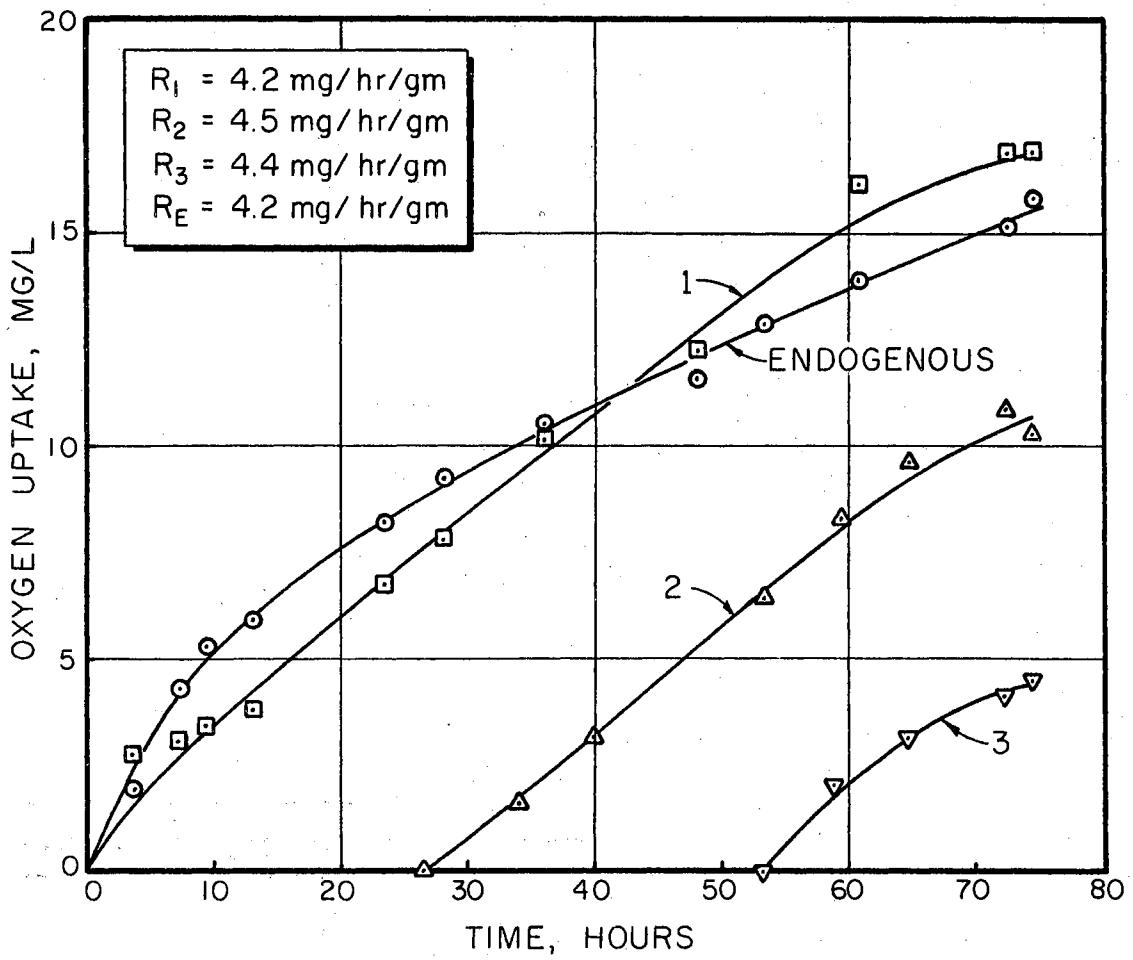

Figure 20. Cumulative oxygen uptake for the open, non-buffered system with negligible response to glucose loading of 100 mg/l.

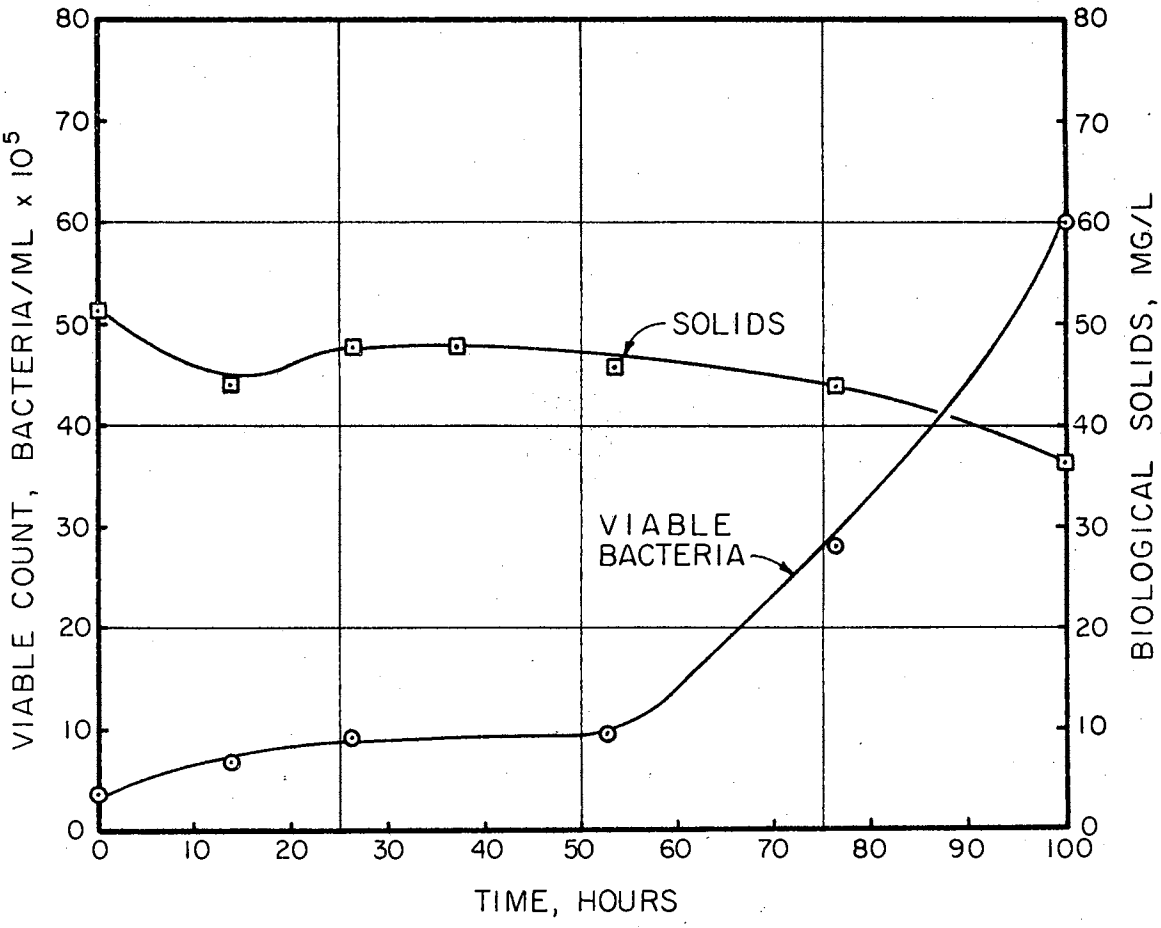

Figure 21. Relationship between biological solids and viable bacteria for the open, non-buffered system with negligible response to glucose loading of 100 mg/l.

i.e., pH, DO concentration, and biological solids content. The DO concentration exhibited a continual increase due to reaeration by mixing. There was little or no removal of the applied organic loading (100 mg/l glucose) and the COD of the system remained relatively constant after exhibiting a slight initial increase which may have been due to cell lysing. The ORP values for this system were quite different from any of the other previously shown systems. Initially, the ORP was 120^{+} mv but rapidly decreased to 15- mv and remained relatively stable the remainder of the time. The change in the high initial pH was negligible when compared to any of the previous systems having the similar initial pH.

The oxygen uptake for this system is shown in Figure 20. It can be seen that the addition of the exogenous substrate had little or no effect on the rate of respiration at any time during the experiment. The endogenous rate of respiration was 4.2 mg/hr/gm solids, while the other three flasks exhibited an average rate of 4.3 mg/hr/gm solids. These data also indicate a lack of biological activity in the system.

The relationship between the biological solids concentration and viable bacteria is shown in Figure 21. It is interesting to note that for the first fifty hours, the biological solids remained relatively constant while the viable bacteria exhibited a slow increase. Undoubtedly, this slow increase in viable bacteria was a reason for the

slow response of the system. However, after fifty hours, the viable bacteria increased while the biological solids decreased. As previously mentioned, visual and microscopic examination of the samples from the system during the continual decrease in biological solids showed that this decrease was primarily due to algal cell reduction.

During this experiment as well as all of the previous experiments, a distinct odor similar to that of hydrogen sulfide was detectable after approximately 72 hours of operation. Also, there was an apparent increase in the amount of slime material in the system. The odor of hydrogen sulfide as well as the increase in slime material of the systems may have been due to various products released as a result of algal and bacterial cell lysing and/or products of dark heterotrophic metabolism of a photoautotrophic developed system.

3. Response of a Pure Culture of Chlorella pyrenoidosa to Glucose in the Dark using a Warburg Respirometer

Glucose utilization and the effects of glucose on the rate of respiration of Chlorella pyrenoidosa in the dark are shown in Table V. The following three types of cultured systems were investigated: 1) algae grown photosynthetically in a buffered (1 M phosphate buffer, pH = 7.0) algal growth medium, 2) algae grown photosynthetically in an unbuffered algal growth medium, and 3) algae acclimated to a glucose-algal growth medium. A comparison of the responses of all three systems shows that the largest

TABLE V

PURE CULTURE RESPONSE TO GLUCOSE IN THE DARK

Previous Condition	Culture Age Days	Added Substrate (S_i) mg/l	pH_i	pH_F	Time Hours	RO_2 mg O_2/hr/gm	Anthrone mg/l	COD mg/l
1. Photosynthetically cultured with algal growth medium with buffer	13	-	7.6	7.6	61.50	2.65	3.8	13.75
		150	7.6	7.6	23.58	13.55	141.5	149.5
		150	7.6	7.6	36.58	10.62	71.4	137.5
		150	7.6	7.6	61.50	7.46	54.7	123.7
2. Photosynthetically cultured in algal growth medium	14	-	8.75	9.4	72.17	0.83	3.3	23.6
		150	8.75	9.1	23.42	4.53	107.4	196.5
		150	8.75	9.0	31.17	4.14	80.4	208.1
		150	8.75	9.0	72.17	3.03	93.5	202.3
3. Photosynthetically cultured in algal growth medium	21	-	8.5	9.2	45.08	2.5	10.0	
		50	8.5	9.0	45.08	6.23	10.2	
		100	8.9	8.9	40.42	5.78	71.5	
		100	8.5	9.0	45.08	5.84	68.1	
		125	8.5	9.0	45.08	5.48	103.4	
		100	8.9	8.9	53.42	5.56	61.0	
		100	8.9	8.9	61.50	4.48	54.0	
4. Photosynthetically cultured in algal growth medium with glucose	26	-	8.3	9.0	30.00	2.26	15.0	30.0
		150	8.3	9.0	58.17	2.15	129.7	181.0

increase in the respiration rate (R_{O_2}) was observed in the buffered system while the acclimated system exhibited no increase in rate as a result of the addition of an exogenous substrate. It is interesting to note that the endogenous rates of respiration for systems 1, 3, and 4 (Table V) were very similar. However, the observed low rate in system 2 may be attributed to the fact that large barometric changes occurred during this experiment.

A comparison of the rates of respiration at various times throughout the experiments for the non-acclimated systems shows that the increase in the rate of respiration was only temporary, and that the system returned to a slower rate or possibly to the endogenous rate.

The results for all systems indicate that Chlorella pyrenoidosa was capable of glucose utilization in the dark but did not significantly reduce the COD of the systems. The rate of substrate utilization in the dark was slow and more notably in the system acclimated to the substrate.

It can be seen that there was an increase in pH during the experiments with the non-buffered systems, while no change was detected in the buffered system. Although the pH did not seem to affect the endogenous rate of respiration, there was some indication that it may have affected the rate of respiration in the presence of an exogenous substrate.

CHAPTER V

DISCUSSION OF RESULTS

The response of a laboratory oxidation pond population to an organic substrate has been examined under the following conditions: 1) in a light-dark cycle, 2) in complete darkness, and 3) in a pure culture using a Warburg respirometer. The observed results are discussed below.

A. Biological Response in a Light-dark Cycle

The results of these studies were presented in Figures 2 through 4. A comparison of the recovery rates of the dissolved oxygen concentration for all three systems clearly indicates that the recovery rate was dependent on the biological solids concentration as well as the applied organic loading. Although it may be conceivable that oxygen was continually being produced during the lighted periods, the step-like recovery was not observed until the rate of oxygen production by the algae exceeded the rate of oxygen consumption of the microbial population. In regard to the removal rate of the applied organic load, it can be said from these studies that it is independent of the initial loading conditions but dependent on the dissolved oxygen concentration. At higher organic loading conditions

Wu (16) has shown that there was a decrease in the removal rate. From a comparison of the oxidation-reduction potential (ORP) values and the dissolved oxygen concentration, it appears that the ORP is not as sensitive in indicating anaerobic conditions as the dissolved oxygen.

B. Biological Response in Complete Darkness

The results of these studies were presented in Figures 5 through 18. Although three different experimental conditions were examined, that is, an open system with and without mixing and a closed system with mixing, a comparison of the overall results allows the following generalizations to be drawn: There was an initial lag period in the rate of substrate removal as well as in the oxygen uptake of the systems. This lag may be attributed to the fact that because the microbial population had been developed photoautotrophically, the needed enzymes for heterotrophic metabolism had to be synthesized before substrate utilization. Another reason for this lag period could also be attributed to a change in biological predominance in the system caused by the presence of the exogenous substrate. However, the results from microscopic and colony type examination during the experiments supports the former explanation. In all systems there was a good correlation between the biological solids concentration and the viable bacterial counts, both of which exhibited a maximum at the time when most or all of the exogenous substrate was removed. Although it was previously mentioned that algae were capable of heterotrophic

growth in the dark but at a slow rate, the results indicated that the bacteria present are primarily responsible for the reduction of the organic substrate (32, 41, 45).

A comparison of the ORP values shows that all of the systems exhibited one or a combination of the following properties: 1) a dynamic potential, i.e., a potential that changes with time, which is indicative of biological activity, 2) a stable positive potential, which may be indicative of a lack of chemical or biological activity and/or the presence of dissolved oxygen, and 3) a stable negative potential indicating the presence of products of anaerobic or facultative decomposition and/or hydrogen sulfide and other reduced compounds.

Pertaining to the glucose and COD removal, the open systems exhibited a faster removal of the applied organic loading than did the closed systems. Although both systems showed a slow increase in the bacterial population, it was apparent that the dissolved oxygen concentration played a rate-limiting role in the substrate removal of the closed system. Wu (16) has also observed this slow substrate removal in a laboratory scale oxidation pond under similar conditions. The amount of metabolic intermediates and/or end products produced by the microbial populations in either system were somewhat insignificant.

The oxygen uptake curves for the open systems (Figures 9 and 10) were very similar in that there were three distinct phases shown, a lag phase, a linear phase, and the

endogenous phase, during the experiments. A comparison of the average rates of respiration for both systems indicates that the system loaded with 150 mg/l glucose (Figure 10, R_{avg} = 23.0 mg/hr/gm solids) was biologically more reactive than the system loaded with 100 mg/l glucose (Figure 9, R_{avg} = 10.9 mg/hr/gm solids). Although oxygen uptake data was obtained for the closed non-buffered and buffered systems, Figures 14 and 16, respectively, only the initial twenty-five hours was of any value because the batch systems (Figures 13 and 15) exhibited anaerobic conditions during the remainder of the experiments. However, it is interesting to note the two completely different responses of the system. The non-buffered system exhibited three different rates (14.5, 17.4, and 21.0 mg/hr/gm solids) during the experiment, while the buffered system never did show any constant oxygen uptake rate. A comparison of the endogenous respiration curves of both systems does, however, indicate that the rate of endogenous respiration approximated first order decreasing kinetics.

Inasmuch as the initial pH of all of the systems ranged from values of 8.0 to 10.5, it is felt that there were no inhibitory effects on the microbial population because it had been developed under the prevailing conditions. However, the initial pH value could have a definite effect on the type of microbial population which was developed in the system. Although it does seem possible as well as probable that these high pH values would inhibit

substrate removal to some extent as well as growth, but as previously mentioned, it is felt that the rates of substrate removal and growth are dependent on the conditions prior to the addition of the organic substrate.

In contrast to the previously discussed open systems, the results from the open system with negligible response (Figures 19 through 21) were quite different despite the fact that the method of developing the system was the same in all cases. Undoubtedly, the slow increase in the bacterial population was a reason for the negligible response to the applied organic load.

C. Pure Culture Response of Algae to Glucose in the Dark

The primary purpose in these studies was to provide a more complete understanding of the precise role of the algal population when subjected to an exogenous substrate in the dark. A comparison of the overall results in Table V indicate that there was at least a two-fold increase and possibly as much as a six-fold increase in the respiration rate (R_{O_2}) in the systems that had been photoautotrophically cultured. This effect has also previously been observed with cultures of Chlorella (47). In addition, the increase in the rate of respiration was shown to be relatively independent of the amount of exogenous substrate as well as the age of the culture. Although it has been shown by Nielsen (22) that the rate of respiration of Chlorella pyrenoidosa was not inhibited for pH ranges of 3.0 to 11.0, the results of these studies indicate that there were some

inhibitory effects on the endogenous respiration and a more noticeable effect when an exogenous substrate was added. Pertaining to the substrate removal of Chlorella pyrenoidosa in the dark, it may be concluded that although glucose was utilized at a slow rate, the total COD was not significantly reduced.

CHAPTER VI

CONCLUSIONS

Based on the experimental evidence presented, the following conclusions may be drawn:

1. In spite of periods of anaerobiosis during substrate removal, there were no apparent inhibitory effects on the reoxygenation of the systems due to photosynthesis once the substrate was removed.

2. During active substrate removal the system will exhibit a dynamic oxidation-reduction potential (ORP).

3. The relative rates of substrate removal were much slower in the dark systems than in the systems receiving light, and that the bacteria present were primarily responsible for substrate removal.

4. Mixed populations developed photoautotrophically exhibit a sluggish response to an added organic substrate in the dark, whereas a pure culture of algae grown photosynthetically exhibits an immediate response to the added organic substrate in the dark.

5. The amount of oxygen utilized by algae in the dark is relatively insignificant when compared to that of a mixed population in the presence of an external oxidizable substrate.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE WORK

In view of the previous experimental evidence, the following suggestions are offered for future work:

1. A study on the effects of pH on algal respiration in the presence of an external oxidizable substrate.

2. Studies should be made to establish the extent of heterotrophy in the various algal species commonly found in oxidation ponds, and to detail the role of the bacteria as competitors for the organic compounds normally present.

3. There is a need to establish a more defined heterotrophic growth pattern of various algal species in the dark, and to elaborate what biochemical changes occur in the basic cell constituents.

SELECTED BIBLIOGRAPHY

1. Caldwell, D. H., "Sewage Oxidation Ponds," Sewage Works Journal, 18, 433-458 (1946).

2. Smallhorst, D. F., "The History of Oxidation Ponds in the Southwest," Proceedings, Oklahoma Water Pollution Control Association, 21-32 (1961).

3. "Survey Shows Present Status of Oxidation Ponds and Sewage Lagoons," Public Works, 90, 90-92 (1959).

4. Porges, R., "Industrial Waste Stabilization Ponds in the United States," Journal Water Pollution Control Federation, 35, 456-468 (1963).

5. Bassham, J. A., "Photosynthesis: Energetics and Related Topics," Advances in Enzymology, 25, 39-117 (1963).

6. Arnon, D. I., "The Role of Light in Photosynthesis," Scientific American, 203, 105-118 (1959).

7. Kok, B., and A. Jagendorf, "Photosynthetic Mechanisms of Green Plants," National Academy of Sciences Publication 1145, National Research Council, Washington, D. C. (1963).

8. Giese, A. C., Photophysiology, Academic Press, New York (1964).

9. White, A., P. Handler, and E. L. Smith, Principles of Biochemistry, McGraw-Hill Book Company, Inc., New York (1964).

10. Varma, M. M., and M. J. Wilcomb, "Effect of Light Intensity on Photosynthesis," Water and Sewage Works, 110, 191-194 (1963).

11. Lubbers, R. H., and D. Parikh, "The Effects of Algal Concentration, Luminous Intensity, Temperature and Diurnal Cycle or Periodicity upon Growth of Mixed Algal Cultures from Waste Stabilization Lagoons as Determined on the Warburg Apparatus," Proceedings 21st Industrial Waste Conference, Purdue University, Lafayette, Indiana, 348-367 (1966).

12. Oswald, W. J., H. B. Gotaas, H. F. Ludwig, and V. Lynch, "Algae Symbiosis in Oxidation Ponds. III," Sewage and Industrial Wastes, 25, 692-705 (1953).

13. Kutyurin, V. M., M. V. Ulubekova, and N. H. Nazarov, "Effect of Oxygen Concentration on the Photosynthetic Rate and Respiration of Aquatic Plants," Chemical Abstracts, 61, 8631c (1964).

14. Stone, A. R., and W. E. Abbott, "Diurnal Variation in the Dissolved Oxygen Content of Polluted Waters," Water and Sanitary Engineering, 1, 334-340 (1951).

15. Copeland, B. J., "Oxygen Relationships in Oil Refinery Effluent Holding Ponds," Ph.D. Thesis, Oklahoma State University (1963).

16. Wu, Y. C., "Effect of Organic Loading on Reaeration in Semi-quiescent Waters," Masters Thesis, Oklahoma State University (1967).

17. Gloyna, E. F., and D. Thirumurthi, "Suppression of Photosynthetic Oxygenation," Water and Sewage Works, 114, 83-88 (1967).

18. Gibbs, M., "Fermentation," Chapter 5, 91-97, in R. A. Lewin, Physiology and Biochemistry of Algae, Academic Press, New York (1962).

19. Herman, E. R., and E. F. Gloyna, "Wastes Stabilization Ponds. II. Field Practices," Sewage and Industrial Wastes, 30, 646-651 (1958).

20. Silva, P. C., and G. F. Papenfuss, "A Systematic Study of the Algae in Sewage Oxidation Ponds," California State Water Pollution Control Board Publication 7, Sacramento, California (1953).

21. Rohlich, G. A., and G. P. Fitzgerald, "An Evaluation of Stabilization Pond Literature," Sewage and Industrial Wastes, 30, 1213-1224 (1958).

22. Nielsen, E. S., "Influence of pH on the Respiration in Chlorella pyrenoidosa," Physiologia Plantarium, 8, 106-115 (1955).

23. Oswald, W. J., "Light Conversion Efficiency of Algae Grown in Sewage," Journal Sanitary Engineering Division ASCE, 86, 71-95 (1960).

24. Pipes, W. O., "pH Variation and BOD Removal in Stabilization Ponds," Journal Water Pollution Control Federation, 34, 1140-1150 (1962).

25. Ludwig, H. F., W. J. Oswald, H. B. Gotaas, and V. Lynch, "Algae Symbiosis in Oxidation Ponds. I," Sewage and Industrial Wastes, 23, 1337-1355 (1951).

26. Oswald, W. J., H. B. Gotaas, H. F. Ludwig, and V. Lynch, "Algae Symbiosis in Oxidation Ponds. II," Sewage and Industrial Wastes, 25, 26-37 (1953).

27. Towne, W. W., A. F. Bartsch, and W. H. Davis, "Raw Sewage Stabilization Ponds in the Dakotas," Sewage and Industrial Wastes, 29, 377-396 (1957).

28. Bartsch, A. F., "Algae as a Source of Oxygen in Waste Treatment," Journal Water Pollution Control Federation, 33, 239-249 (1961).

29. Burlew, J. S., "Algae Culture from Laboratory to Pilot Plant," Carnegie Institution of Washington, Publication 60, Washington, D. C. (1953).

30. Clark, J. W., and W. Viessman, Water Supply and Pollution Control, International Textbook Company, Scranton, Pennsylvania (1965).

31. Bristol-Roach, B. M., "On the Carbon Nutrition of Some Algae Isolated from Soil," Annals of Botany (London) 41, 509-517 (1927).

32. Myers, J., "Physiology of the Algae," Annual Review of Microbiology, 6, 157-180 (1951).

33. Lewin, R. A., Physiology and Biochemistry of Algae, Academic Press, New York (1962).

34. Fogg, G. E., The Metabolism of Algae, John Wiley & Sons, Inc., New York (1953).

35. Myers, J., and H. Samejima, "Heterotrophic Growth of Chlorella pyrenoidosa," Journal of General Microbiology, 18, 107-117 (1958).

36. Pipes, W. O., "Algae Growth Rate," Water and Sewage Works Reference Number, 10, 328-332 (1961).

37. Steers, E., and Ellner, P. D., "Urea as a Carbon Source for Chlorella and Scenedesmus," Archives of Biochemistry and Biophysics, 59, 534-535 (1955).

38. Gotaas, H. B., and W. O. Pipes, "Utilization of Organic Matter by Chlorella Grown in Sewage," Applied Microbiology, 8, 163-169 (1959).

39. Merz, R. C., R. Zehndfennig, and J. R. Klima, "Chromatographic Assay of Extracellular Products of Algal Metabolism," Journal Water Pollution Control Federation, 34, 103-115 (1962).

40. Allen, M. B., "General Features of Algal Growth in Sewage Oxidation Ponds," California State Water Pollution Control Board Publication 13 (1955).

41. Parker, B. C., H. C. Bold, and T. R. Deason, "Facultative Heterotrophy in Some Chloroccacean Algae," Science, 133, 761-763 (1961).

42. Finkle, B. J., D. Appleman, and F. K. Fleischer, "Growth of Chlorella vulgaris in the Dark," Science, 111, 309 (1950).

43. Lewin, R. A., "The Utilization of Acetate by Wild Type and Mutant Chlamydomonas dysosmos," Journal of General Microbiology, 11, 457-459 (1954).

44. Pearsall, W. H., and R. P. Bengry, "The Growth of Chlorella in Darkness and in Glucose Solution," Annals of Botany (London), 4, 365-377 (1940).

45. Avilov, I. A., "Utilization of Various Carbon Sources by Algae of the Genus Chlorella in the Dark," Leningrad Universitet Vestnik, Seriia Biologii. No. 3, 62-64 and 66, Vol. 18 (15) (1963).

46. Myers, J., "A Study of the Pigments Produced in Darkness by Certain Green Algae," Plant Physiology, 15, 575-588 (1940).

47. Gibbs, M., "Respiration," Chapter 4, 61-90, in R. A. Lewin Physiology and Biochemistry of Algae, Academic Press, New York (1962).

48. Stainier, R. Y., M. Doudoroff, and E. A. Adelberg, The Microbial World, 2nd Ed., 456, Prentice Hall Publishing Company, Englewood Cliffs, New Jersey.

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

50. Beckman Operating and Maintenance Instruction Manual, Beckman Instruments, Inc., Fullerton, California.

51. Gaudy, A. F. Jr., "Colorimetric Determination of Protein and Carbohydrate," Industrial Water and Wastes, 7, 17-22 (1962).

VITA

John Thomas Solook

Candidate for the Degree of

Master of Science

Thesis: LIGHT AND DARK MICROBIAL RESPONSE IN SEMI-QUIESCENT WATERS

Major Field: Sanitary and Public Health Engineering

Biographical:

Personal Data: Born July 31, 1943, at Jamesburg, New Jersey, the son of John E. and Florence M. Solook.

Education: Attended Jamesburg High School, Jamesburg, New Jersey; completed requirements for the Bachelor of Science degree from the University of Missouri at Rolla, Rolla, Missouri, in May, 1966; completed requirements for the degree of Master of Science in Sanitary and Public Health Engineering at Oklahoma State University in May, 1968.

Professional Experience: Field Engineer, Howard, Needles, Tammen & Bergendoff, Research Assistant, Oklahoma State University.

Membership in Professional Societies: American Society of Civil Engineers, Water Pollution Control Federation.