

COMPLETION REPORT  
OXYGEN DIFFUSION IN SEMIQUIESCENT WATERS  
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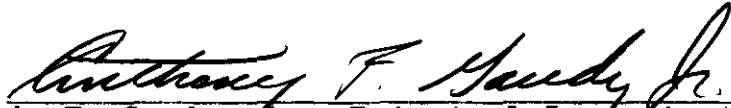
PREFACE

In accord with the joint goals of the Oklahoma Water Resources Research Institute, the Center for Water Research in Engineering, and the Bioengineering and Water Resources Division of the School of Civil Engineering, of Oklahoma State University, this research project was designed with the dual aim of accomplishing useful research and training graduate student researchers in important areas pertinent to the development and use of the nation's water resource. In addition to the independent value of the research findings of this project, the work has allowed the principal investigator to gain needed information on oxygen diffusion processes and mechanisms which have application and useful ramifications to other research projects underway in the Bioengineering laboratories. Thus, the experimental results obtained through the conduct of this project will have broader utility when integrated with and analyzed in light of findings of other ongoing research projects. The project has also permitted two former students, Dr. D. F. Kincannon and Dr. M. Ramanathan, now members of the faculty, to participate in research and training in this important area. Concerning the graduate students, after graduation Y. C. Wu (see Master's thesis Appendix I) was employed as a pollution control engineer by a state health department. J. T. Solook (see Master's thesis Appendix II) received a military commission and his duties are in the area of pollution control. O. V. Natarajan (see Ph.D. thesis Appendix III) is planning to join a university faculty and his work will be in the area of environmental engineering.

The accompanying document represents a detailed report on the completion of the project, and embodies pertinent information suggested in OWRR "Reporting Guidelines." It consists of the summary report, which includes an introduction, statement of experimental approach, review of project findings, discussion and summary, conclusions, and

recommendations. The three theses (Appendices I, II, and III) are referenced as sources of detailed information in the review of the results section of the report. It is planned that portions of the work will be submitted for journal publication.

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A. F. Gaudy, Jr., Principal Investigator  
Edward R. Stapley Professor of Civil  
Engineering; Director, Center for Water  
Research in Engineering

## COMPLETION REPORT

### OXYGEN DIFFUSION IN SEMIQUIESCENT WATERS

by

A. F. Gaudy, Jr., and O. V. Natarajan

#### ABSTRACT

Laboratory investigations were undertaken with the aim of gaining an insight into the net relative contribution of algal and physical reaeration in polluted waters under semi-quiescent conditions such as exist in oxidation ponds. Separate studies of physical oxygenation and oxygen stripping, biological deoxygenation (bacterial and algal), and biological aeration (algal) were conducted under both batch and continuous flow conditions. A considerable portion of the work involved the use of bench scale oxidation ponds (30 and 40 liter reaction volume) which could be opened or closed to the atmosphere. Various organic loadings were applied, and parameters used to assess system performance included dissolved oxygen concentration, oxidation reduction potential, pH, biological solids concentration, substrate analysis (anthrone test), and chemical oxygen demand. The results indicated that for operation under aerobic conditions, the algae contribute approximately two times as much to the dissolved oxygen resource of oxidation ponds as does the physical aeration process. Results have shown that aerobic conditions could be maintained at higher organic loadings in ponds which were closed to the atmosphere, indicating that a greater amount of DO was lost due to stripping during daytime operation (supersaturation) than was gained by physical aeration during night operation (undersaturated conditions).

#### KEY WORDS

oxygenation - deoxygenation - oxidation ponds - biological oxygenation - oxygen transfer - algal organotrophy

#### INTRODUCTION

The use of oxidation ponds as a unit process for the removal of organic pollution has in recent years been viewed as an alternate to activated sludge and trickling filtration processes. The low construction cost of

oxidation ponds, coupled with their dual physical and biological mode of aeration provides an attractive alternative to activated sludge and trickling filtration in locales where land values are not high. Their popularity among design engineers and pollution control regulatory authorities is from time to time subject to quite severe fluctuation. Some pollution control engineers condemn their use, while others staunchly defend their use.

Other than the economic factors involved, there are two key technical or scientific considerations which would seem to determine their ultimate utility and future status as an alternate secondary treatment process. The first consideration involves the problem of the separation of the suspended solids in the pond mixed liquor before the treated liquor is allowed to enter a receiving stream. The second concern involves the lack of knowledge concerning the net contribution of the algae to the oxygen supply in the pond. If the algae do not make a significant enough contribution to the net organic removal to overcome some of the pollution problems that they themselves can cause, then the essential premise for their use (inexpensive oxygen supply) breaks down, and the added operational expenditure of mechanical aeration or diffused air (thereby approaching an activated sludge) would surely seem warranted. It was felt that this concern represents a more critical aspect than solids separation insofar as the general or wide applicability of oxidation ponds is concerned, since if the algae do not supply enough  $O_2$  for the energy requirements of the organotrophic mode of metabolism responsible for removal of the organic compounds in a waste water, the process might not be highly recommended and the solids separation problem would not be encountered.

The present work was indeed designed as a multipurpose project, but the overriding aim of the work was to gain a more basic quantitative assessment of the contribution made by the algae to aeration in oxidation ponds. It is felt

that such information constitutes a needed input in the formation of engineering decisions concerning selection of unit processes in the design of pollution control facilities. Also, it was reasoned that the basic information obtained should be of use in understanding and interpreting changes in the oxygen balance in other semiquiescent bodies of water, e.g., lakes and reservoirs, which are not normally intended as biological pollution control facilities but which are nonetheless subject to the same mechanistic principles.

#### Experimental Rationale

It was reasoned that more progress toward accomplishment of the goals outlined above could be attained by controlled laboratory studies of a basic scientific nature than by field studies which are, in the main, not subject to acceptable scientific control. While field studies which relieve the investigator of the need to extrapolate from laboratory to field conditions could be designed, the underlying reasons for the results (which are of the same paramount significance in engineering research as in purely scientific research) might be less subject to delineation, and the work would not be of use to a wide spectrum of workers in the field or of any lasting significance. Accordingly, laboratory studies were designed to gain insight into the major forces tending to increase the dissolved oxygen and those tending to decrease the dissolved oxygen resources in oxidation ponds. These are listed below:

#### 1. Biological Deoxygenation

(a) Oxygen utilization due to the energy requirements of the aerobic heterotrophs in utilizing the organic carbon source represented by the pollutant in waste water: This process proceeds in both the light and dark periods.

(b) Oxygen utilization due to heterotrophic metabolism of the algae: This process would be expected to occur

during the dark period; also during this period the algae might be expected to assimilate organic carbon source.

## 2. Biological Oxygenation

(a) Production of oxygen due to the photoautotrophic metabolism of the algae: This would occur during the light period.

## 3. Gas Transfer across the Liquid-air Interface (Pond Surface)

(a) Physical oxygenation, transfer of  $O_2$  from atmosphere to pond, when the pond's dissolved oxygen is below the saturation level.

(b) Physical deoxygenation, transfer of  $O_2$  from pond to atmosphere, when the pond's dissolved oxygen is above the saturation level.

(c) Transfer of carbon dioxide in or out of the pond: The carbon dioxide content of the water can affect the oxygen resource, since it is a carbon source for the algae which produce oxygen. Carbon dioxide can also exert an effect on the efficiency of the pond, since the  $CO_2$  fixed in the algae becomes organic carbon not unlike the organic carbon in the waste which the pond was designed to remove.

Experimentation pertinent to the above aspects was conducted in batch and in continuous flow reactors operated on a lighting schedule intended to simulate day and night conditions, under total dark conditions, and in especially designed experimental ponds which were closed to the atmosphere but subject to light penetration. Analytical parameters employed in the investigation included dissolved oxygen, specific substrate analysis, chemical oxygen demand (COD), oxidation-reduction potential (ORP), pH, biological solids, and oxygen uptake. The experimental procedures and analytical methods employed for all experiments are

delineated in detail in two Master's theses and one doctoral thesis which comprise Appendices I, II, and III of this report. These also include a detailed description of the research results of the project, and discussion and conclusions drawn from the results. This report is intended to summarize the research findings and conclusions, and to point out and cross-reference the materials contained in the theses, but it is not intended to replace the detailed findings and discussions of the three theses which comprise the main body of the report. In the following "review of results" section, frequent reference is made to figures and tables in the three appendices. In all cases the reference is identified by figure or table number and appendix number.

#### REVIEW OF RESULTS

- I. Studies on Discontinuous Systems (Batch Ponds)
- A. Response to Single Organic Loadings at Various Concentrations of Organic Carbon Source

Constituents of the synthetic wastes employed in these studies are given in Table IV of Appendix I, and in Tables III and IV of Appendix II. Organic loadings (glucose) from 100 to 600 mg/l were applied, and the response was observed for a period of seven days (see Figures 12-29, Appendix I). Three different modes of operation were used: (1) synthetic medium with no added bicarbonate ion, twelve hours on-twelve hours off lighting cycle (system #1); (2) synthetic medium with bicarbonate ion added, twelve hours on-twelve hours off lighting cycle (system #2); and (3) synthetic medium with bicarbonate ion added, no light provided (system #3) (see also Table IV, Appendix I). Bicarbonate ion was added in some systems to provide a supplemental carbon source for the algae. Table I represents an attempt to summarize certain "key" data of Figures 12-29. For each system the overall biological solids production and the COD removal efficiency after two days' operation, the minimum DO recorded, the time to attain minimum DO, and the time to recover to the

TABLE I

Influent Glucose mg/l	Solids Production mg/l			COD Removal in 2 Days %			Minimum DO mg/l			Occurrence Minimum DO, Day			% Recovery (Period in Days)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
100	73	90	65	73	82	50	3.0	3.3	3.0	1	2	1	100 (3)	100 (3)	90 (5)
200	225	275	200	82	90	76	1.3	0.9	0.5	2	2	2	100 (6)	100 (4)	40 (7)
300	350	400	240	69	77	55	0.4	0.7	0.3	3	3	2	100 (6)	100 (6)	23 (7)
400	310	370	240	61	72	70	0.5	0.8	0.3	5	4	5	100 (7)	100 (7)	20 (7)
500	330	400	200	64	58	64	0.0	0.0	0.0	4	6	4	9 (7)	24 (7)	0 (7)
600	370	490	350	59	56	41	0.0	0.0	0.0	3	3	1	0 (7)	trace(7)	0 (7)

Data taken from Figures 12-29 of Appendix I.

Numbers 1, 2, and 3 designate modes of operation:

System 1 - 12 hr light, 12 hr dark, no added bicarbonate

System 2 - 12 hr light, 12 hr dark, bicarbonate added

System 3 - not lighted, bicarbonate added.



initial dissolved oxygen concentration, and/or percent recovery at the end of the experiment are given.

It can be seen that as the organic loading was increased there was an increase in biological solids production. In most systems the COD removal period lasted two days; as the organic loading was increased, the removal efficiency decreased. Anaerobiosis was prevalent at loadings of 500 and 600 mg/l glucose. The time required for the systems to recover dissolved oxygen increased with increased organic loadings. Concerning biological solids production, the system which received light and which contained sodium bicarbonate (system #2) produced more biological solids than did the system which received light but contained no added sodium bicarbonate (system #1). However, system #1 produced much more solids than did system #3, which contained sodium bicarbonate but received no light. There were no large differences in dissolved oxygen concentration between systems #1 and #2; however, much more oxygen was produced in systems #1 and #2 than in system #3, which received no light. The oxidation reduction potential of system #2, to which sodium bicarbonate was added, recovered to high values more effectively than did system #1. However, in system #1 the ORP values recovered more rapidly than in system #3. The trends given above held true in general over the entire range of loadings which were studied. In systems which received light, anaerobiosis, as measured by dissolved oxygen concentration, existed essentially until the end of the 7-day period for systems loaded above 400 mg/l. Systems receiving a load of 400 mg/l or less recovered completely in seven days or less. In systems which did not receive light, however, there was only a slight recovery in dissolved oxygen concentration at a loading level of only 200 mg/l glucose.

Pertaining to one of the most important parameters assessed, COD removal rate, there were no great differences between systems #1 and #2; however, the removal rates in

both of these systems were much greater than those observed in system #3, which received no light. This result would be expected at the higher loadings, where dissolved oxygen or oxygen tension might be expected to play a rate-limiting role in COD or substrate removal. However, it may be seen in Figures 12-17 (Appendix I) that the same trend, i.e., greater removal rate in systems receiving light than in the dark system, were observed for systems in which the dissolved oxygen concentration did not decrease to levels low enough to limit the rate of COD removal.

Similar studies at loadings of 100, 150, and 200 mg/l glucose verified these results (see Figures 2, 3, and 4, Appendix II). A series of studies under dark (no lighting) conditions was performed using mixed populations of algae and bacteria (see Appendix II, page 25 ff). In this series of experiments, open stirred reactors and open non-stirred (quiescent) reactors were employed. In addition, studies were conducted in stirred reactors which were closed to the atmosphere. Comparison of the results of the open and closed systems indicated that dissolved oxygen played an important rate-limiting role with regard to glucose and COD removal.

#### B. Response to Organic Loadings Applied at 3-Day Intervals (Four Feeding Cycles)

The results of these studies, employing organic loadings of 100 and 200 mg/l glucose, are shown in Figures 30-35 of Appendix I, and the COD removal efficiencies are summarized in Table VII (Appendix I). This series of experiments demonstrated increased COD removal efficiency due to photosynthetic oxygenation in systems which were subject to a twelve hours on-twelve hours off lighting cycle compared with the "dark" systems which depended solely upon physical reaeration for replenishment of the oxygen resource. At a loading of 100 mg/l glucose, a minimum dissolved oxygen residual of approximately one mg/l was maintained in both lighted systems (with and without bicarbonate). However,

at the 200 mg/l level, the system which was not supplemented with sodium bicarbonate experienced zero or extremely low DO concentrations during much of the experimental period. For the system containing bicarbonate (see Figure 34, Appendix I), higher DO levels were maintained.

### C. Response to Organic Loadings Applied Daily (Seven Feeding Cycles)

The results of these studies are shown in Figures 36-39 of Appendix I for daily loadings of 50 and 100 mg/l glucose. At the 50 mg/l loading level, all systems (i.e., bicarbonate-supplemented, 12-hr lighting; no bicarbonate supplement 12-hr lighting; and bicarbonate-supplemented, not lighted) experienced anaerobic conditions (zero DO) within two days. The dark system remained anaerobic throughout the 7-day period of operation, and the oxidation reduction potential (ORP) gave no indication of eventual recovery to aerobic conditions. In the lighted system which was not supplemented with bicarbonate ion, dissolved oxygen was absent at all times after the second day, but the ORP did not reach the low level attained in the dark system, and after the fourth day the ORP gradually rose to approximately -80 mv. The lighted system which received the bicarbonate ion supplement regained an aerobic condition after six days. The return of dissolved oxygen was preceded by a rapid rise in ORP. These results suggest that the oxidation reduction potential can be employed as a means of predicting imminent return to aerobic conditions in oxidation ponds which have turned anaerobic. Since the lighted system supplemented with bicarbonate ion exhibited the most favorable response at a loading of 50 mg/l glucose, another experiment at daily loadings of 100 mg/l glucose was made (see Figure 39, Appendix I). At this loading level the system became anaerobic during the second day, and there was no indication of recovery of aerobic conditions.

## II. Studies in Continuous Flow Systems

Continuous flow studies were made in specially-designed

laboratory oxidation ponds. Detention times employed in the ponds were ten days and twenty days. Mixed populations of bacteria and algae were employed; bacterial seed was obtained from sewage. In order to separate oxygenation and deoxygenation due to physical and biological processes, studies were conducted in experimental ponds which were closed to the atmosphere but which allowed light to enter the reaction liquor. In addition, experimentation was conducted in open ponds in which there were no algae present (i.e., operation with physical gas transfer alone).

#### A. Open Ponds With Algae

##### 1. Studies at a Detention Time of Ten Days

Two series of studies were made in open ponds employing a detention time of ten days. In the first series the mixed liquor reaction volume was thirty liters, and the medium used was that shown as #3 in Table II of Appendix III. In the second series of runs at this detention time, the volume of the mixed liquor was forty liters, and medium #6 of Table II in Appendix III was employed.

The organic loadings employed in the first series of runs were 100, 300, and 600 mg/l glucose. The 600 mg/l glucose loading produced anaerobic conditions. The effluent COD from the system receiving 100 mg/l glucose (Figure 40, Appendix I) attained a relatively stable value at the fourth day of operation, and the pond was approximately 80 per cent efficient with respect to COD. The dissolved oxygen, as well as the ORP, reached a minimum value on the second day of operation and recovered thereafter, reaching a more or less uniform level after the fifth day of operation. The dissolved oxygen (measured at the end of the light period) attained a steady value of approximately 14 mg/l. The system which received 300 mg/l glucose (Figure 41, Appendix I) yielded essentially the same response as was observed for the 100 mg/l loading. Relatively steady values for effluent COD, dissolved oxygen, and oxidation reduction potential were attained after five days of operation. COD removal

efficiency was approximately 85 per cent, and the dissolved oxygen at the end of each light period was approximately 8 mg/l. However, when the pond was subjected to an organic loading of 600 mg/l glucose (Figure 42, Appendix I), the initial DO was consumed in less than two days and dissolved oxygen was absent thereafter. The oxidation reduction potential dropped from +300 mv at the beginning of the experiment to -100 mv on the fourth day of operation. The ORP stayed at this level for the remainder of the experiment. It is interesting to note that, even though anaerobic conditions prevailed, the COD removal efficiency was slightly above 80 per cent. Figure 45 of Appendix I shows the results when the pond received this high glucose loading under conditions of continuous lighting. It is seen that even under continual daytime conditions, the dissolved oxygen in the pond could not be restored.

A second series of experiments using a 10-day detention time was conducted in systems open to the atmosphere. In this series of experiments organic loadings of 100, 150, and 250 mg/l glucose were employed. Also, a change in the sampling procedure was initiated. In this series, dissolved oxygen measurements were made at both the beginning and at the end of the light periods. In Figure 7 of Appendix III the response to a continuous loading of 100 mg/l glucose is shown. It is seen that under relatively stable conditions of operation the dissolved oxygen at the end of the light period was approximately 10 mg/l. The average effluent filtrate COD was equal to the influent COD. In addition, the biological solids level was approximately 90 mg/l, indicating that additional organic matter (other than that arising from the synthetic waste) was produced in the system. When the loading was increased to 150 mg/l glucose (see Figure 8, Appendix III), the average dissolved oxygen level at the end of the light period was close to 5 mg/l, and at the beginning of the light period (or end of the dark period) it was approximately 0.4 mg/l. The COD removal

efficiency was 46 per cent, and approximately 90 mg/l of biological solids were produced. For the system which received 250 mg/l glucose (Figure 9, Appendix III), the dissolved oxygen at the end of the dark period was always zero; however, at the end of each light period, approximately 10 mg/l of DO were present. The system was 76 per cent efficient with respect to COD removal.

It should be pointed out that the results of the continuous flow pond experiments using the 10-day detention time shown in Appendices I and III are not in agreement with respect to COD removal. For the aerobic systems of Appendix I (100 and 300 mg/l glucose feed), COD removals in the range of 80-85 per cent were recorded, whereas for comparable systems reported in Appendix III (100, 150, and 250 mg/l glucose feed), COD removal increased from zero to 76 per cent as the loading level increased. However, dissolved oxygen levels at the end of the light period were comparable (e.g., 8 mg/l at 300 mg/l glucose - Appendix I, and 10 mg/l at 250 mg/l glucose - Appendix III). The difference in the volume of the reactor and the synthetic waste previously noted would not appear to have contributed to the difference in COD removal efficiency. The incident light intensity for the experiments in Appendix III was slightly above 450 foot candles and was uniform across the surface of the pond. However, the light intensity reported (nominally) as 450 foot candles for experiments in Appendix I represents the surface intensity directly below the light. Since in these experiments three gro-lux lights were spaced along the tank whereas for the experiments in Appendix III lights were more closely spaced, it was reasoned that the overall surface intensity was lower for the experiments shown in Appendix I. Subsequent determination of light intensity showed that for these experiments the light intensity at various points along the axis of the tank varied from 150 to 400 foot candles. For the experiments of Appendix III, the surface light intensity was at (or close to) the value at

which incident intensity no longer exerts an influence on algal photosynthetic activity, i.e., it was above the so-called saturation level. The latter experiments seem more realistic with respect to light intensity, since surface values in excess of the saturation values are normally recorded during summer months in Oklahoma (5000-8000 ft-c). It should also be pointed out that the suspended solids (biological solids) concentrations recorded during the experiments shown in Appendix I were much higher than for experiments of Appendix III.

Observations of the color of the mixed liquor during both sets of experiments indicated that there was a greater proportion of algae present in the system during the experiments reported in Appendix III. Also, the anthrone analyses indicate that glucose was removed, i.e., most of the effluent COD for experiments reported in Appendix III was due to metabolic intermediates or end products which were most probably produced by the algae and were not usable substrates for the bacteria. Although the apparent ecological balance and the efficiency of COD removal for both sets of systems was drastically different at the lower organic loading level, it is significant to note that both sets of experiments indicate that for maintenance of aerobic conditions and acceptable COD removal efficiencies, the influent organic loading at the 10-day detention time is in the range 250-300 mg/l of organic carbon source. Expressed on the basis of areal loading, both sets of experiments yield essentially the same allowable loading, i.e., 62 lbs. COD/acre/day, and 63 lbs COD/acre/day for studies reported in Appendices I and III, respectively.

The possible deleterious effects on COD removal efficiency at lower organic loadings due to a phenomenon which may be termed overproduction of algae is delineated more fully in the discussion section of Appendix III.

## 2. Studies Employing a 20-Day Detention Period

In the first series of experimental runs employing the detention period of twenty days (Appendix I), systems were subjected to glucose loadings of 100, 300, 600, and 1000 mg/l (see Figures 46-52, Appendix I).

The initial dissolved oxygen in the pond which received 100 mg/l glucose (Figure 46, Appendix I) decreased to approximately one mg/l at the end of the second day of operation. However, after the fourth day, the dissolved oxygen level at the end of the light period was approximately 14 mg/l, and it remained at this level for the remainder of the operational period. The oxidation reduction potential followed the same trend as the dissolved oxygen level. The effluent COD (filtrate) attained a somewhat steady level after the fourth day, and the efficiency of COD removal amounted to approximately 85 per cent. Biological solids level attained a relatively stable value at approximately 240 mg/l. The oxidation pond system responded favorably to a continuous glucose loading of 300 mg/l (Figure 47, Appendix I). The biological solids reached a steady level of approximately 530 mg/l, and COD removal was approximately 88 per cent. The dissolved oxygen at the end of the light period was 7.5 mg/l. At a loading of 600 mg/l (Figure 48, Appendix I) the dissolved oxygen in the system dropped to zero for two days, and thereafter reached a value of approximately one mg/l at the end of each light period. The COD removal efficiency was slightly above 80 per cent. Biological solids concentration attained a relatively steady level, approximately 760 mg/l. When the oxidation pond system was loaded at a level of 1000 mg/l glucose (Figure 51, Appendix I), anaerobic conditions were attained in two days, and the system never recovered to an aerobic condition during the period of operation. The COD removal efficiency was close to 70 per cent, and the biological solids level was approximately 530 mg/l. Operation at the same loading level under conditions of continuous lighting did not restore aerobic conditions.



## B. Closed Systems with Algae

In this series of experiments (see Appendix III) airtight ponds, closed to the atmosphere, were employed, and continuous loading levels of 100, 150, and 250 mg/l glucose were employed at a detention time of ten days. In addition, 500 mg/l glucose was continuously fed to a system employing a 20-day detention period.

The dissolved oxygen in the system subjected to a continuous loading of 100 mg/l glucose (Figures 10, 11, 12, Appendix III) was at all times at supersaturated levels (even at the end of the dark period). The average daily change in dissolved oxygen levels was approximately 12 mg/l. COD removal efficiency (based on filtrate COD) was approximately 59 per cent. At a loading level of 150 mg/l glucose (Figures 13, 14, Appendix III) the response was similar to the one observed at the previous loading. The daily dissolved oxygen change was 14 mg/l, the COD removal efficiency was 61 per cent, and the biological solids concentration was 59 mg/l. Two studies were made at a loading level of 250 mg/l glucose (Figures 15, 16, 17, Appendix III). In the first experiment (Figures 15 and 16), dissolved oxygen in the unit gradually decreased until on the eighteenth day zero DO was attained at the end of the dark period. Following this, an attempt was made to regenerate the system by providing continuous lighting for a 3-day period followed by another 3-day period during which the lights were left on for fifteen hours in each twenty-four. On the seventh day the system was again operated at the original lighting cycle of twelve hours on and twelve hours off. Figure 17 of Appendix III shows the performance of the "regenerated" system after the regular lighting schedule was initiated. After the regeneration period, the average daily dissolved oxygen change was 13.5 mg/l. Biological solids concentration was 73 mg/l in both systems, i.e., Figures 15 and 16, and Figure 17. The COD removal in the first portion of the study was 86 per cent, and in the regenerated system, 80 per cent.

The closed system which was operated with a detention period of twenty days at the relatively high organic loading of 500 mg/l glucose attained anaerobic conditions (Figure 18, Appendix III).

#### C. Open Systems Without Algae

These systems were operated in total darkness, and there was no opportunity for biological aeration. The system which was operated at a loading level of 50 mg/l glucose (Figure 19, Appendix III) remained aerobic and contained at all times between three and four mg/l dissolved oxygen. COD removal efficiency was nearly 70 per cent. When the feed concentration was increased to 80 mg/l glucose (Figure 20, Appendix III), the dissolved oxygen level near the influent end of the pond was approximately 0.3 mg/l, indicating that at this loading level the system was near its capacity with respect to maintenance of aerobic conditions. At this loading level, 76 per cent of the influent COD was removed. At a loading level of 120 mg/l glucose (Figure 21, Appendix III), approximately one mg/l DO was maintained in the pond effluent and the removal efficiency was approximately 81 per cent.

#### D. Gas Transfer by the Physical Process

Reaeration as well as deaeration studies were conducted in the laboratory oxidation ponds in order to determine the mode of kinetics for both processes, and the values of the kinetic constants. It was found that both processes went forward in accordance with first order decreasing rate kinetics, and that the velocity constants for reaeration and deaeration were the same. Results for experiments of this type are presented in Figures 22-25 in Appendix III, and Figures 5 and 6 in Appendix I.

#### E. Bacterial Deoxygenation Studies

Deoxygenation by heterogeneous bacterial populations was investigated, employing substrate concentrations which had been previously found to exist in the oxidation ponds

during "balanced" or relatively steady operation during the continuous flow studies. Investigations were conducted in the experimental ponds as well as in standard BOD bottles (see Figures 26-33, Appendix III). At the substrate concentrations employed, increasing first order rates of deoxygenation, typical of the kinetics which exist in a logarithmic growth phase, were observed. The first order rates were calculated, and it was found that the values increased with increasing substrate concentration for studies conducted in the ponds as well as those conducted using BOD bottles. The velocity constants calculated from the BOD bottle data were on the average 25 per cent lower than the rate constants calculated from data collected in the ponds.

#### E. Deoxygenation by Algae (*Chlorella pyrenoidosa*)

Although some preliminary studies were conducted using what might be termed heterogeneous populations of algae, a decision was made to use a pure culture of a particular species, *Chlorella pyrenoidosa*, since it is one commonly found in oxidation ponds. Also, a considerable amount of information is available on its photosynthetic and organotrophic metabolism. In addition, it was easier to check for evidence of contamination by bacteria for this single algal species rather than in a heterogeneous mixture of algae.

Oxygen uptake and other related parameters for this culture were investigated for various initial concentrations of biological solids (Figures 34-41, Appendix III). At the highest initial biological solids concentration (Figure 34, Appendix III) 896 mg/l, the oxygen uptake curve followed zero order kinetics during the period of active substrate removal. When lower initial algal concentrations were used, e.g., 215 and 113 mg/l (see Figures 37 and 38, Appendix III), the oxygen uptake curves were more characteristic of curves indicating the existence of a logarithmic growth phase for the algae. For systems employing initial biological solids concentrations between the highest and lowest values used,

e.g., 448 and 400 mg/l (see Figures 35 and 36, Appendix III), the rate of oxygen uptake approached zero order kinetics and only a slight curvature of the accumulated oxygen uptake curve was noted. In all experiments both the COD and carbohydrate tests for substrate were made, and there was no indication that metabolic intermediates and/or end products accumulated during the substrate removal period. It should be noted that the algal cells used in this experiment had been previously grown up under photosynthetic conditions and then immediately subjected to conditions of organotrophic metabolism. It is noteworthy that the cells could switch from photosynthetic to organotrophic metabolism with considerable ease. Experiments conducted under endogenous conditions (absence of any carbon source) are shown in Figures 39, 40, and 41 of Appendix III. After a brief interval in which first order decreasing rate kinetics were observed, the oxygen uptake curve assumed linearity, i.e., zero order kinetics prevailed.

#### SUMMARY, DISCUSSION, AND CONCLUSIONS

This section of the report is intended to point out in summary fashion some aspects of the analysis of the results and interpretation placed upon the results which led to rather general conclusions concerning basic kinetic and mechanistic modes of operation in oxidation ponds. More detailed discussion and conclusions drawn from the various portions of the work can be found in appropriate chapters in Appendices I, II, and III; particular attention is drawn to the discussion section of Appendix III.

Comparison of experiments run in the open and in the closed ponds and calculations made to approximate a carbon balance for the ponds provided evidence that atmospheric  $\text{CO}_2$  was fixed by the algal population in the open pond, and that at low loadings (e.g., 100 mg/l glucose) the effect of carbon fixation by the algae was to make of the oxidation pond a reactor for growing algae rather than a reactor for treating the synthetic waste water. Approximately 100 per

cent more carbon was present in the effluent than was present in the influent. At a loading of 150 mg/l, approximately 25 per cent more carbon was present in the effluent than in the influent, whereas when the loading was increased to 250 mg/l glucose, the overall carbon content of the effluent was approximately half that of the influent. Thus it may be discerned that the use of lower organic loadings in oxidation ponds instead of providing a safety factor, actually militates against the success of the oxidation pond as a secondary treatment process. It was also found that closing the pond to the atmosphere significantly increased the purification efficiency at the lower loadings, and there was a moderate increase at the higher loadings. Provision of a gas-impermeable transparent cover for field installations could prevent the transfer of carbon dioxide from the atmosphere into the pond, and it could also prevent the escape of valuable dissolved oxygen from the pond during daylight hours when a condition of supersaturation with respect to DO exists. Although the provision of an impermeable membrane does not seem impossible from an engineering point of view, it cannot be recommended without further study at the field pilot plant level.

The results obtained in the laboratory investigation indicated that an open pond which did not contain algae could be operated at a loading level of 80 mg/l glucose with maintenance of aerobic conditions at all times, while a comparable pond which was operated under the twelve hours on-twelve hours off lighting cycle could accept a loading of 250 mg/l glucose with maintenance of aerobic conditions in the pond. From these data it would appear that oxygenation from the biological source contributed approximately twice as much oxygen to the  $O_2$  resource in the pond as was contributed from the physical aeration mechanism. An analysis of the similarities and differences between the laboratory conditions and field conditions indicated that the proportional contributions of biological aeration and physical

aeration observed in the laboratory studies could be extrapolated to the field condition, and the general conclusion that for field installations the net contribution to the oxygen resource of an oxidation pond by the algae is approximately twice that by physical aeration has been made. There would, of course, be times when the proportional contribution would be greater or less than 2:1, e.g., seasonal changes (light intensity and duration and temperature) could shift the ratio; however, it is suggested that the 2:1 figure concluded on the basis of the laboratory investigations provides a good average estimate for ponds loaded at reasonable levels expected to provide for aerobic conditions at all times.

Based upon the results of this investigation, the following conclusions seem warranted:

1. Oxidation ponds operated at relatively low loadings cannot be said to be in biological balance, since a significant portion of the algal carbon source,  $\text{CO}_2$ , is not provided by the bacteria, and unless an adequate means of removing the algae from the pond effluent is provided, the pond can contribute more carbon to the receiving stream than is removed from the waste water.

2. In general, due to physical oxygen exchange processes across the liquid interface of the pond, more oxygen can be lost to the atmosphere than is gained from it. This situation arises because the velocity constant for oxygenation is approximately equal to the velocity constant for stripping of oxygen from the pond, and the driving force (the difference between the DO concentration and the saturation value) very often exceeds the driving force for undersaturated conditions.

3. The adverse conditions caused by a net gain of  $\text{CO}_2$  from the atmosphere and a possible net loss of dissolved oxygen to the atmosphere might be alleviated by covering the ponds with a gas-impermeable, light-penetrable material, e.g., a "Saran"-type membrane attached to a floating grid.

This conclusion is based on the finding that closed ponds increased the purification efficiency significantly at lower organic loadings and moderately at higher loadings.

4. It seems reasonable to predict that in field installations the algae contribute, on the average, approximately twice as much oxygen to the oxygen resource of the pond as does physical reaeration.

5. The similarity between the kinetic modes of oxygen uptake and substrate removal observed in other studies performed in this laboratory using heterogeneous microbial populations and in the present studies using Chlorella under heterotrophic conditions suggest that the biological solids concentration exerts a general effect on the kinetics of substrate removal and cell growth under heterotrophic conditions.

In general, it is felt that the conclusions which it has been possible to make through the conduct of this research work have fulfilled the primary objective of the research project, i.e., to gain an insight into the net relative contribution to the oxygen resource in semiquiescent waters made by the process of biological aeration and by physical aeration across the surface of the quiescent body of water. The work was not intended to serve as a basis for either recommending or not recommending the use of oxidation ponds as a secondary treatment process for organic waste waters. There are, in addition to the scientific and engineering aspects, various aesthetic, social, and economic considerations which come into play in determining the optimum selection of a treatment process for any particular situation or locale. There are some locales where aesthetic conditions may not be particularly important, and where it may not even be desirable or necessary to maintain aerobic conditions. However, these aspects are usually considered to be important in most locations where oxidation ponds are used, and the finding that biological reaeration provides only double the amount obtained by simple physical

transfer from the atmosphere does not appear to the investigators to provide a sufficient margin of gain to suggest that oxidation ponds provide a comparable alternative to activated sludge and trickling filtration processes for the removal of organic carbon.

The conduct of this research project has also suggested some avenues of possible future research which may prove to yield fruitful results. For example, studies are now under way in the laboratory into the mechanistic and kinetic mode of substrate removal by Chlorella under strictly heterotrophic conditions. Also, the results of the present study indicate that a pilot plant scale field study of open and closed oxidation ponds (see suggestion in conclusion #3) might provide interesting and useful results. Also, the work has provided an investigational interest in the possible use of algae for the removal of excess nitrogen and phosphorus rather than carbon in waste waters, and has engendered preliminary investigations into possible engineering solutions for the problem of the separation of the algae from a mixed liquor.



Y. C. Wu  
Master's Thesis

EFFECT OF ORGANIC LOADING ON REAERATION IN  
SEMI-QUIESCENT WATERS

APPENDIX I

**EFFECT OF ORGANIC LOADING ON REAERATION IN  
SEMI-QUIESCENT WATERS**

By

**YEUN CHENG WU**

**Bachelor of Science**

**Taiwan Provincial Cheng Kung University**

**Tainan, Taiwan, Republic of China**

**1963**

**Submitted to the faculty of the Graduate  
School of the Oklahoma State University  
in partial fulfillment of the  
requirements for the degree  
of  
MASTER OF SCIENCE  
May, 1967**

**EFFECT OF ORGANIC LOADING ON REAERATION IN  
SEMI-QUIESCENT WATERS**

**Thesis Approved:**

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**Thesis Adviser**

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**Dean of the Graduate School**

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## CHAPTER I

### INTRODUCTION

The purposeful addition of waste to ponds began in ancient times in the Orient and Europe. Today, in many parts of the world, ponds are purposefully fertilized with organic wastes as well as with inorganic fertilizer in order to encourage the growth of algae, thereby increasing the yield of fish. Edminston (1) has described the technology and technical philosophy of fish culture ponds and their development from ancient to modern times. Purification of sewage in fish ponds has been a recognized art in Germany for many years (2). In America, fish ponds have not been used for sewage treatment (3).

The first stabilization or oxidation ponds in the United States were not apparently built as treatment devices, but for the purpose of withholding wastes from receiving streams where their presence would be objectionable. However, the waste purification potential of such ponds was quickly realized. Since Gillespie's (4) description of the ponds of Santa Rosa, California, which were built in 1924, there has been a succession of papers describing one or several specific pond installations (5,

6, 7, 8) and a number of papers and articles which attempt to place pond design on an increasingly rational basis (9, 10, 11, 12). A comprehensive review on stabilization ponds has been published by Fitzgerald and Rohlich (13).

According to a report of the American Society of Civil Engineers published in 1957, oxidation ponds have been widely employed for the treatment of domestic and industrial wastes throughout the United States and in many countries of the world (14). The number of oxidation ponds presently employed to treat purely industrial wastes in the United States and elsewhere in the world is not accurately known.

The name "oxidation pond" is a more recent term used to describe the storing of waste water in artificial reservoirs for the purpose of reduction of organic loading by natural processes. Such ponds have also been called "stabilization lagoons," "stabilization ponds," and simply "lagoons."

The oxidation pond is a shallow, earthen storage tank in which raw or partially treated waste water is held for a period of time, usually from ten to thirty days (15). Dense growths of algae develop in the ponds and produce a large amount of oxygen which is utilized in satisfaction of the energy requirement for heterotrophic bacteria. The organic material in the waste water serves as carbon source for the heterotrophic bacteria and the carbon dioxide produced as an end product of bacterial metabolism serves as a hydrogen acceptor in the metabolic processes of the photo-

synthetic algae. The algal synthesis product may be expressed by the general formula  $CH_2O$ , which has been termed "primary cell" material (16).

It should also be remembered that oxidation ponds may be employed as a pre-treatment process in which the pond serves as a surge tank or reservoir to equalize the effect of peak loadings on a sewage treatment plant. It can also serve to dilute concentrated waste, and can provide an additional settling basin for sewage treatment (17).

The rising cost of secondary treatment of waste waters, the increasing population, industrialization, urbanization, etc., together with the general drive for improved health standards and esthetic character contribute to the magnitude of the waste water problem, and the need to provide adequate waste water treatment at economical costs. For small communities, the use of oxidation ponds for secondary treatment of wastes appears to hold the most promising economic answer. Also, for larger towns where other secondary treatment processes have already been in operation for many years, the use of oxidation ponds as a possible tertiary treatment measure would appear to hold promise.

Although oxidation ponds have been in use for a number of years as a second treatment process (10, 18), much information remains to be uncovered concerning the amount of organic loading that can be successfully treated, and

there is much still to be learned concerning the engineering possibilities for creation of the most favorable conditions for the photosynthetic processes. Some general surveys of the algae occurring in oxidation ponds have been made (19, 20, 21), the most extensive being reported by Silva and Papenfuss (22). However, a study of successive organic loadings on oxidation ponds does not appear to have received extensive attention in previous studies.

The purpose of the present study was the investigation of effects of organic loading in oxidation ponds. Controlled laboratory experiments were conducted in both batch, or discontinuous, systems and in continuous flow culture systems. In these studies both organic loading and detention time were varied. One of the most important aspects of the study was the determination of the amount of oxygen made available by the photosynthetic process. Dissolved oxygen concentration was monitored in all experiments since it is perhaps the most critical parameter for determining or predicting the conditions in an oxidation ponds.

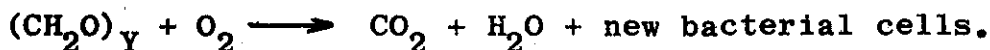


## CHAPTER II

### THEORY AND MECHANISM OF BIOLOGICAL WASTE TREATMENT IN OXIDATION PONDS

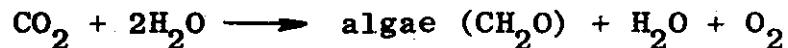
#### A. The Role of Photosynthesis

A diagram of the biological processes prevalent in oxidation ponds is shown in Figure 1. In oxidation ponds two biological phases will exist, i.e., the bacterial phase and the algal phase. The bacterial phase may consist of three different biological systems: 1) aerobic bacterial metabolism, under essentially aerobic or under totally aerobic conditions, 2) an acid-forming bacterial phase, and 3) a methane-forming bacterial phase (3). It should be noted that the acid-forming and methane-forming phases exist under anaerobic or possibly facultative conditions. In shallow ponds with an active algal phase in operation, the photosynthetic production of oxygen does much to assure that the aerobic bacterial phase exists in the system. In aerobic ponds the overall bacterial metabolism may be represented as follows:



In the above equation  $(\text{CH}_2\text{O})_Y$  represents the organic matter in the waste water. This material is decomposed

fairly rapidly in water due to its availability as food for microorganisms. Complex organic material in waste water is converted into simple substances which may be readily utilized by the bacteria. A considerable portion of the original carbon sources in the waste water is converted to new bacterial cells, and the portion from which energy is extracted to produce the new bacterial cells may be roughly equated to the amount of  $\text{CO}_2$  which is produced as an end product of aerobic metabolism. This aerobic process would soon come to a halt if oxygen were not continuously supplied to the system. Oxygen may be supplied by biological means in accordance with the following equation:



From a chemical standpoint, the growth of algae may be characterized by two overall processes, i.e., photosynthesis and secondary synthesis. In the photosynthetic process, carbon dioxide is converted to carbohydrate and oxygen is produced. Illumination is required for the overall photosynthetic reaction. The secondary synthesis involves the conversion of carbohydrate to other biochemical compounds which the algae need in order to reproduce and grow; e.g., such compounds as lipids, proteins, nucleic acids, etc. The cyclic patterns characterizing such metabolic processes are indicated by the double circles in Figure 2 (23).

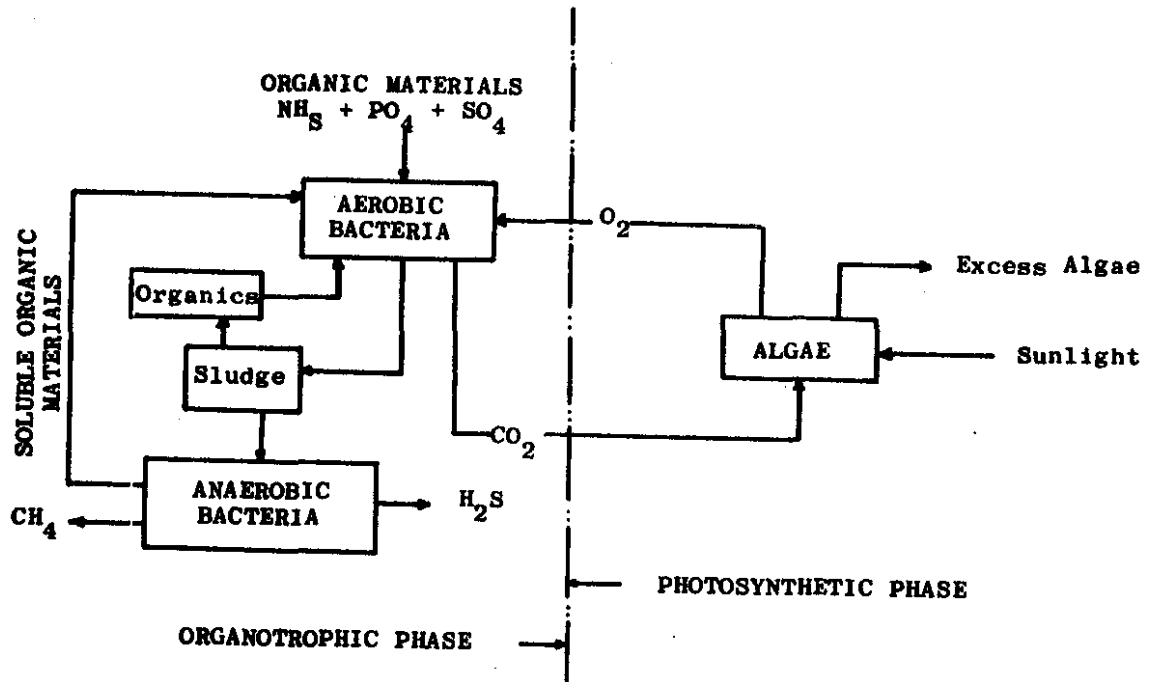


Figure 1. Schematic Representation of Major Biological Cycles Which may occur in Oxidation Pond.

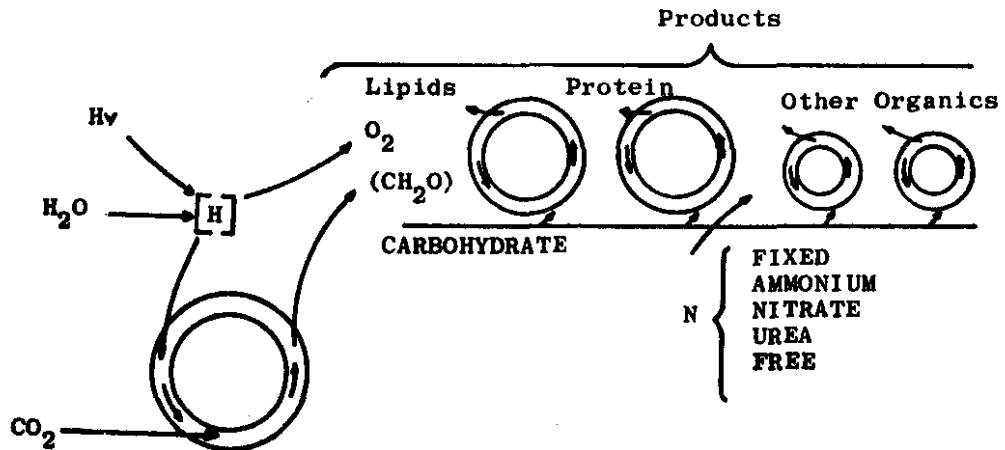


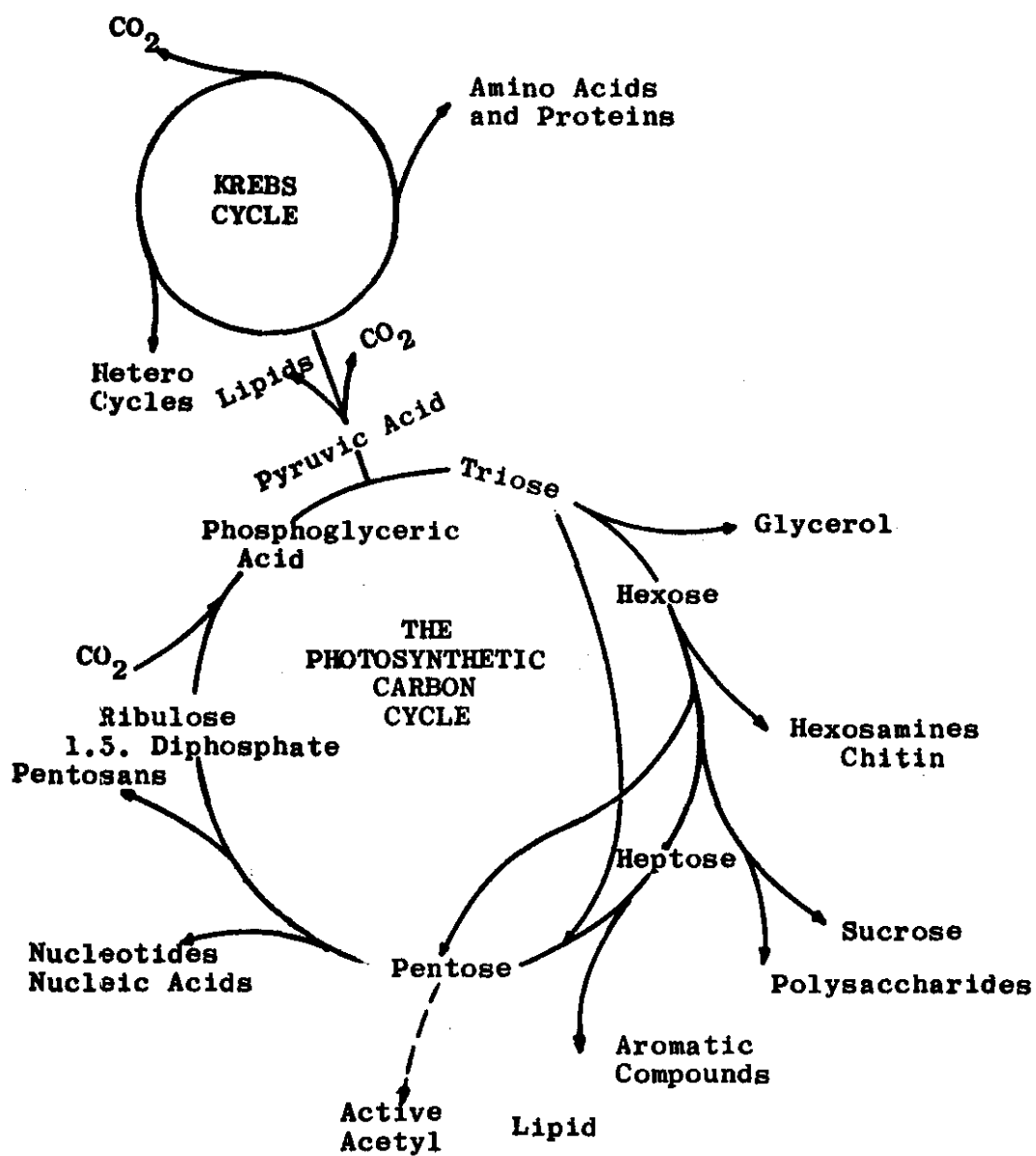
Figure 2. Simplified Diagram of Algal Cell Synthesis.

## B. The Path of Carbon Photosynthesis

The pathway of carbon fixation in photosynthesis may be considered as a series of reactions starting with  $\text{CO}_2$  and ending with the fixing of the carbon in  $\text{CO}_2$  into a carbohydrate storage compound (24). In its most simple form, the reaction can be given as follows:

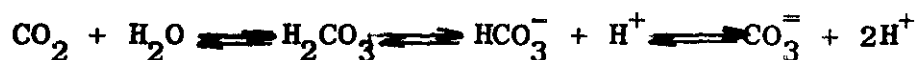


where  $(\text{CH}_2\text{O})$  represents the carbohydrate and  $(\text{H})$  represents the reducing agents or the "reducing power" derived from the light reaction. The energy stored in the form of carbohydrate can be recovered by the algae in the form of ATP synthesized in oxidative phosphorylation processes during respiration. Figure 3 shows a general metabolic diagram depicting the cyclic synthesis of essential intermediates needed by the algae (25). It is seen that the only carbon compound which enters the cycle is  $\text{CO}_2$ . The first stable product of carbon assimilation in photosynthesis is 3-phosphoglyceric acid. Some of the carbon which is fixed may be oxidized by the Krebs cycle, and much of the ATP which is obtained by the algae in the absence of light most probably arises through the functioning of this oxidative pathway. The major source of ATP, however, is photophosphorylation. The key process in the pathway of carbon in photosynthesis is quite naturally the fixation of carbon dioxide. In aqueous solution, carbon dioxide is present in various forms in equilibrium with each



**Figure 3.** Carbon Compound Synthesis from Carbon Dioxide After the Path of Carbon In Photosynthesis By J. A. Bassham, 1957.

other (26). This equilibrium may be represented as follows:

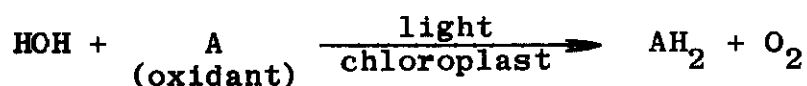


In the first reaction,  $\text{CO}_2$  combines with the five-carbon acceptor, ribulose diphosphate, to form two molecules of phosphoglyceric acid. The reaction may be described as follows:

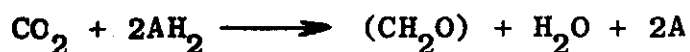
Ribulose-1, 5-diphosphate  $\xrightarrow{\text{CO}_2}$  2-carboxy, 3-keto-1, 5-diphosphate  $\longrightarrow$  2 phosphoglyceric acid. Basshams and Calvin (27) have found that the reductive carboxylation occurs in the light and produces two molecules of 3-phosphoglyceric acid (PGA). The triosephosphates which are produced are either combined to form hexose or are utilized directly in the regeneration of ribulose diphosphate. The net effect of the cycle shown in Figure 3 is the conversion of three molecules of carbon dioxide to triosephosphate; this reaction is brought about at the expense of six molecules of  $\text{NADPH}_2$  and nine molecules of ATP.

While some of the ATP needed by the algae is undoubtedly produced through the oxidative phosphorylation during the oxidation of some of the carbohydrate compounds which are produced, the major source of ATP and of reducing power ( $\text{NADPH}_2$ ) comes about during the light period and involves the activation of pigments such as chlorophyll A, chlorophyll B, and carotenoids which provide the initial

production of "assimilation power" (ATP and NADPH<sub>2</sub>) required for the assimilation of carbon dioxide which takes place in the dark, as shown in Figure 3. These very important light reactions involving the chlorophylls are not yet well understood, and a detailed discussion of the various theories which have been proposed is somewhat beyond the scope of the present discussion. However, it may be stated that one of the primary functions of the light reaction is the photolysis of water. In 1937, R. Hill (28) presented evidence which suggested that the effect of light is to cause a splitting of water with a consequent reduction of a hydrogen acceptor. If the hydrogen acceptor is designated as some oxidant (A) the overall reaction may be written as follows:



In the above equation AH<sub>2</sub> represents reducing power which the algae use to fix CO<sub>2</sub> in accordance with the following reaction:



The Hill reaction explains in some respects the production of reducing power needed to fix carbon dioxide, but tells little concerning the production of energy in the form of ATP which is needed to drive the synthetic reaction.

Arnon (29) has proposed that the primary light reaction is the activation of an electron in chlorophyll

raising it to a higher energy level. This higher energy level is very unstable, and when the electron falls back to a lower level, the energy released can be trapped as ATP. A detailed description pertaining to the electron flow mechanism can be found in the literature (30).

C. Influence of External Factors on Rate of Photosynthesis in Oxidation Ponds

Although there is much yet to be learned concerning the precise chemical mechanisms involved in the photosynthetic process, it is possible to gain information which can be used in the engineering design of oxidation ponds by studying the external factors which exert some control over the photosynthetic process. Some of the most important factors which should be considered are: carbon dioxide concentration, light intensity, and temperature.

1. The Effect of CO<sub>2</sub> Concentration

Blackman and Smith (31) found that at high light intensity and at constant temperature the rate of photosynthesis in water was proportional to the concentration of carbon dioxide. However, above a certain concentration, further increases in carbon dioxide concentration have no effect on the rate of photosynthesis. Warburg (32) working with Chlorella found that the rate of photosynthesis was proportional to the carbon dioxide



concentration within the range of 0.05 to 10.0 mg/l (32). Above the upper concentration, further increases in carbon dioxide resulted in smaller and smaller increases in photosynthesis. Brown noted that for any given light intensity, a point is reached where increasing the CO<sub>2</sub> concentration does not affect the rate of photosynthesis; however, if a higher light intensity is used, a higher CO<sub>2</sub> concentration can be utilized (33).

## 2. The Effect of Light Intensity

The first research on the influence of light intensity on the photosynthetic process appears to be that of Daubeny in 1886. He concluded that photosynthesis was directly proportional to the light intensity (34). Oswald (35) reported that the effective range of light intensity for algal production in oxidation ponds lies between 400 ft. candles and 800 ft. candles.

## 3. The Effect of Temperature

It is well known that the rate of many chemical reactions is considerably affected by temperature. The photosynthetic activity of chloroplasts is readily lost at temperatures above 45°C. Thus, chloroplasts exhibit a higher degree of thermolability than is characteristic of many enzyme systems (31). Matthali found that with increasing temperature photosynthesis increased to a maximum between temperatures of 15 and 25°C. and thereafter

fell off as the temperature was further increased (36). Precise information concerning the effect of temperature on the operation of oxidation ponds is not abundantly available. It is difficult to separate the effects of temperature on the photosynthetic process and on the heterotrophic metabolic processes as they affect overall oxidation pond efficiency; also, in the field, changes in temperature are very often associated with changes in light intensity. It is known (21) that temperatures of 8-13°C. do not necessarily interfere with the production of large algal crops, nor do they hinder the effective operation of ponds with respect to BOD removal. Oxidation ponds are successfully operated in North Dakota during periods in which they are covered by a layer of ice (8).

## CHAPTER III

### MATERIALS AND METHODS

#### A. Experimental Apparatus

##### 1. Batch Unit

The experimental ponds used in these studies were constructed in accordance with the following specifications:

Materials: Plate glass fixed by aluminum frames

Width : 28.5 cm

Length : 48.6 cm

Depth : 27.2 cm

Total surface area of pond: 1387 cm<sup>2</sup>

Total volume of the pond : 36 liters.

The ponds were illuminated by two light sources:

Three gro lux lamps (F15t8-GRO, Sylvania) were placed transversely across the pond at a distance of three inches from the water surface. In addition, the ponds were placed directly below two soft white fluorescent ceiling lights (40W). The surface of the water was three feet from the ceiling lights. These light sources combined yielded an incident light intensity of 450 ft. candles at the surface of the pond. Each pond was equipped with a rubber sampling siphon which was used to transfer samples to BOD bot-

ties for determination of dissolved oxygen.

## 2. Continuous-flow Unit

The bench scale oxidation ponds used in the continuous-flow studies were of the same dimensions as those used in the batch studies. A side view of the experimental setup is shown in Figure 4. Feed was admitted to the oxidation ponds from a 10-liter constant head feed tank; the flow was metered through a 10 ml biuret attached to the line from the constant head feed tank. The oxidation ponds were fitted with three outlets, as shown in the figure. The placement of the outlets allowed sampling at three depths in the tank.

## B. Seeding Population

### 1. Heterogeneous Microbial Seed

The heterogeneous microbial seed used in most studies herein reported was obtained from the primary clarifier effluent of the municipal waste water treatment plant in Stillwater, Oklahoma. In a few experiments, seed was obtained from a laboratory activated sludge unit, and this is noted in the protocol for the individual experiment.

### 2. Algal Seed

In order to ensure development of an algal population which would be somewhat typical of that found in an oxidation pond, and in order to enhance the possibilities

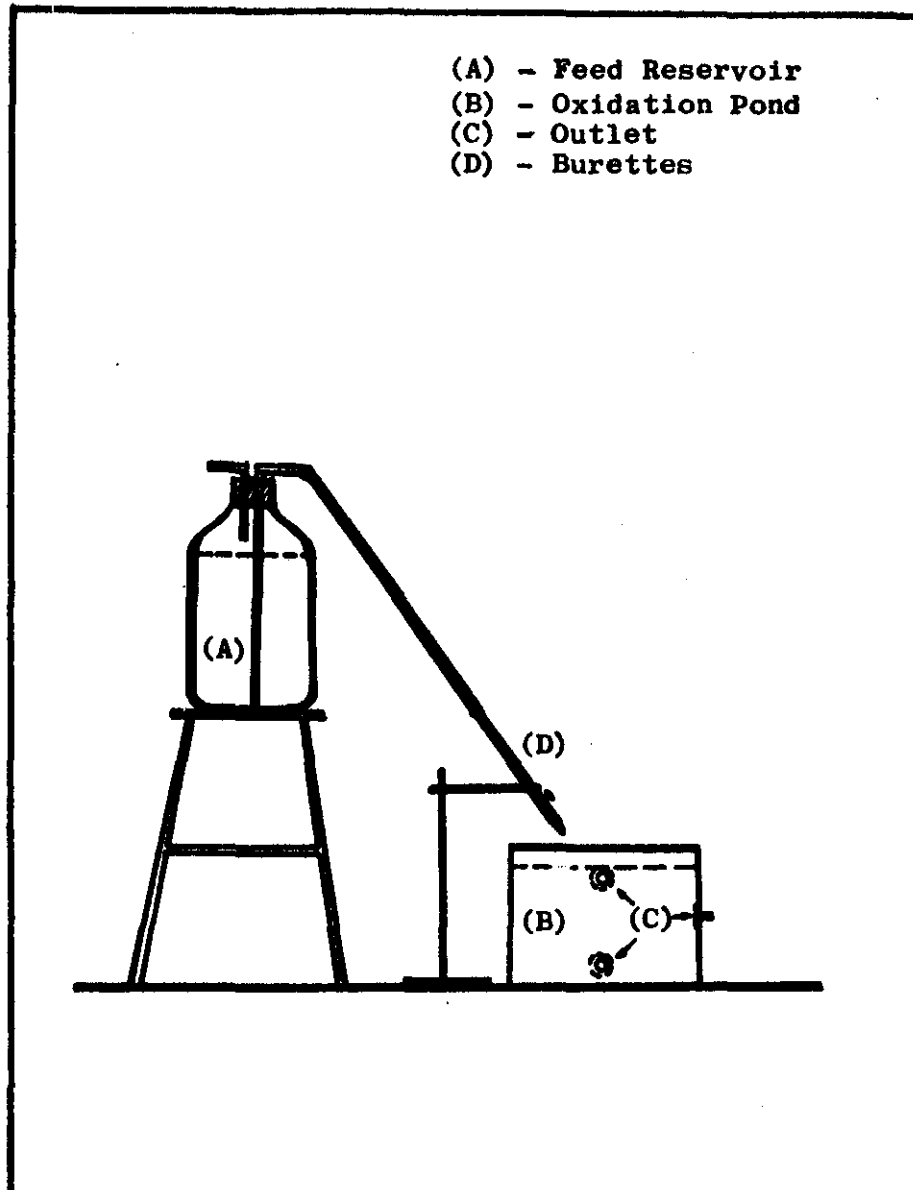


Figure 4. Schematic Representation of the Continuous Flow Oxidation Pond.

of the predominance of a dispersed algal culture, the ponds were seeded with a pure culture of Chlorella pyrenoidosa. The culture was obtained from the Department of Agronomy at Oklahoma State University. A stock algal seeding population was maintained in the laboratory in one of the batch oxidation ponds which was fed a high concentration of algal growth medium (see next section for description of medium). No attempt was made to maintain a pure culture of Chlorella in the stock seeding material or in the oxidation pond studies. This stock algal seed was maintained primarily to ensure a healthy algal seed at the initiation of each experiment and one for which sedimentation would be minimal.

### C. Synthetic Wastes

#### 1. Standard Synthetic Waste

The chemical constituents of the standard synthetic waste used throughout these studies are shown in Table I.

TABLE I  
STANDARD SYNTHETIC WASTE

Constituent	Concentration
Glucose	1000 mg/l
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	500 mg/l
Mg(SO <sub>4</sub> )·7H <sub>2</sub> O	100 mg/l
FeCl <sub>3</sub> ·6H <sub>2</sub> O	0.5 mg/l
MnSO <sub>4</sub> ·H <sub>2</sub> O	10 mg/l
CaCl <sub>2</sub> ·2H <sub>2</sub> O	7.5 mg/l
Trace Elements (Tap Water)	100 ml/l
*1.0M potassium phosphate (pH 6.8)	40 ml/l
*K <sub>2</sub> HPO <sub>4</sub> : 107	grs/0.5 l
KH <sub>2</sub> PO <sub>4</sub> : 52.7	grs/0.5 l

## 2. Algal Growth Medium

The chemical constituents of the medium used to promote the growth of algae are shown in Table II.

TABLE II  
STOCK ALGAL GROWTH MEDIUM<sup>(37)</sup>

Constituent	Concentration
Potassium Sulfate	75 gm/l
Ammonium Nitrate	150 gm/l
pH Salt Mixture	75 gm/l

It is seen in Table II that 75 gm/l of "pH salt mixture" were used. The chemical constituents of this mixture are given in Table III.

TABLE III  
STOCK pH SALT MIXTURE<sup>(37)</sup>

Constituent	Concentration
Dipotassium Phosphate	24.0 gm/l
Sodium Chloride	22.5 gm/l
Magnesium Sulfate (hydrate)	12.5 gm/l
Calcium Phosphate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	5.6 gm/l
Ferric Citrate	2.06 gm/l
Manganese Sulfate	0.37 gm/l
Potassium Iodide	0.06 gm/l
Copper Sulfate	0.03 gm/l
Zinc Chloride	0.02 gm/l
Cobalt Chloride	0.004 gm/l

## D. Analytical Techniques

### 1. Removal of Organic Matter

#### (a) Chemical Oxygen Demand (COD) Test

The chemical oxygen demand (COD) test is widely accepted as a measure of the pollutional strength of waste waters. It is based on the principle that organic matter

can be oxidized to carbon dioxide and water under the standard conditions of the test. In the present study the COD technique was used to measure the substrate remaining in solution after passing the mixed liquor through a membrane filter. In all studies the COD test was run in accordance with procedures given in the 11th edition of Standard Methods (38), and in all cases the silver sulfate catalyst was employed.

(b) Anthrone Test

In addition to measuring removal of organic matter by the COD test, the anthrone test was employed to determine the amount of total carbohydrate remaining in the filtrate. The test was run according to procedures given by Gaudy and co-workers (39, 40). The use of the anthrone test in addition to the COD test afforded the possibility of determining whether the microbial population produced from the original carbohydrate substrate any noncarbohydrate metabolic intermediates which were elaborated into the medium.

## 2. Biological Solids Determination

Biological solids concentration was determined by the membrane filtration technique, as described in Standard Methods for the Examination of Water and Waste Water (38). In all cases the membrane filter pore size employed was 0.45  $\mu$  (Millipore Filter Corporation, Bedford, Mass.).



### 3. Dissolved Oxygen Determination

Dissolved oxygen was determined by the azide modification of the Winkler test as outlined in Standard Methods for the Examination of Water and Waste Water (38).

### 4. Other Analyses

#### (a) pH

pH was monitored throughout all studies using a Beckman pH meter (9600 Zeromatic with Standard Electrodes and Holder), which was maintained in accordance with the Beckman operating and maintenance instruction manual (41).

#### (b) Oxidation Reduction Potential

The use of oxidation reduction potential in assessing the performance of biological treatment units was first reported in 1914 (42). The value of the analysis in the operation of biological treatment units is somewhat controversial; however, in systems of known composition, the ORP and the dissolved oxygen analysis together give an indication of the degree of aerobiosis in the system. In these studies the oxidation reduction potential was measured in accordance with the procedure outlined in the Beckman operating and maintenance instruction manual (41).

### E. Experimental Protocol

The types of experiment conducted in the present study may be placed in the following three broad categories:

1. Studies on Physical Reaeration in the Laboratory Oxidation Pond

The protocol for these studies was not particularly complicated, and it is felt that the detailed technique is appropriately given along with the results which are presented in the next chapter.

2. Studies on Reaeration due to Photosynthesis

In these studies the optimum concentration of sodium bicarbonate for algal photosynthesis was determined under two conditions. In one set of experiments, the batch units which were exposed to the atmosphere were seeded with algae in algal growth medium, and the course of reaeration was determined. In this type of study reaeration was due to a combination of physical reaeration from the atmosphere and the photosynthetic production of oxygen by the algae. In another set of experiments, the algal suspension was sealed in BOD bottles and the reaeration due solely to the photosynthesis was measured. As in the case of the physical reaeration studies, the details of the experimental protocol will be presented along with the results in Chapter 4.

3. Studies on the Effects of Various Organic Loadings on the Experimental Oxidation Ponds

These studies comprised the major research effort. Various types of operation were employed in both discontinuous, or batch, systems and in continuous-flow systems.

## (a) Batch Unit Studies

In the batch studies three types of systems were investigated. The essential differences in systems 1, 2, and 3 are shown in Table IV.

TABLE IV  
COMPOSITION OF BATCH UNIT OXIDATION POND

	System 1	System 2	System 3
Sodium Bicarbonate			
30 gm/l	0 ml	100 ml	100 ml
Seed	100 ml	100 ml	100 ml
Algae	500 ml	500 ml	500 ml
Algal Growth Medium	1500 ml	1500 ml	1500 ml
*Standard Synthetic Wastes	90 ml	90 ml	90 ml
1.0M Potassium Phosphate (pH 6.8)	1200 ml	1200 ml	1200 ml
Tap Water	26,510 ml	26,410 ml	26,410 ml
Light Intensity	450 ft-c	450 ft-c	DARK
Light Periodicity	12 hrs/day	12 hrs/day	0 hrs/day

\*Inorganic constituent of standard synthetic waste added to yield concentrations shown in Table I. Glucose, trace elements and phosphate buffer not included.

It is seen that the only difference between systems 1 and 2 was the omission of sodium bicarbonate in system 1, and that the only difference between systems 2 and 3 is that system 3 was operated in the absence of light. Samples were siphoned from the mid-depth of the experimental ponds twice daily. The samples were siphoned directly into a BOD bottle, and a portion of the sample was allowed to overflow into a 1000 ml beaker. Dissolved oxygen was determined on the sample retained in the BOD bottle and other analyses were run on the portion of sample which overflowed to the 1000 ml beaker. In these studies at

various organic loadings in the oxidation ponds, three different loading conditions were examined. They were as follows: 1) initial organic loading followed by a detention period of seven days, during which samples were taken for analysis; 2) organic loading applied at three-day intervals, with samples taken over a period of twelve days; 3) organic loading applied each day with systems observed over a period of seven days. All experiments were run at  $23^{\circ}\text{C.} \pm 1^{\circ}$ .

#### (b) Continuous Flow Studies

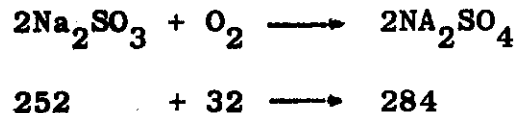
Continuous flow studies in the experimental ponds were performed at inflow rates which yielded detention times of 10 and 20 days. The chemical composition of the synthetic medium was the same as that shown in Table IV for system 2. However, the substrate concentration (glucose) was varied from 100 to 600 mg/l for studies at the 10-day retention time and 100 to 1000 mg/l glucose at the 20-day retention time. The experiments were started by filling the ponds with medium and seeding with 100 ml of heterogeneous bacterial seed and 3000 ml of algal seed. Approximately three days of batch operation were allowed for development of the mixed flora, then feed was admitted continuously and samples were taken at three depths in the pond (see Figure 3). In all cases the lighting intensity was 450 ft. candles and temperature was maintained at  $23^{\circ}\text{C.} \pm 1^{\circ}$ .

## CHAPTER IV

### RESULTS

#### 1. Studies on Physical Reaeration in the Laboratory Oxidation Pond

In oxidation ponds deoxygenation can occur by bacterial respiration and by algal respiration. Reoxygenation can come about by algal photosynthesis and by atmospheric reaeration across the water air surface. In the present study, the physical reaeration characteristics of the experimental oxidation pond were determined using water devoid of algae, thus preventing reaeration by photosynthetic means. The experimental ponds were filled with distilled water (36 liters) and the water was deoxygenated using sodium sulfite. The reaction between sodium sulfite and oxygen may be represented by the following equation:



In accordance with this equation, 7.875 pounds of sodium sulfite or 8.236 pounds of "Santasite" are required to remove one pound of oxygen. For the present experiments the dissolved oxygen which was initially in the water was determined and the amount of "Santasite" was calculated.

The deoxygenation chemical was added in slight excess. After adding sodium sulfite, samples were withdrawn periodically and the course of reoxygenation followed by determining the dissolved oxygen using the Winkler method. In order to maintain a constant volume of water in the oxidation pond, the volume removed at each sampling period was replaced with tap water containing dissolved oxygen at 7.5 mg/l. In this way it was possible to calculate the slight change in dissolved oxygen concentration which would be expected due to mixing the large volume of pond liquor with the rather small (approximately 650 ml) volume of makeup water.

The results are shown in Table V. The column marked "control dissolved oxygen" shows the values of dissolved oxygen concentration in a tank which was previously saturated with DO, and to which sodium sulfite was not added. The remaining columns show the dissolved oxygen and calculated DO deficit for parallel experiments in two oxidation ponds. Figure 5 is an arithmetic and Figure 6 a semilogarithmic plot of the course of reoxygenation in both experimental ponds. It is seen from Figure 6 that physical reaeration follows first order kinetics. Also, it is apparent that comparable rates of reaeration were observed for both tanks.

## 2. Studies on Reaeration due to Photosynthesis

It is noted that these experiments were of a prelim-

TABLE V  
REPLACEMENT OF DISSOLVED OXYGEN BY PHYSICAL REAERATION

Time Hrs	Control DO mg/l	Pond 1 DO mg/l	DO Deficit	Pond 2 DO mg/l	DO Deficit
0	7.56	1.98	5.58	1.39	6.17
1/4	7.53	0.11	7.42	0.00	7.53
3/4	7.56	0.43	7.13	0.27	7.29
2 <sup>30</sup>	7.46	0.94	6.52	0.73	6.73
4	7.48	1.42	6.06	1.59	5.89
6	7.53	2.04	5.49	1.88	5.65
9	7.59	2.73	4.86	2.61	4.98
12	7.64	3.33	4.31	3.11	4.53
22 <sup>30</sup>	7.35	4.64	2.71	4.53	2.82
25 <sup>45</sup>	7.56 <sup>9</sup>	5.08	2.48	5.06	2.50
30	7.35	5.32	2.03	5.38	1.97
36	7.38	6.10	1.28	5.63	1.75
46 <sup>30</sup>	7.45	6.05	1.40	6.13	1.32
53	7.43	6.20	1.23	6.33	1.10
71	7.40	6.60	0.80	6.97	0.43
83	7.38	6.55	0.77	6.75	0.63
95	7.68	6.95 <sup>23</sup>	0.67	7.05 <sup>33</sup>	0.63
107	7.68 <sup>9</sup>	7.05 <sup>23</sup>	0.63 <sup>6</sup>	7.10 <sup>42</sup>	0.58
143	7.46 <sup>88</sup>	7.45	0.01 <sup>46</sup>	7.46	0.00

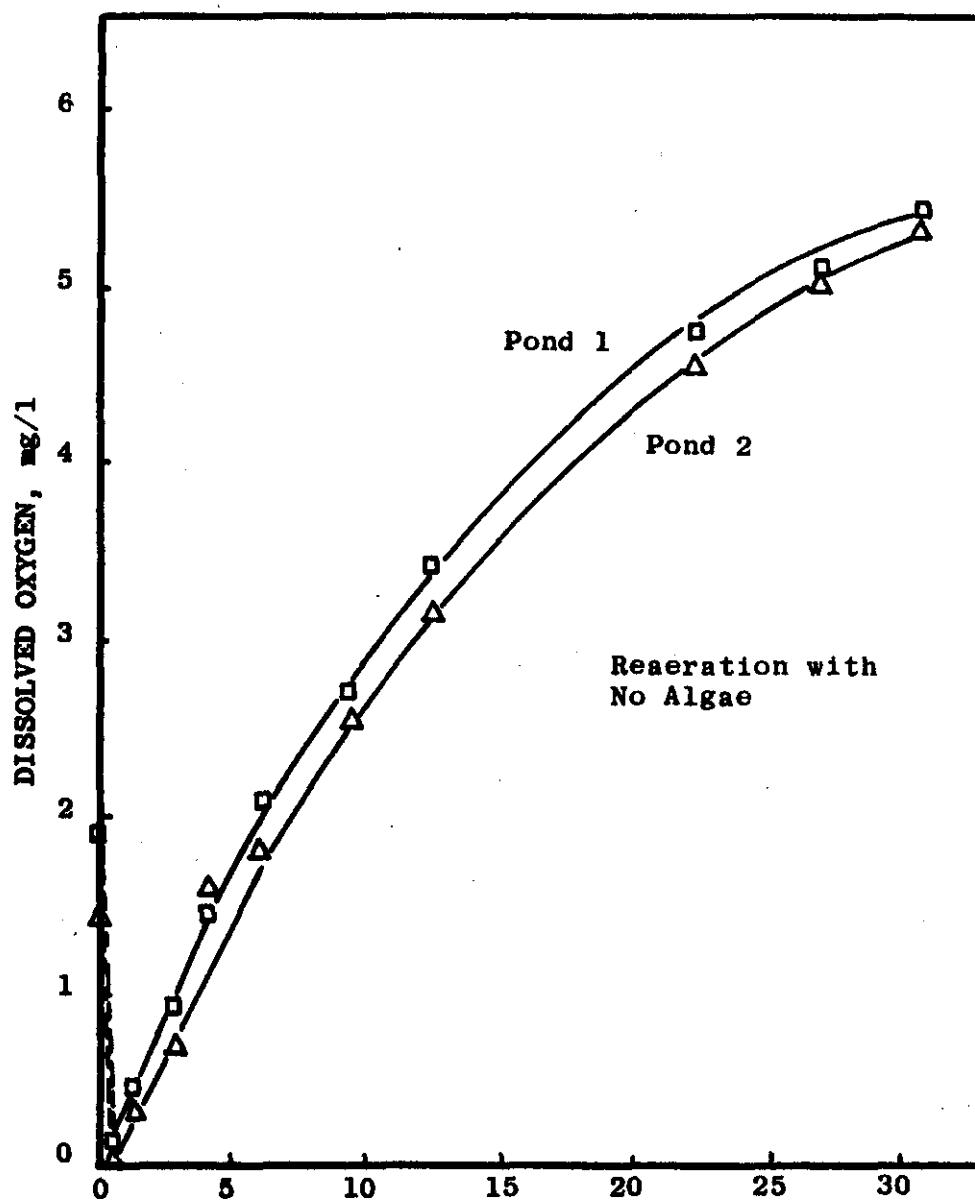


Figure 5. Physical Reaeration in Experimental Pond.



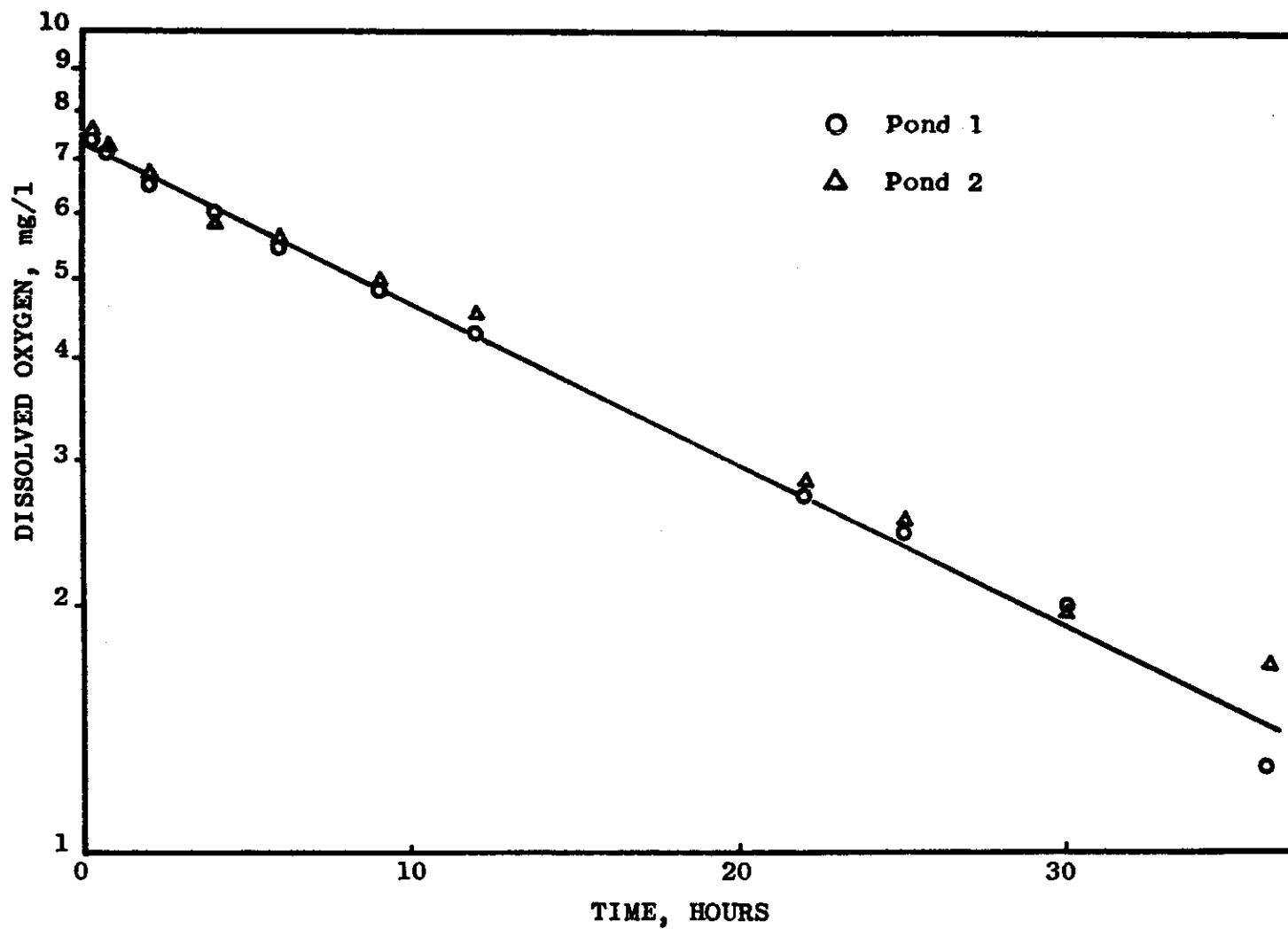


Figure 6. Semi-logarithmic Plot of Dissolved Oxygen vs. Time for Physical Reaeration in Experimental Pond.

inary nature, and were accomplished before the research had progressed to the point where the final algal growth medium had been selected. In the studies reported in this section, the medium which was used corresponded to that shown in Table I, except that glucose was not added. In addition to this medium, varying concentrations of sodium bicarbonate were employed. This was done in order to gain some idea of the proportion of carbon source which might be used in succeeding experiments. Also, the experiments reported in this section of the results were done under a relatively low light intensity (50 ft. candles). It should also be noted that when these experiments were performed, the predominantly Chlorella algal population had not yet been selected. The algal seed used in the present experiments consisted of a mixed population of algae developed from initial seeds obtained from the Botany Department at Oklahoma State University. Medium and algal seed were placed in the laboratory experimental pond; the water was deoxygenated, and the course of changes in dissolved oxygen were followed. The results are given in Table VI and plotted in Figure 7. Using oxygen production as an indirect measure of algal growth, the results shown in Figure 7 indicate that the rate of algal growth is stimulated by the addition of bicarbonate and that 100 mg/l bicarbonate was at least as effective as 300 mg/l.

It was also desirable to perform some preliminary stud-

TABLE VI  
 REPLACEMENT OF DISSOLVED OXYGEN BY PHYSICAL REAERATION  
 AND ALGAL PHOTOSYNTHESIS

Time Hrs.	Pond 1 0 mg/l	Pond 2 100 mg/l	Pond 3 300 mg/l
	Sodium Bicarbonate DO mg/l	Sodium Bicarbonate DO mg/l	Sodium Bicarbonate DO mg/l
0	3.01	2.26	1.97
1/3	2.26	0.65	0.96
5/6	1.96	0.41	0.75
1 2/3	0.88	0.99	1.03
4 2/3	1.07	1.25	1.74
8 2/3	1.15	1.30	2.39
22	2.13	3.08	3.34
28	2.57	3.64	3.39
34	2.90	4.19	3.80
45	3.35	5.29	4.72
51	3.50	5.18	4.82
57	3.75	5.56	5.30
65	4.00	5.60	5.35
76	4.15	6.10	5.50
83	4.30	6.70	5.90
94	4.45	7.15	6.60
106	4.80	7.20	6.75
124	5.15	7.45	7.10
132	5.25	7.60	7.25

Temperature = 22°C. ± 1°

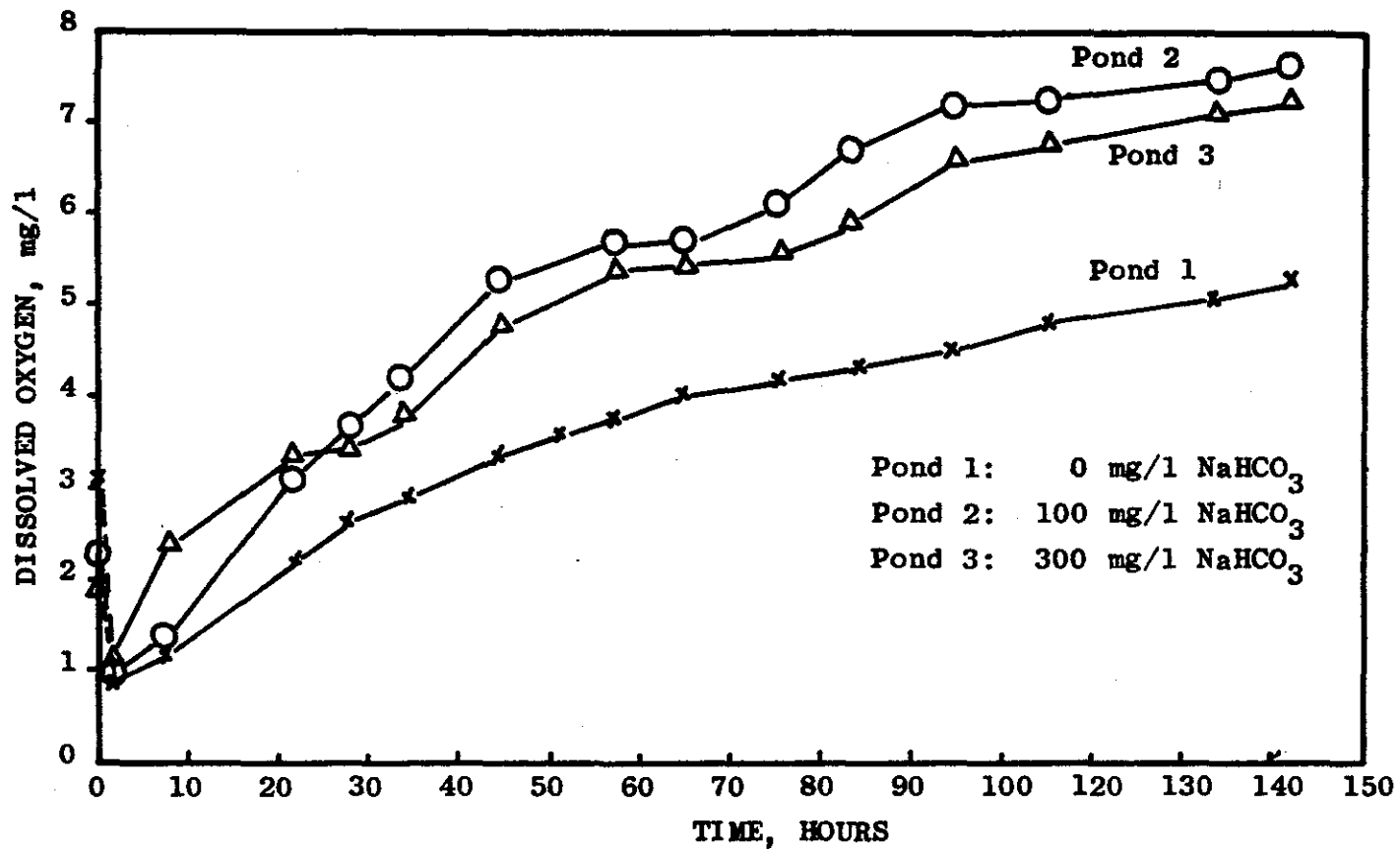


Figure 7. Combined Physical and Algal Reaeration in Experimental Oxidation Pond.

ies in systems in which reaeration was due only to algal photosynthesis. To accomplish this, systems closed to the atmosphere were set up in BOD bottles. In these experiments the same medium and algal seeding material used for the experiments shown in Table VI were employed. The lighting intensity and temperature were also the same. Two series of experiments were set up at varying concentrations of sodium bicarbonate. In another series of experiments in BOD bottles a single concentration of sodium bicarbonate was used and glucose at various concentrations was added.

Figure 8 shows the course of dissolved oxygen production for closed systems devoid of glucose, using three different concentrations of sodium bicarbonate. The increase in dry weight of algal material during the course of the experiment is shown for each system in Figure 9. In both figures there is a striking difference in the performance of systems receiving sodium bicarbonate and the control system to which no carbon source was added. There is also some indication that the presence of sodium bicarbonate in higher concentrations exerted a suppressing effect on the rate of algal growth.

For the experiments shown in Figures 8 and 9, sodium sulfite was not added to deoxygenate the water prior to taking samples. When sodium sulfite was added, its effect was to increase the lag period before rapid oxygen production but it did not seriously affect the oxygen production

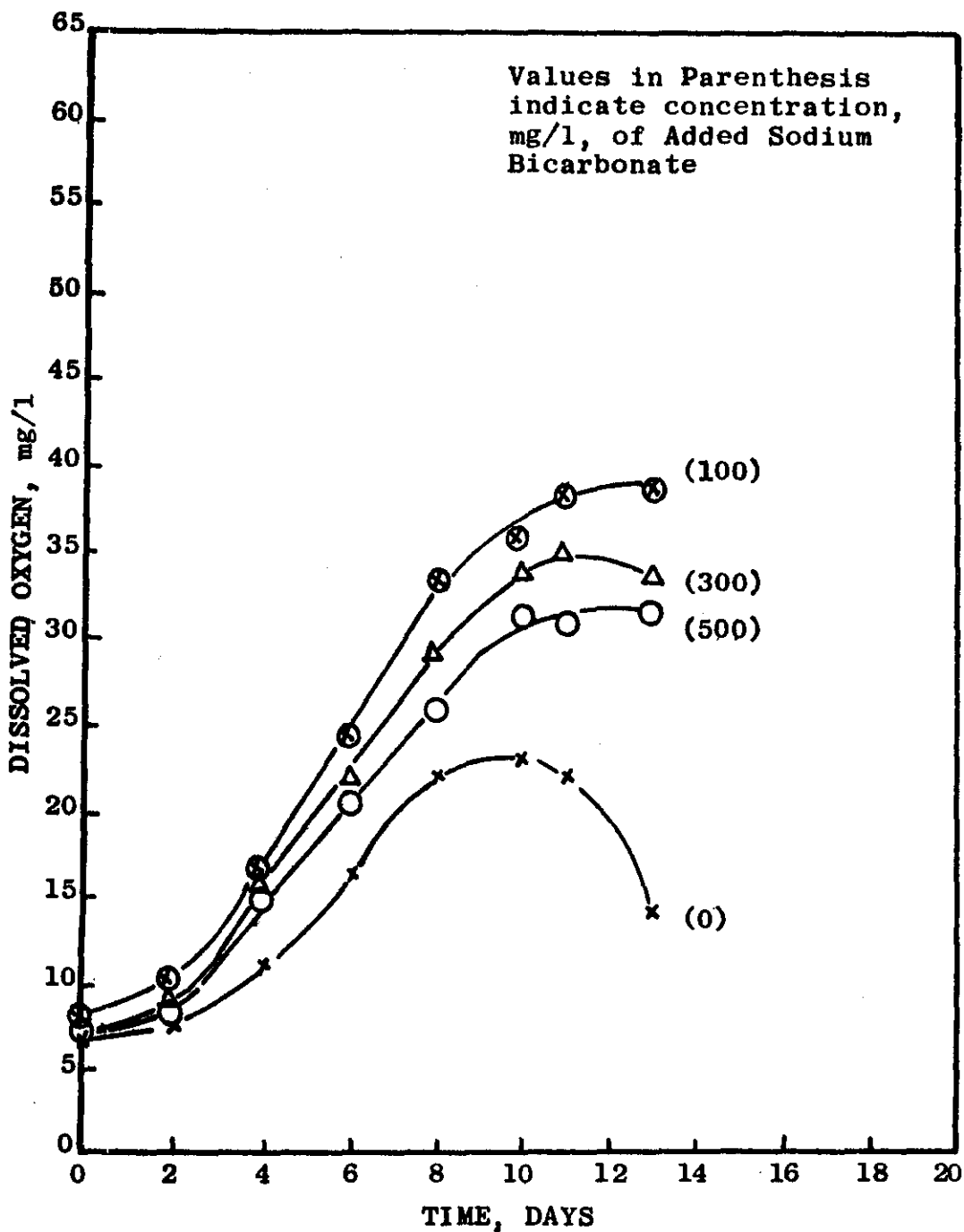


Figure 8. Effect of Sodium Bicarbonate Concentration on Algal Production of Oxygen - No Sodium Sulfite Added.

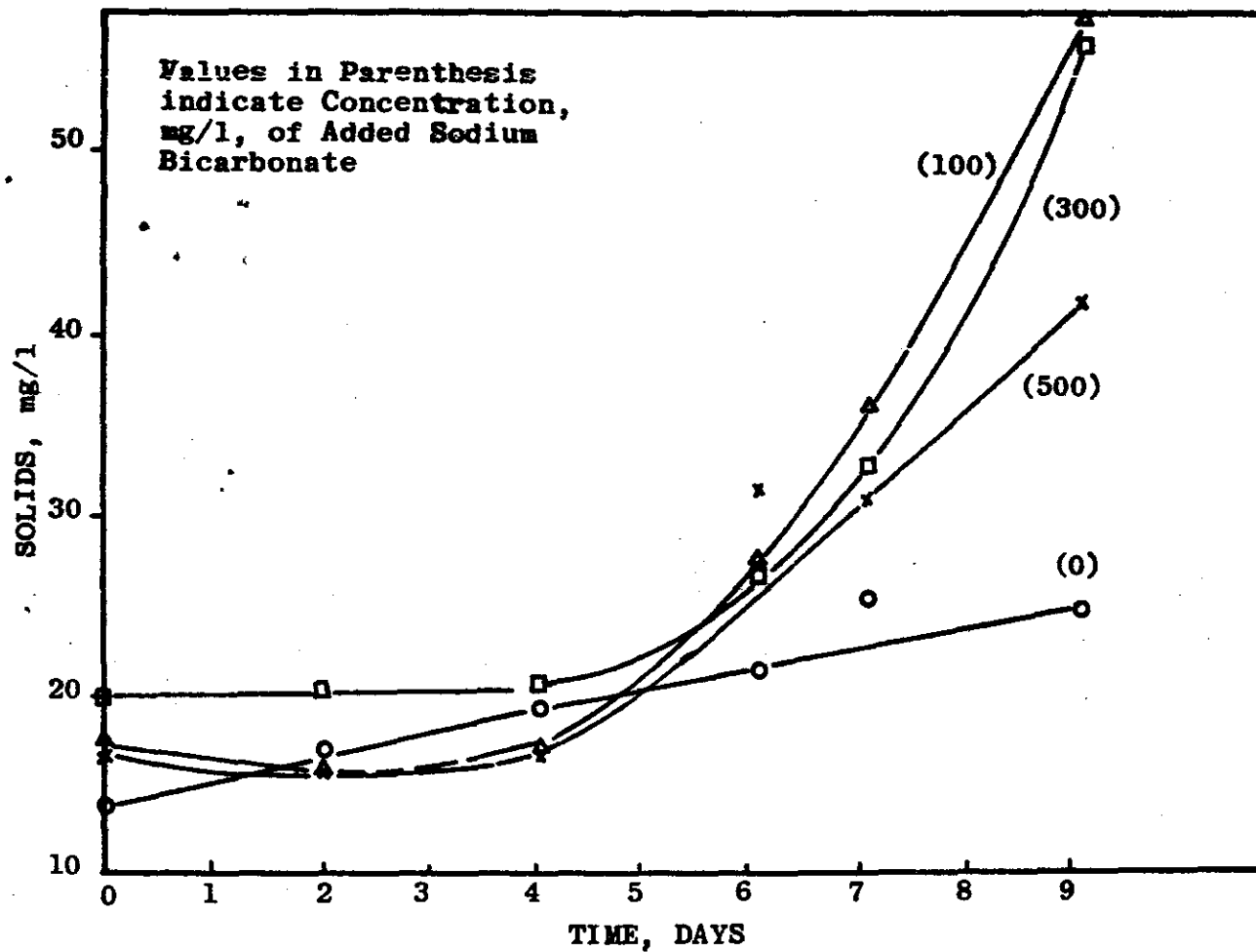


Figure 9. Effect of Sodium Bicarbonate Concentration on Production of Algal Solids

rate after the lag period was over. This can be seen by comparing the results shown in Figure 10 with those in Figure 8. It should be noted that the apparent suppressing effect of higher concentrations of sodium bicarbonate was again evidenced in the experiment shown in Figure 10.

It was desirable to gain some preliminary information pertaining to the course of oxygen utilization and production in closed systems which could be oxygenated by photosynthetic reaeration and deoxygenated by bacterial respiration. In order to gain some insight into this experimental situation, BOD bottle experiments were set up using sodium bicarbonate at 100 mg/l and varying concentrations of glucose. The seeding material consisted of the heterogeneous algal seed used in the previous studies and a mixed population of heterotrophic organisms obtained from a laboratory scale activated sludge plant. The results are shown in Figure 11. It is apparent that the severity of the initial sag in dissolved oxygen concentration was proportional to the amount of glucose added. Even in the system which received no glucose, there was an appreciable decrease in DO concentration during the first two days. It was seen from Figure 8 that there was approximately a two-day lag in oxygen production by the algae, and it is apparent from Figure 11 that during this period of lag in algal oxygen production, the endogenous respiration of the seed material in the BOD bottle was enough to cause a



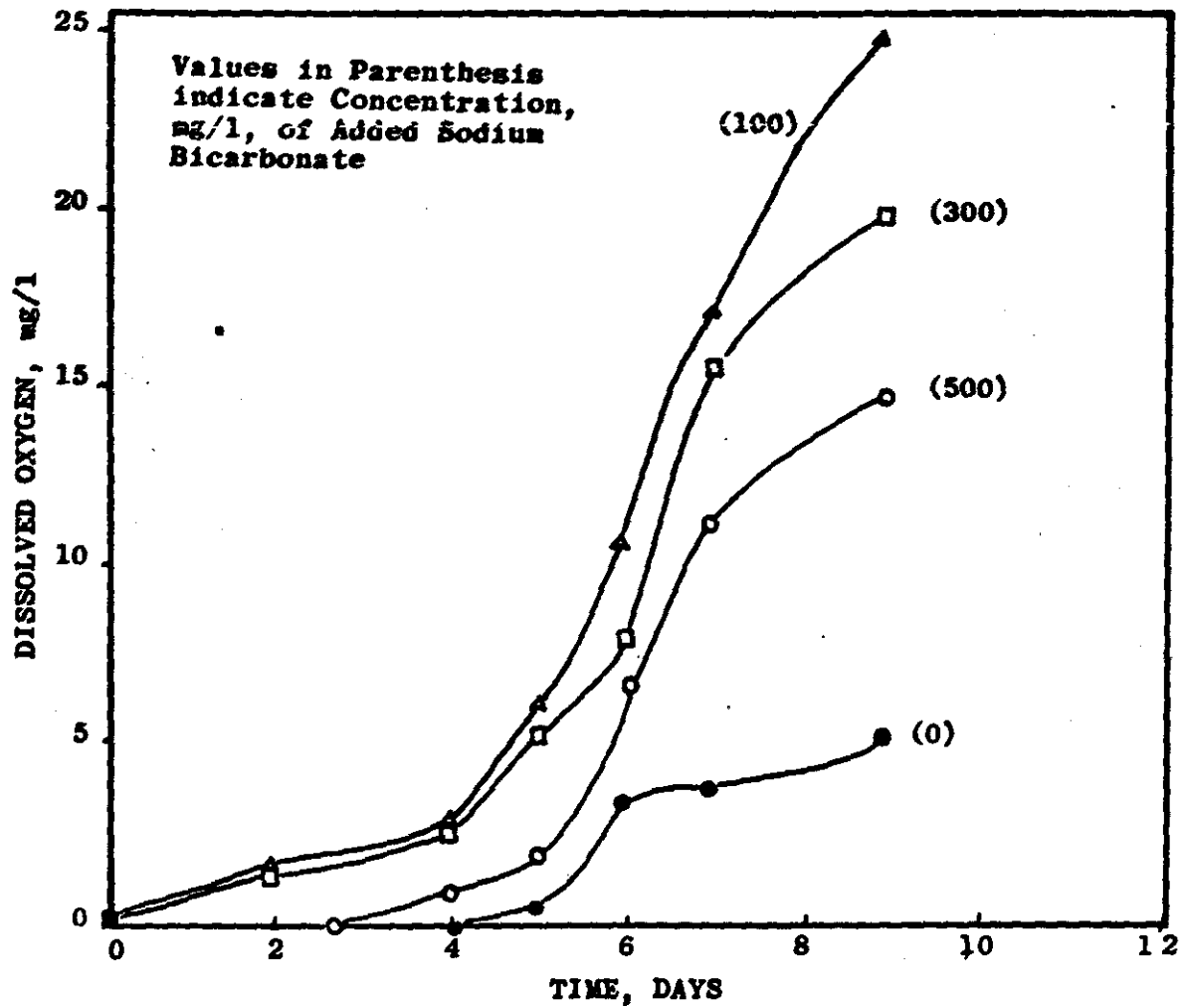


Figure 10. Effect of Sodium Bicarbonate Concentration on Algal Production of Oxygen - Sodium Sulfite Added.

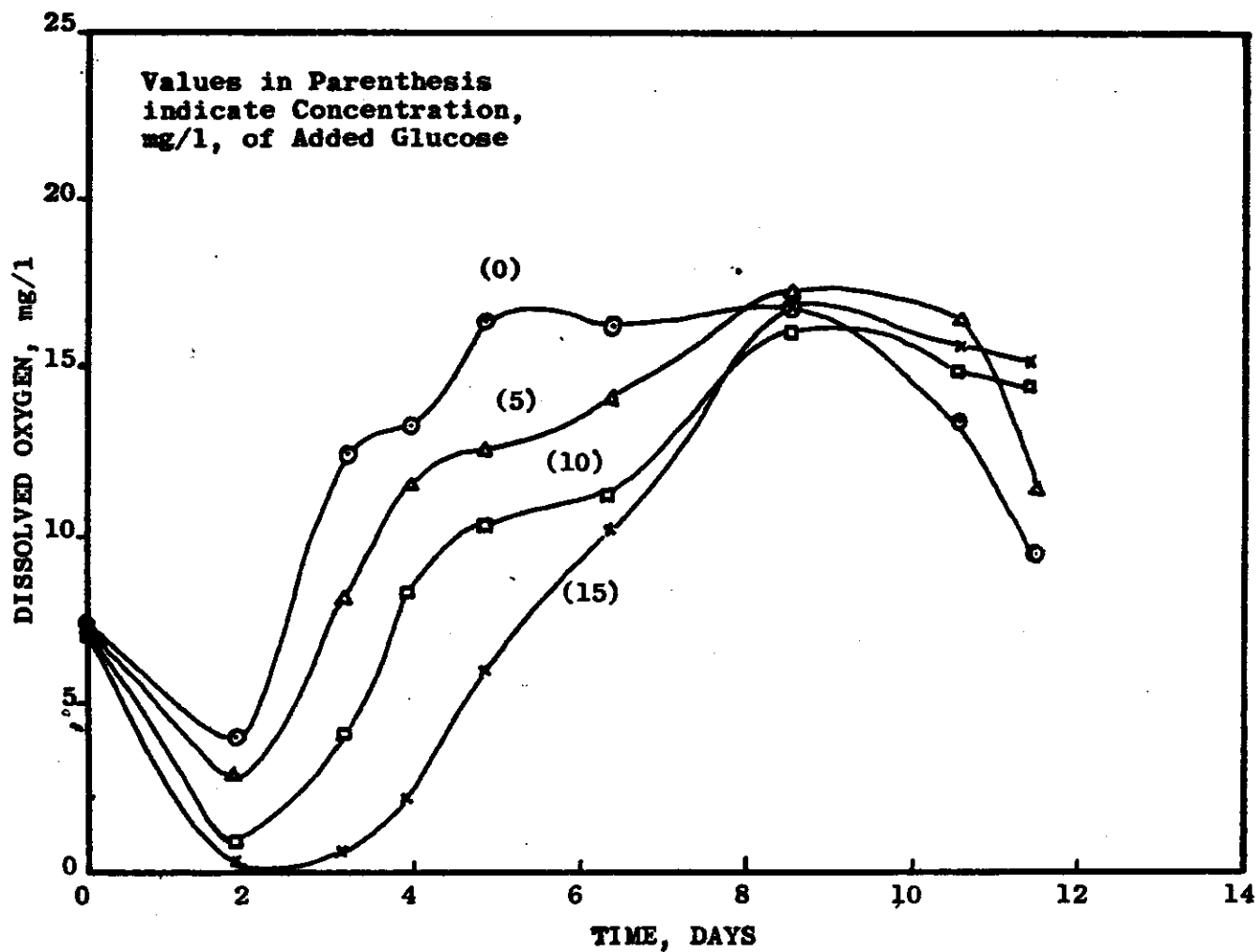


Figure 11. Effect of Various Organic Loadings on Oxygen Production in Closed Systems Containing Heterotrophic Bacteria and Algae.

noticeable decrease in dissolved oxygen concentration. It is also interesting to note the apparent plateau in DO concentration between the fourth and sixth days of the experiment for the systems containing 5 and 10 mg/l glucose. There was also an apparent but slight plateau in DO concentration in the system which received no glucose. The plateau appeared to be somewhat masked in the system containing 15 mg/l glucose..

### 3. Studies on the Effects of Various Organic Loadings on the Experimental Oxidation Ponds

#### A. Batch Unit Studies

##### 1. Initial Organic Loading followed by a Detention Period of Seven Days

In this series of experiments the organic loadings were varied from 100 to 600 mg/l glucose for each of three oxidation ponds defined in Table IV (see Materials and Methods, page 23). The results are shown in Figures 12 through 29.

The 7-day batch studies conducted at a glucose loading of 100 mg/l are presented in Figures 12, 13, and 14, for systems 1, 2, and 3, respectively. It can be seen that the rate of glucose and COD removal for the system which was not illuminated (Figure 14) was considerably slower than for the systems which did receive light. Comparison of biological solids concentrations in all three figures shows quite strikingly the effect of additional algal

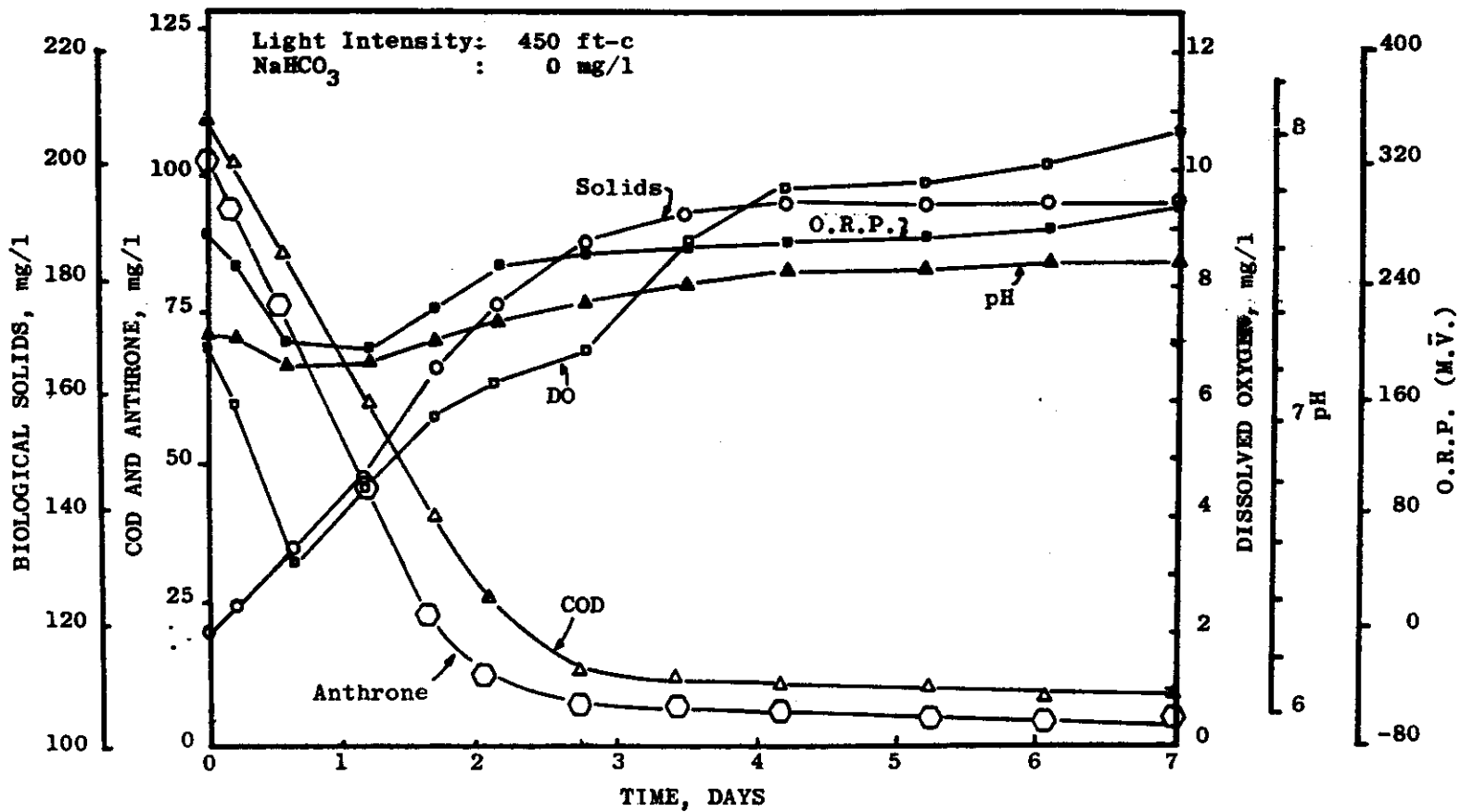


Figure 12. Purification of 100 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

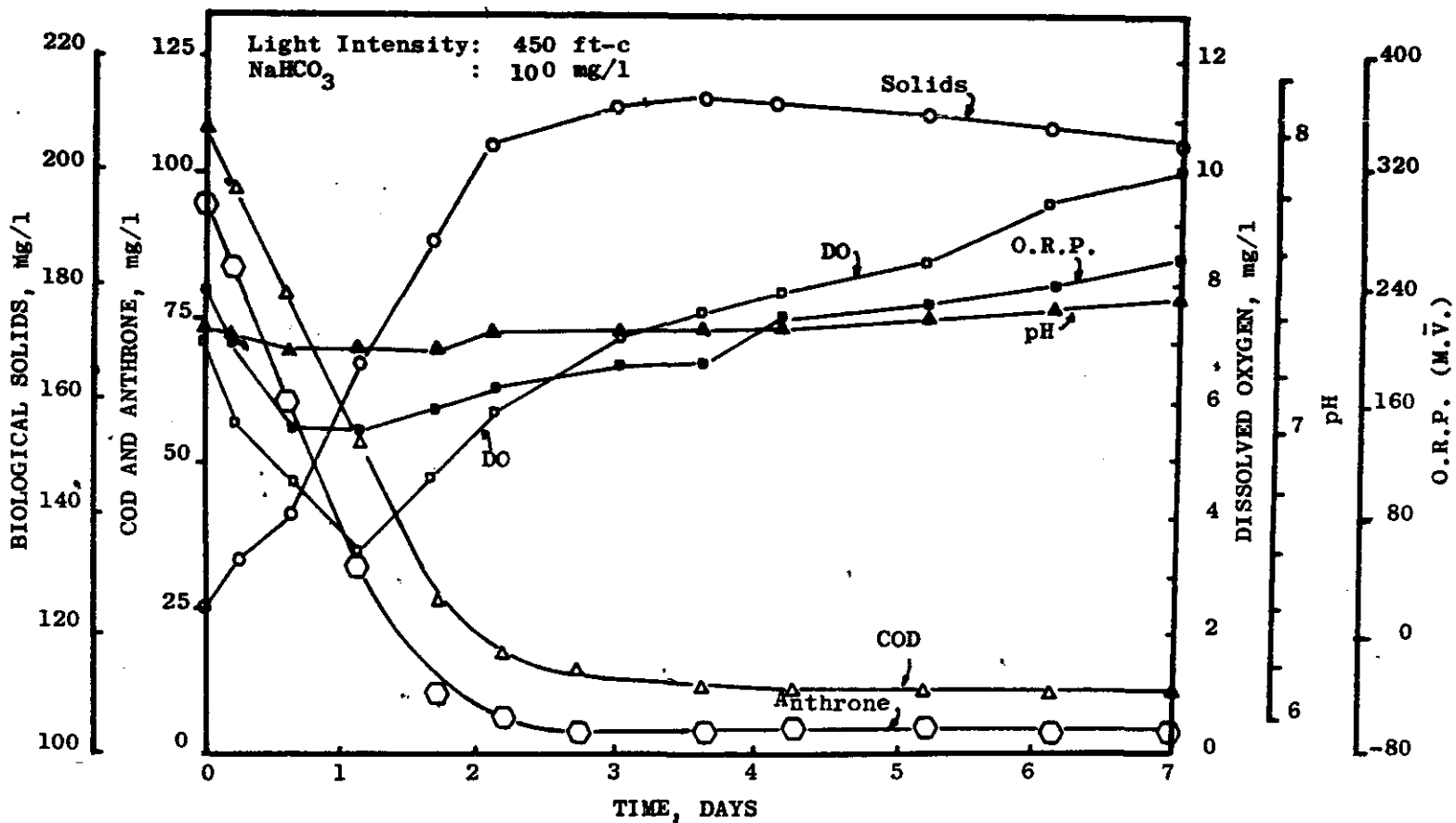


Figure 13. Purification of 100 mg/l Glucose in a Batch Oxidation Period with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

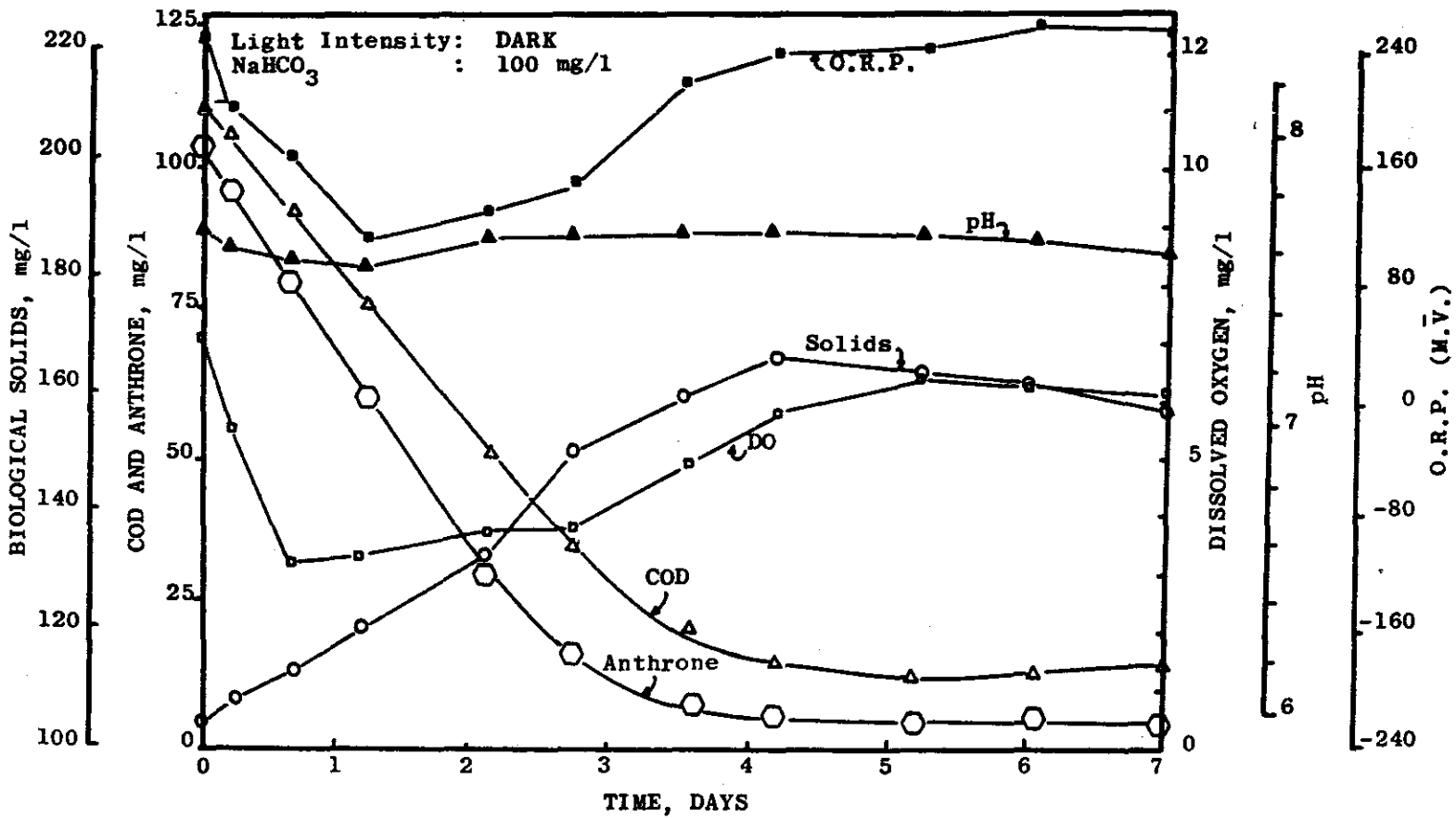


Figure 14. Purification of 100 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

growth caused by the presence of light. It is interesting to note that even in the system which was not subjected to the lighting cycle, the dissolved oxygen did not reach zero, and there was considerable recovery in DO concentration (see Figure 14). The recovery and dissolved oxygen concentration shown in Figure 14 is attributable to physical reaeration. Every attempt was made to shut light out from the experimental oxidation pond; however, the shield was removed for approximately one-half hour in each twenty-four hours (approximately fifteen minutes each sampling period), and light was admitted during this time. Also, since the shield consisted of a cardboard box and the top of the box was approximately three inches from the water surface, four holes approximately three-eighths inch in diameter were made in the top of the shield in order to allow access of air to the atmosphere above the water surface. A small amount of light was continuously admitted through the ventilation holes. It is seen that in all systems the pH underwent slight but predictable changes during the course of substrate removal, and that the presence of algae did not cause an undue increase in pH. This was due to the rather large amount of phosphate buffer in the medium.

Experiments conducted at the 200 mg/l glucose loading level are shown in Figures 15, 16, and 17. These results are in general accord with those shown in the previous three figures. The rates of substrate and COD removal are

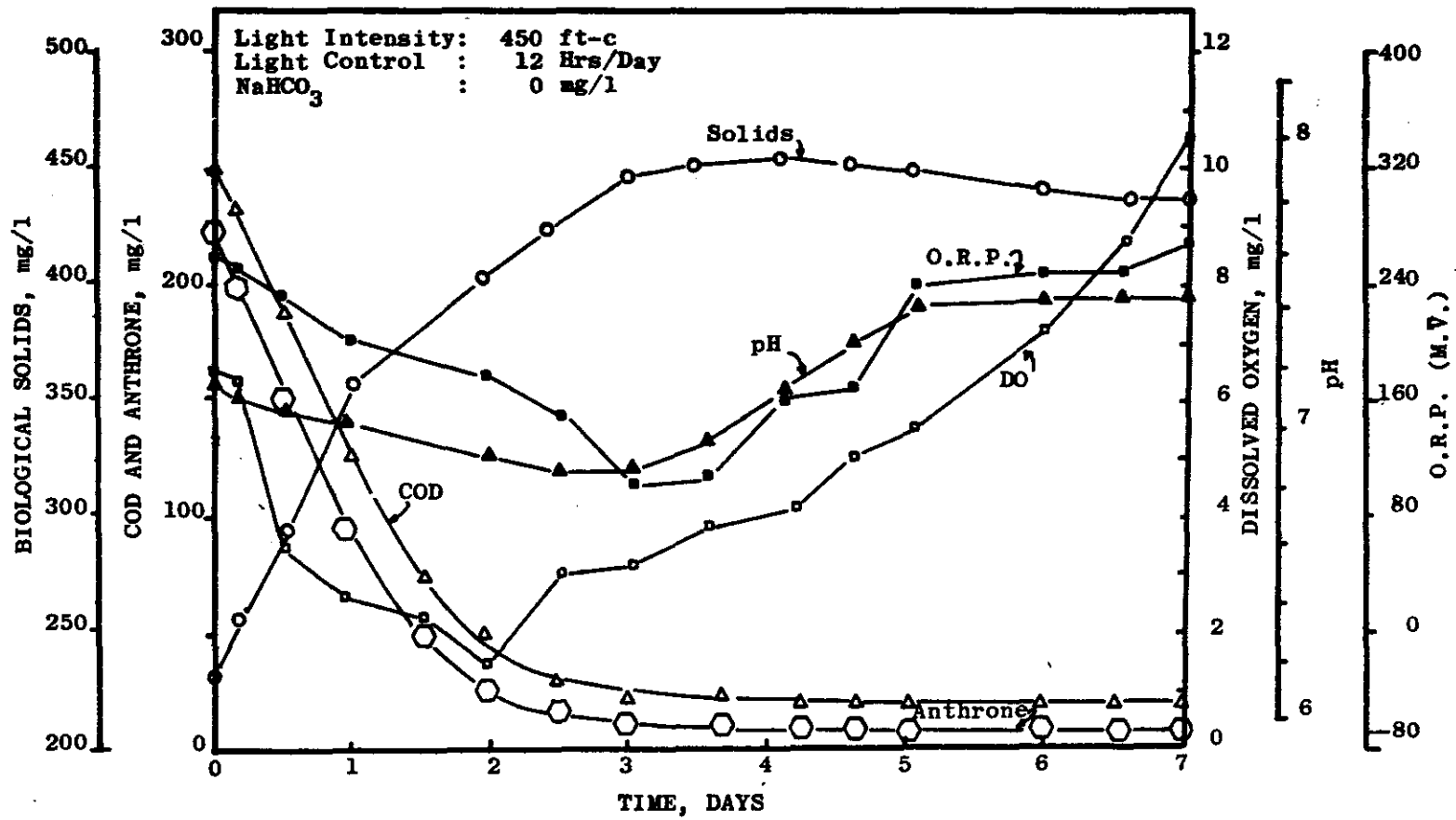


Figure 15. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).



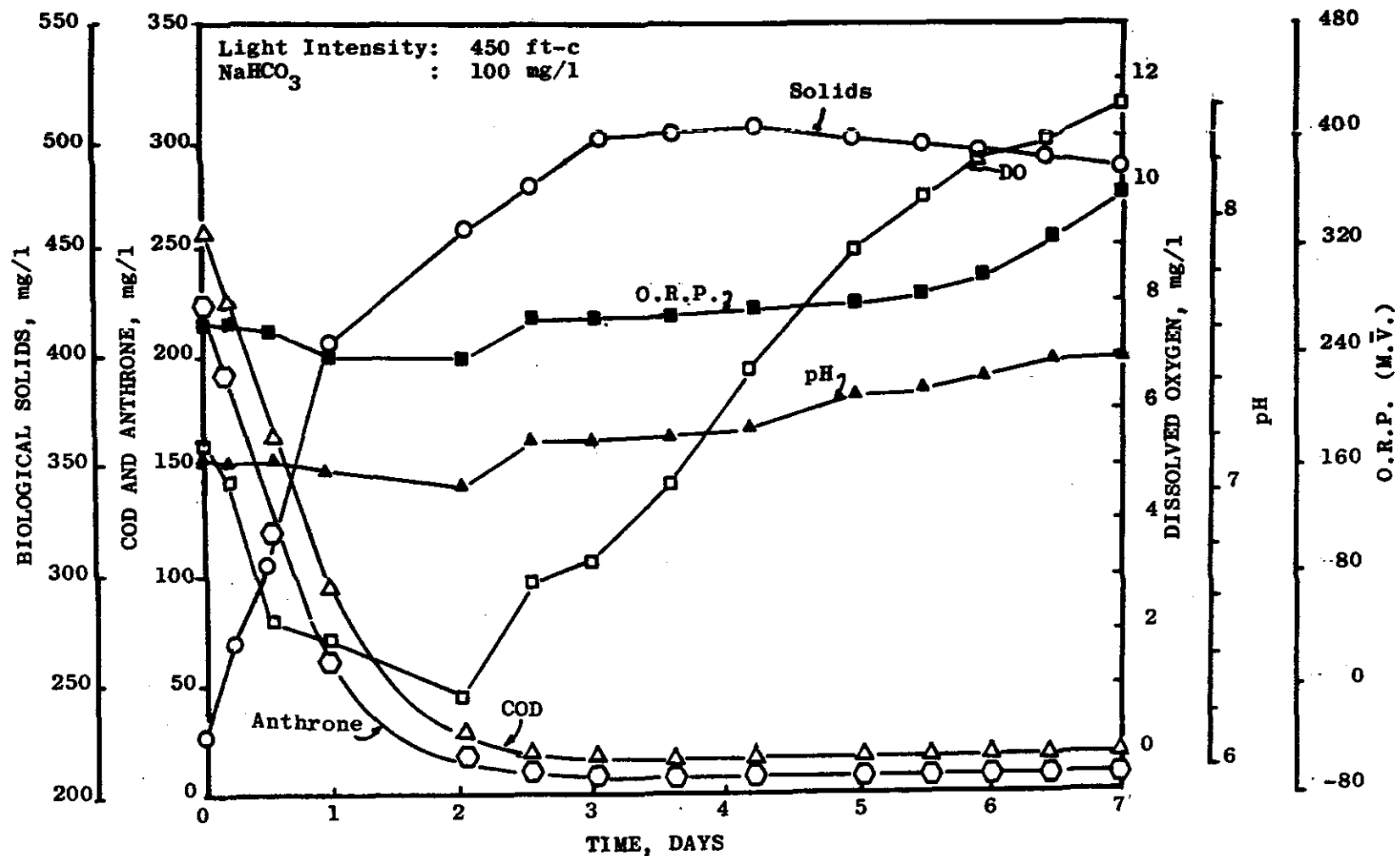


Figure 16. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l of Sodium Bicarbonate (Light Period - 12 Hrs/Day).

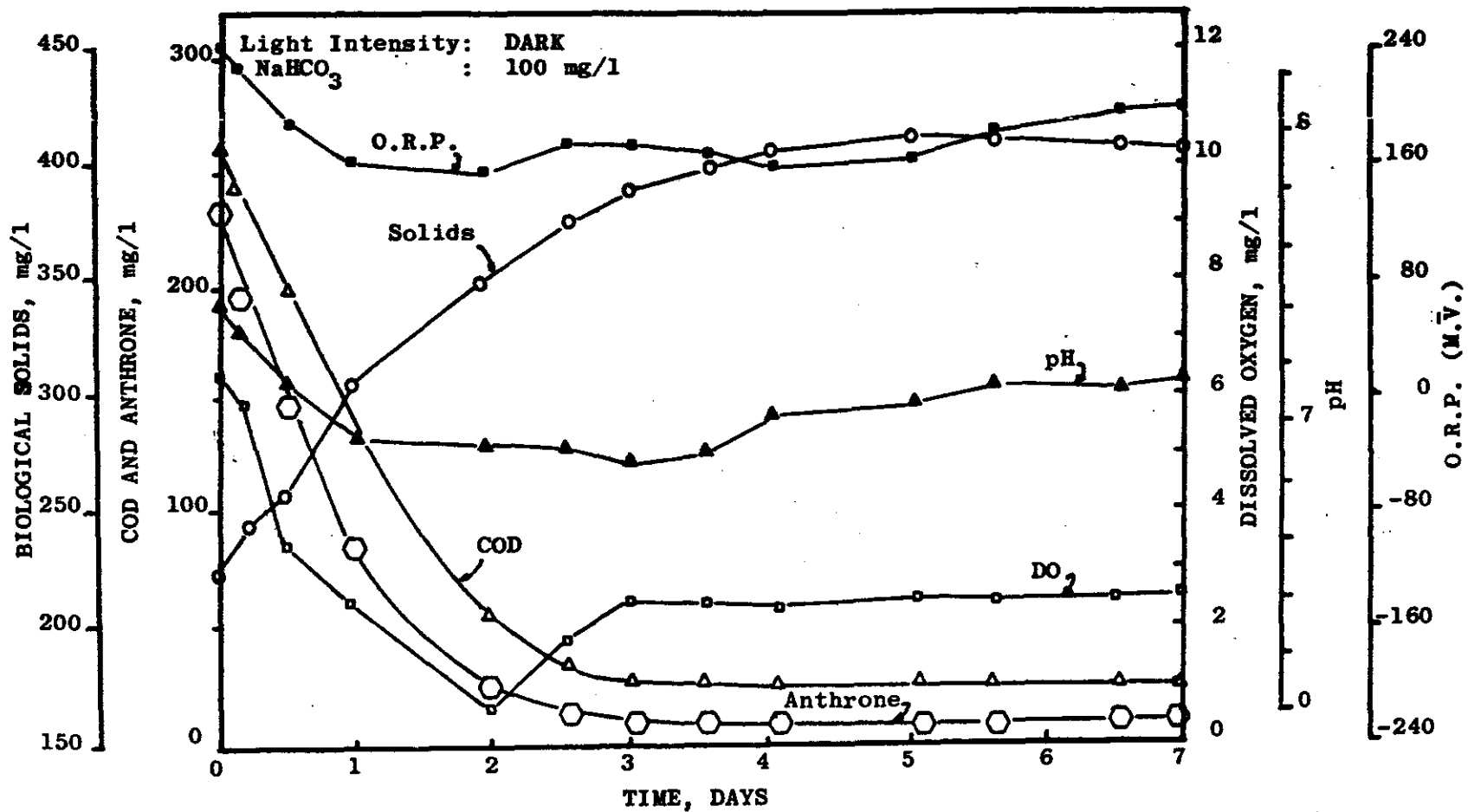


Figure 17. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

somewhat higher; however, this would appear to be due largely to higher initial biological solids concentration employed in the studies performed at the 200 mg/l glucose loading level.

Experiments run at the 300 mg/l glucose loading level are shown in Figures 18, 19, and 20. At this loading level there was a striking increase in the severity of the dissolved oxygen sag, and the dissolved oxygen in the system which was not lighted did not exhibit an appreciable dissolved oxygen recovery. The lack of recovery in the system in which photosynthesis was not permitted, or in any event was negligible, would appear to be due primarily to the endogenous respiration of the biological solids in the system. Again, the rate of removal of COD and substrate was noticeably slower in the system which received no light than in either system which was subjected to the lighting cycle.

The results of studies conducted at the 400 mg/l glucose loading level are shown in Figures 21, 22, and 23. It is seen in this series of experiments that the dissolved oxygen for systems which were subjected to the lighting cycle did not recover to the extent that was observed at lower loading levels, i.e., the severity of the sag was increased. The system which was run in the dark showed a fairly rapid drop in dissolved oxygen concentration, and evidenced little or no recovery. It may also be observed

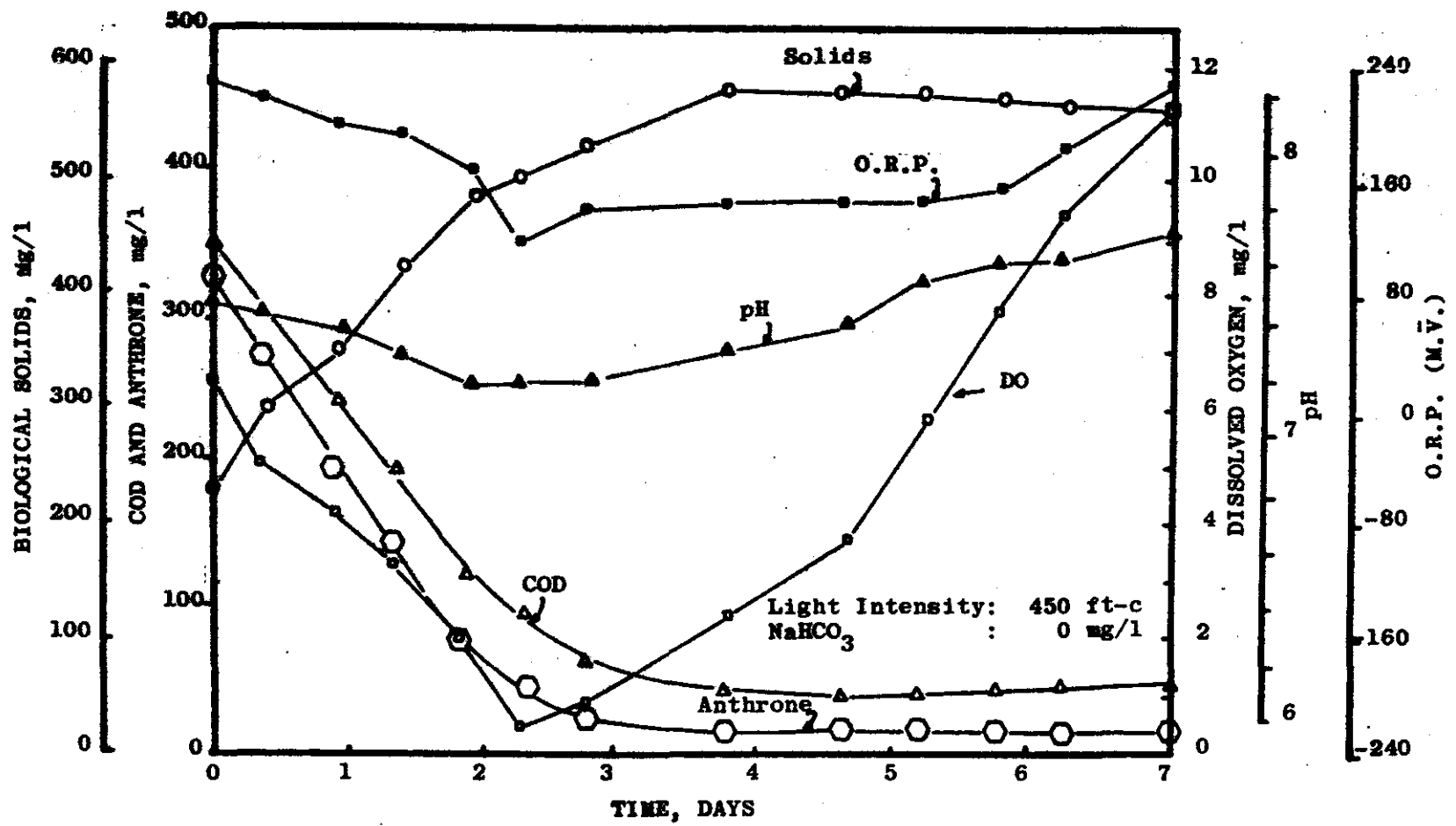


Figure 18. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

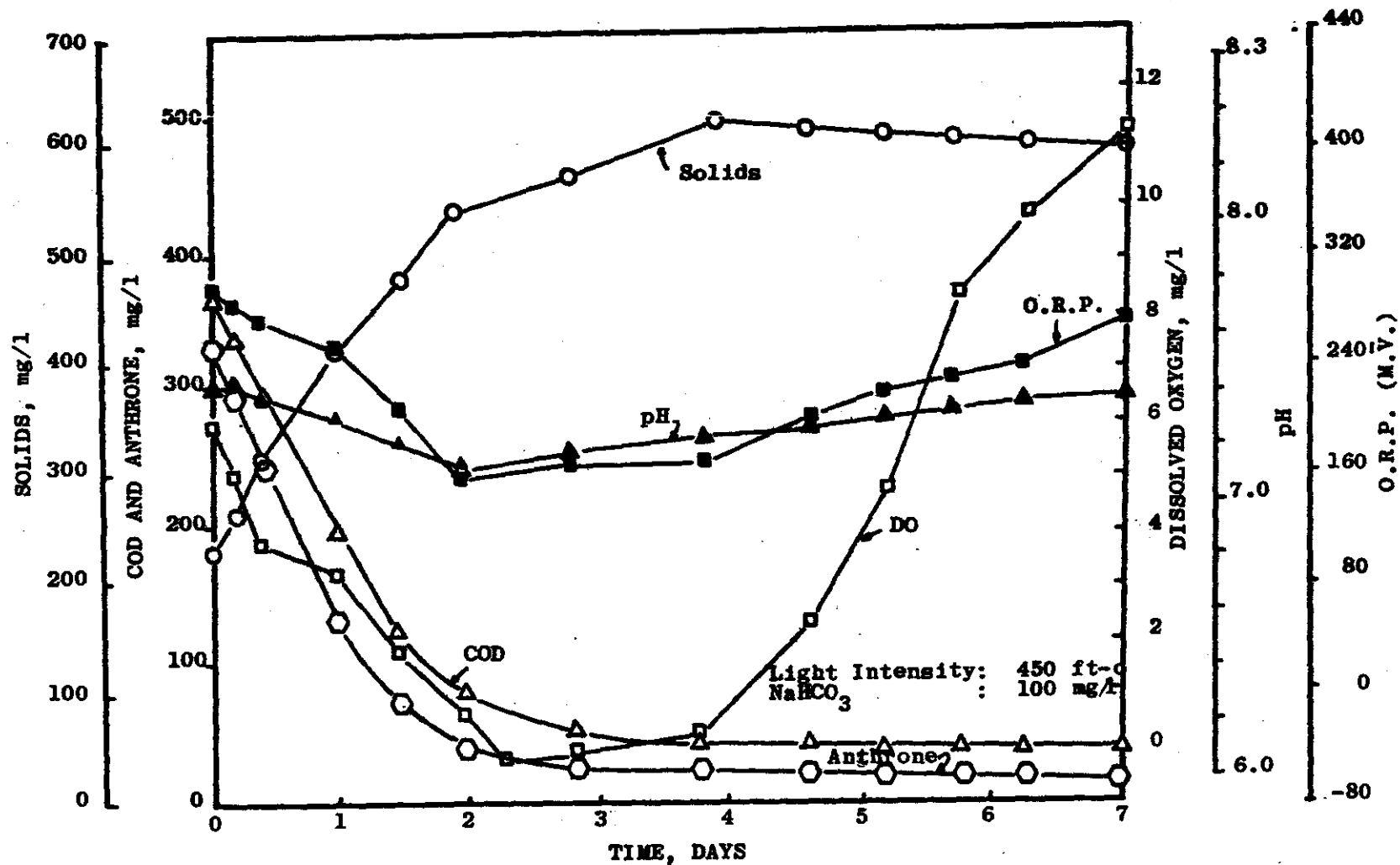


Figure 19. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l of Sodium Bicarbonate (Light Period - 12 Hrs/Day).

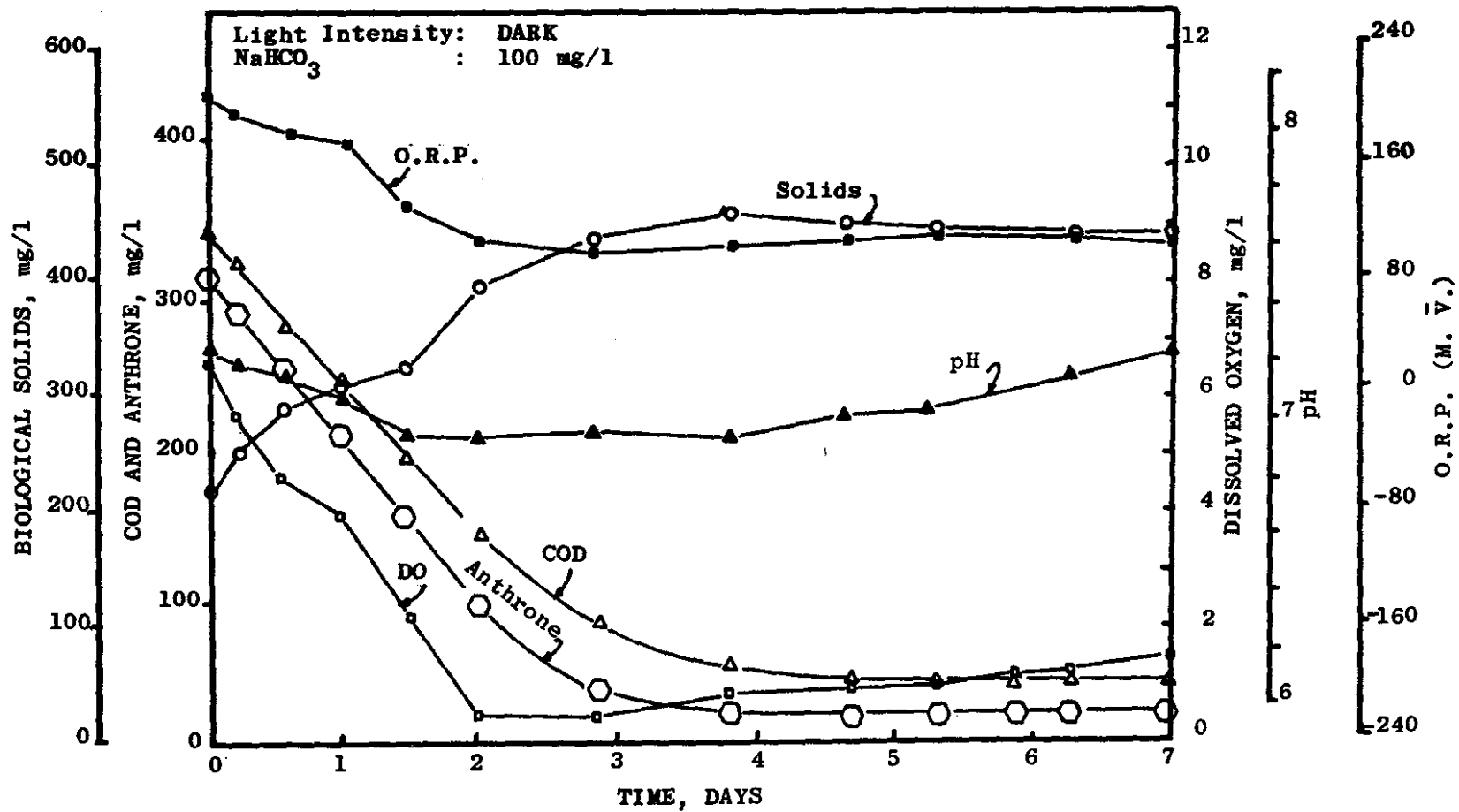


Figure 20. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

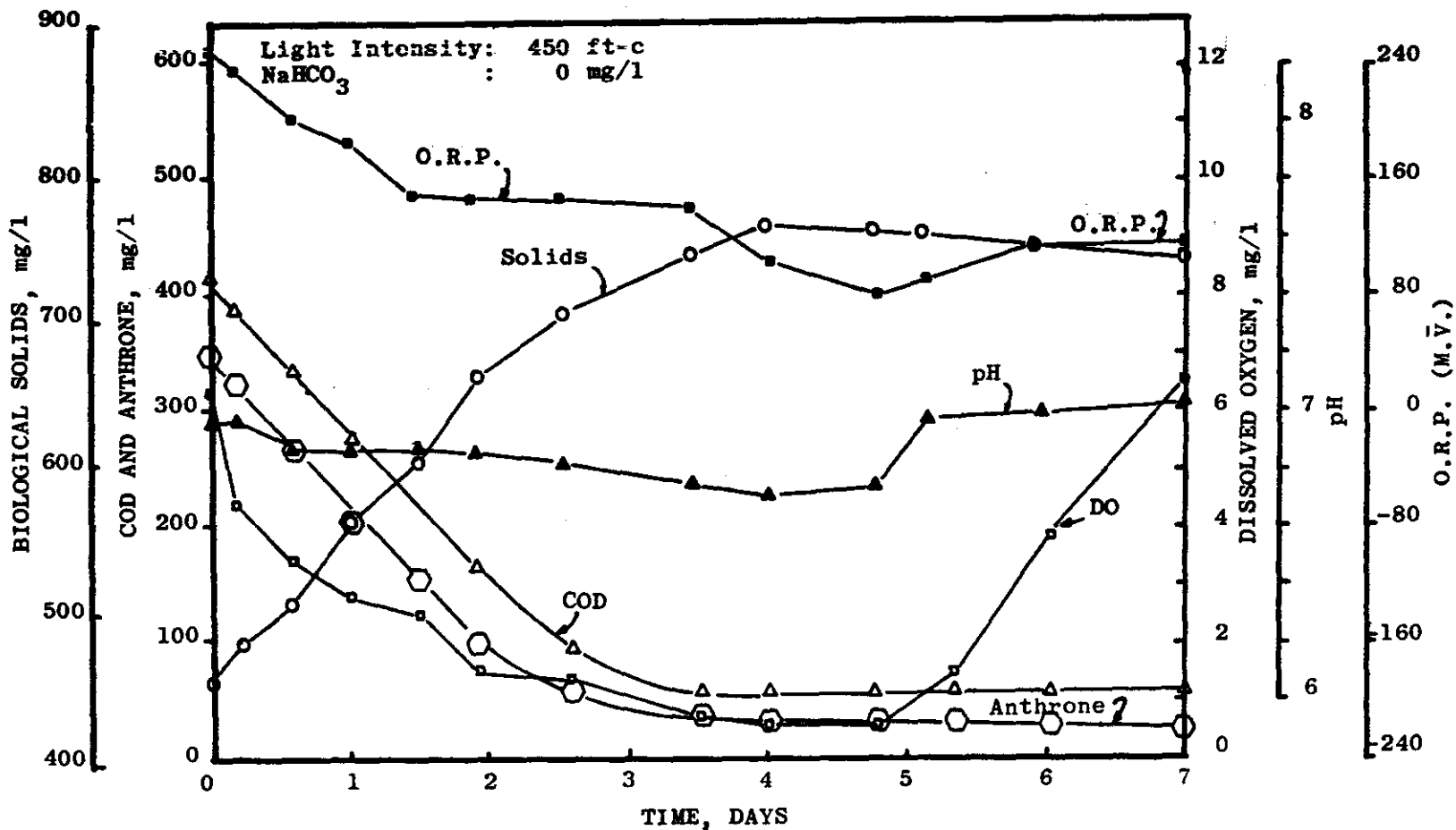


Figure 21. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

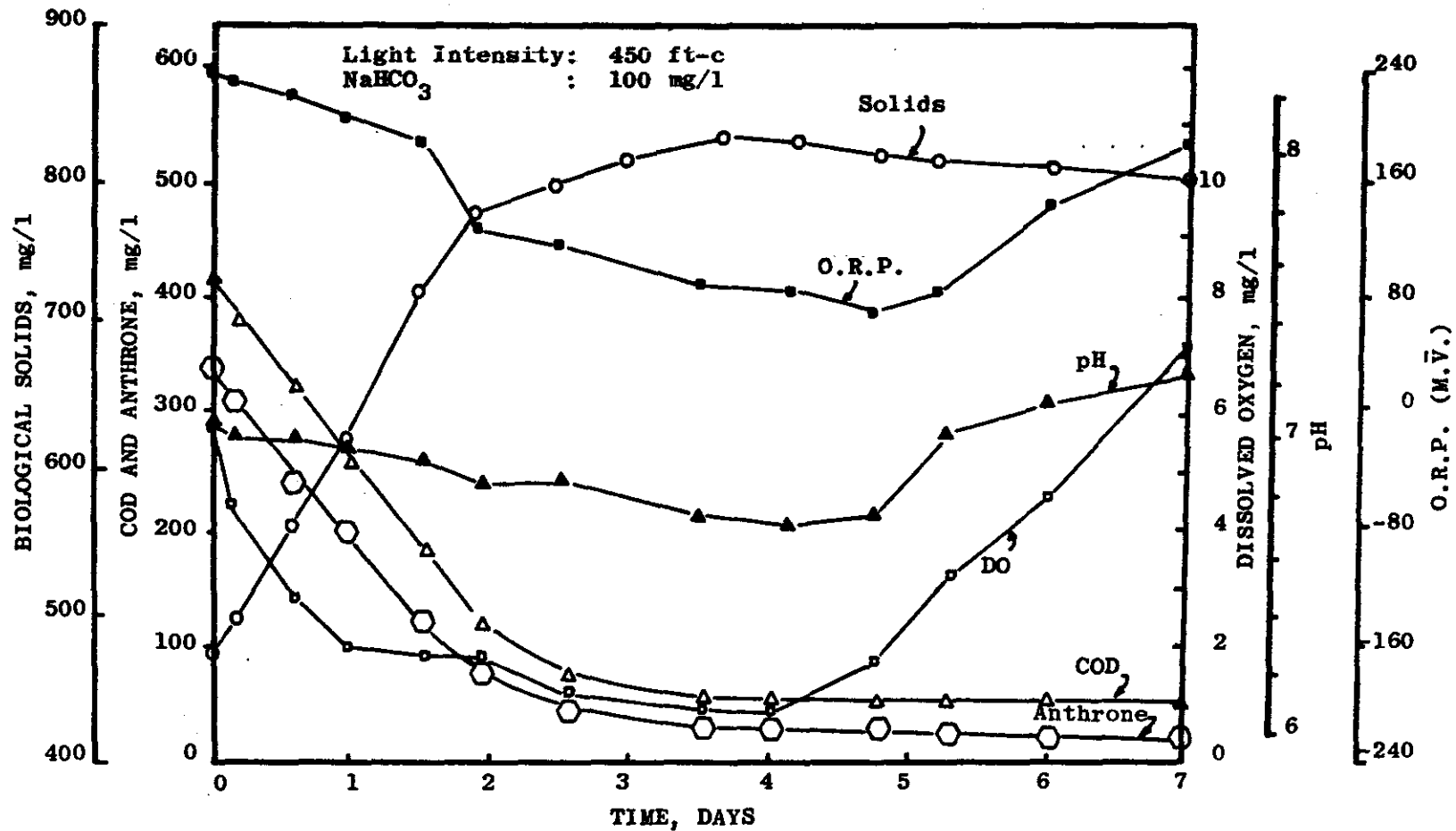


Figure 22. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).



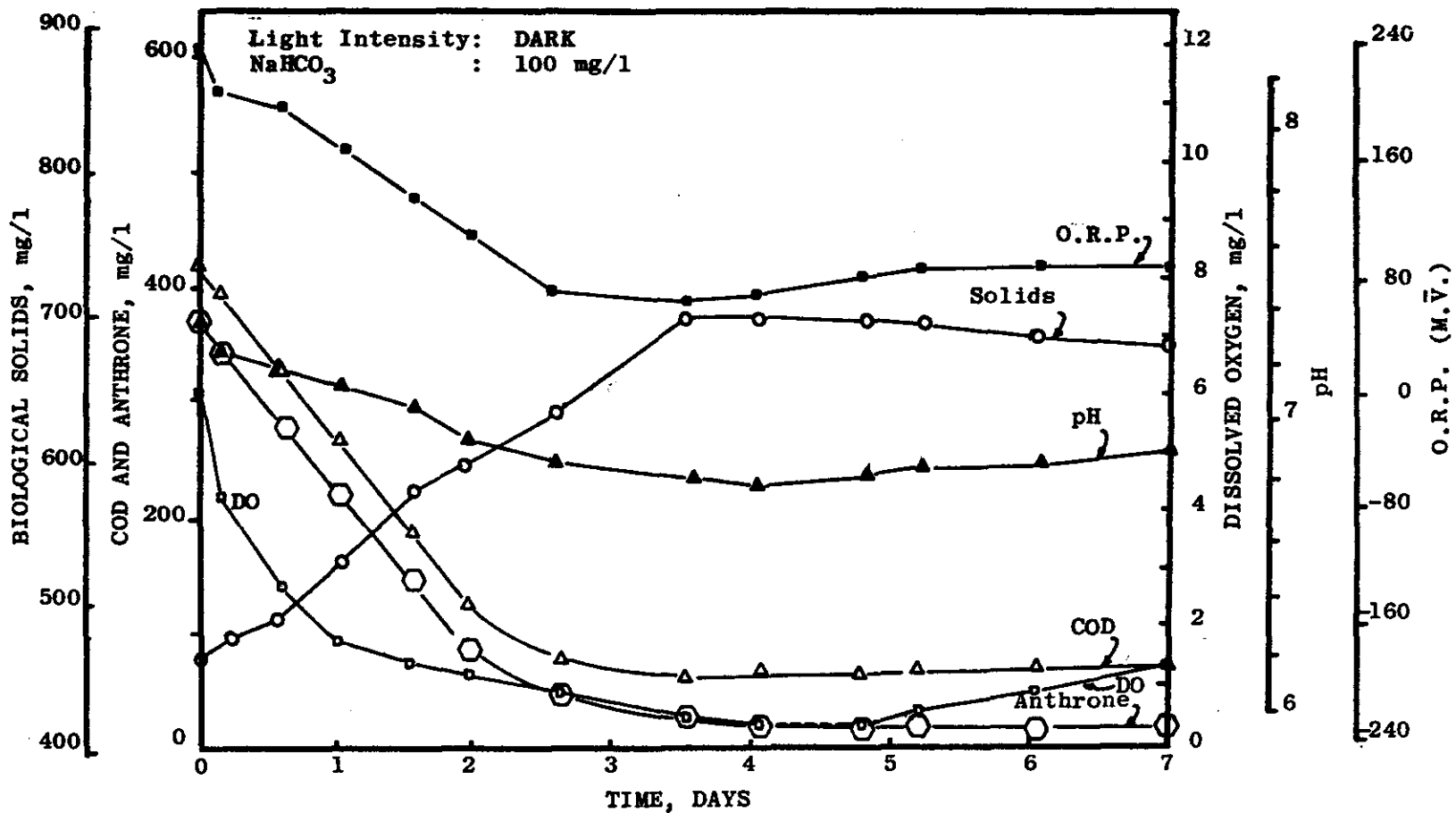


Figure 23. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

that changes in oxidation reduction potential (O.R.P.) could be correlated fairly well to changes in dissolved oxygen concentration.

Experiments at the 500 mg/l glucose loading level are shown in Figures 24, 25, and 26. At this loading level there was a rather serious oxygen deficiency in all three systems. The oxidation reduction potential dipped below zero, and in the systems which received light the attainment of negative O.R.P. marked an apparent change in the predominance or in physiological characteristics of the algal culture in the system. The color of the unit changed from a dark green to a yellowish-green. The dark system exhibited some physical evidence of anaerobiosis, e.g., traces of hydrogen sulfide gas were emitted from the pond surface.

Anaerobiosis was much more pronounced at the 600 mg/l glucose loading level (see Figures 27, 28, and 29). At this loading level there was a severe depression in oxidation reduction potential, and a much more noticeable change in algal characteristics, as evidenced by a color change from dark green to light yellow after the oxidation reduction potential dropped below zero. All systems exhibited typical anaerobic odors during the period of negative oxidation reduction potential.

2. Organic Loading Applied at Three-day Intervals  
with Samples taken over a Period of Twelve Days  
In this set of experiments, two loading levels, 100

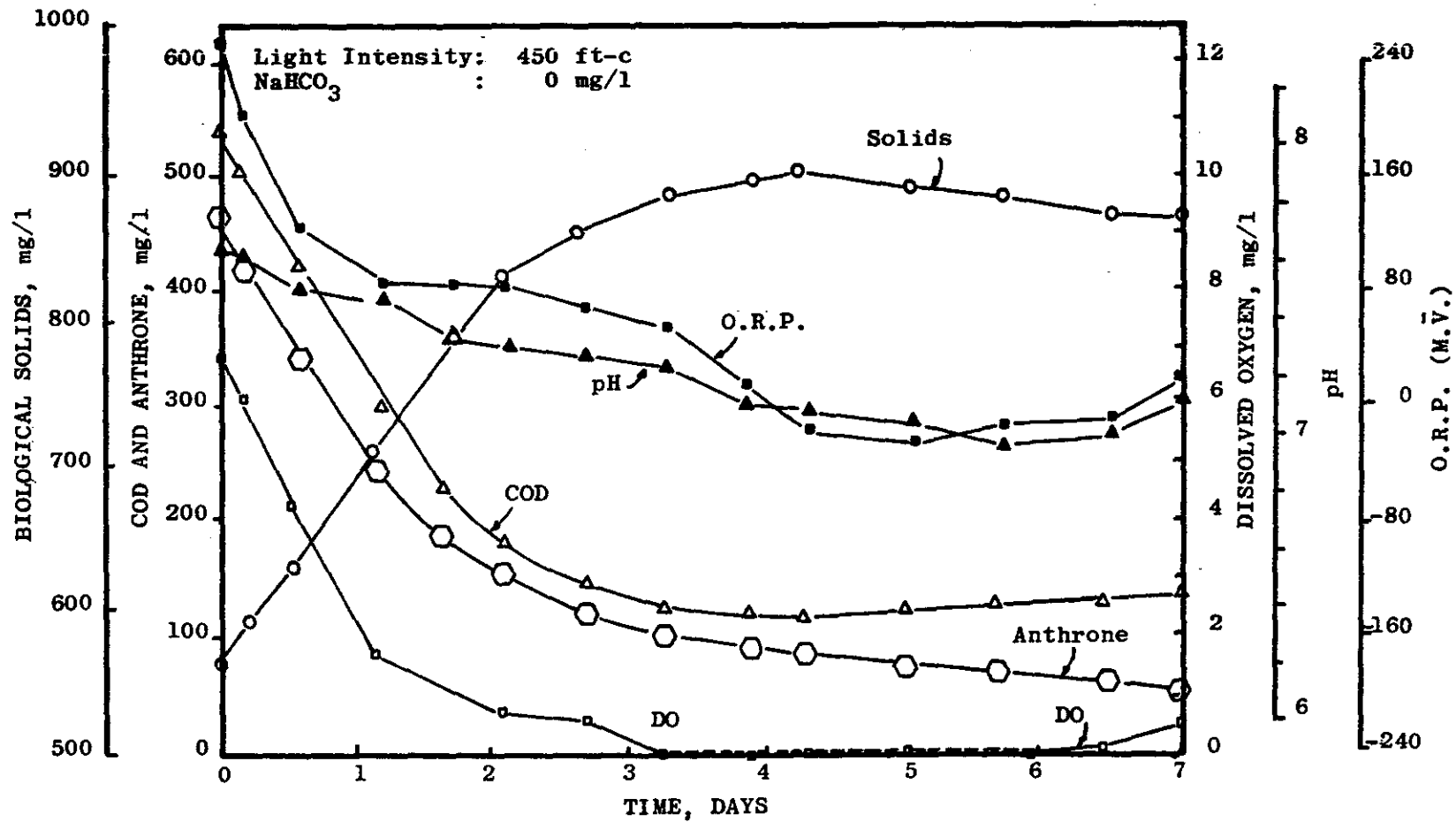


Figure 24. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

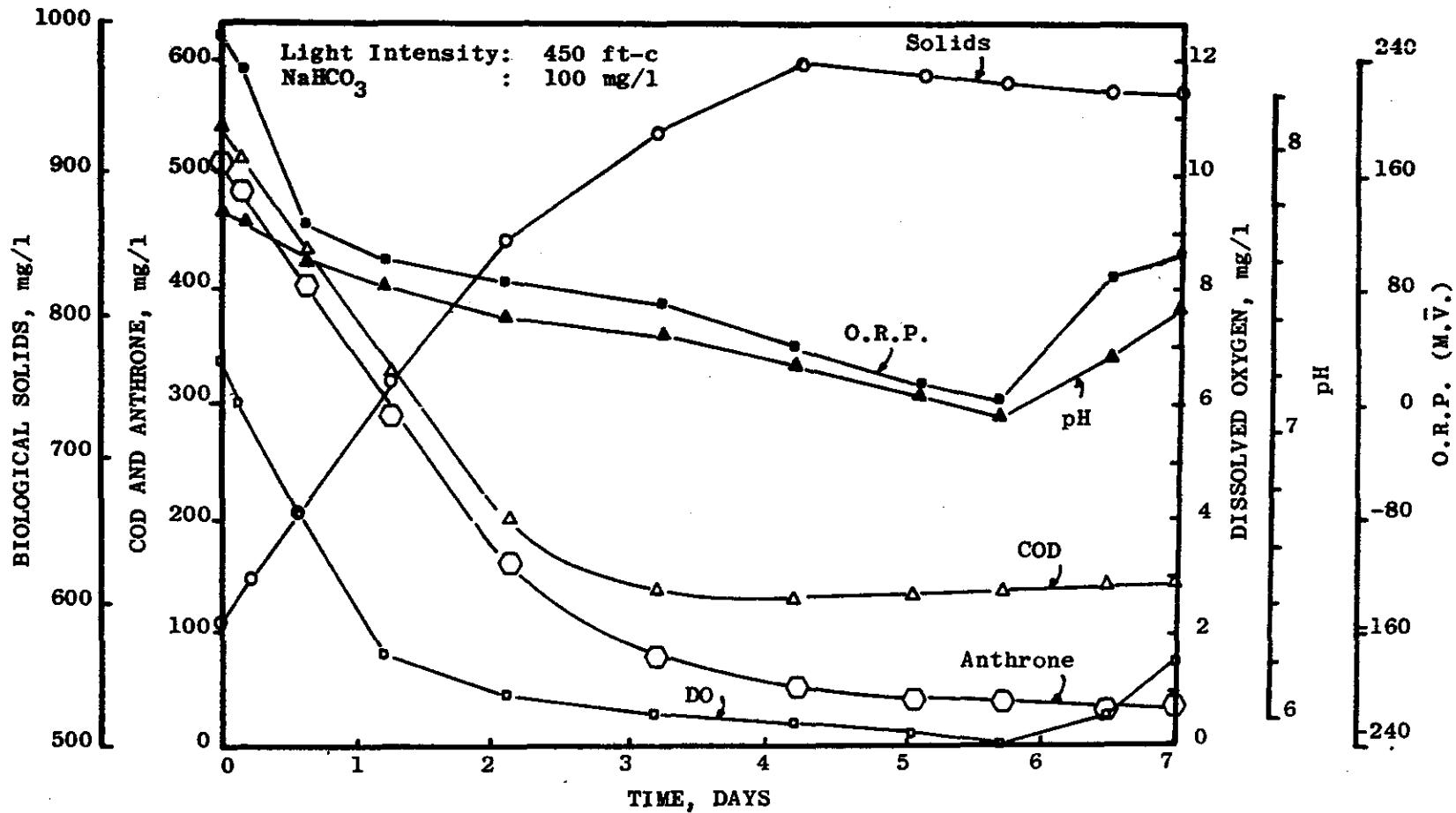


Figure 25. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

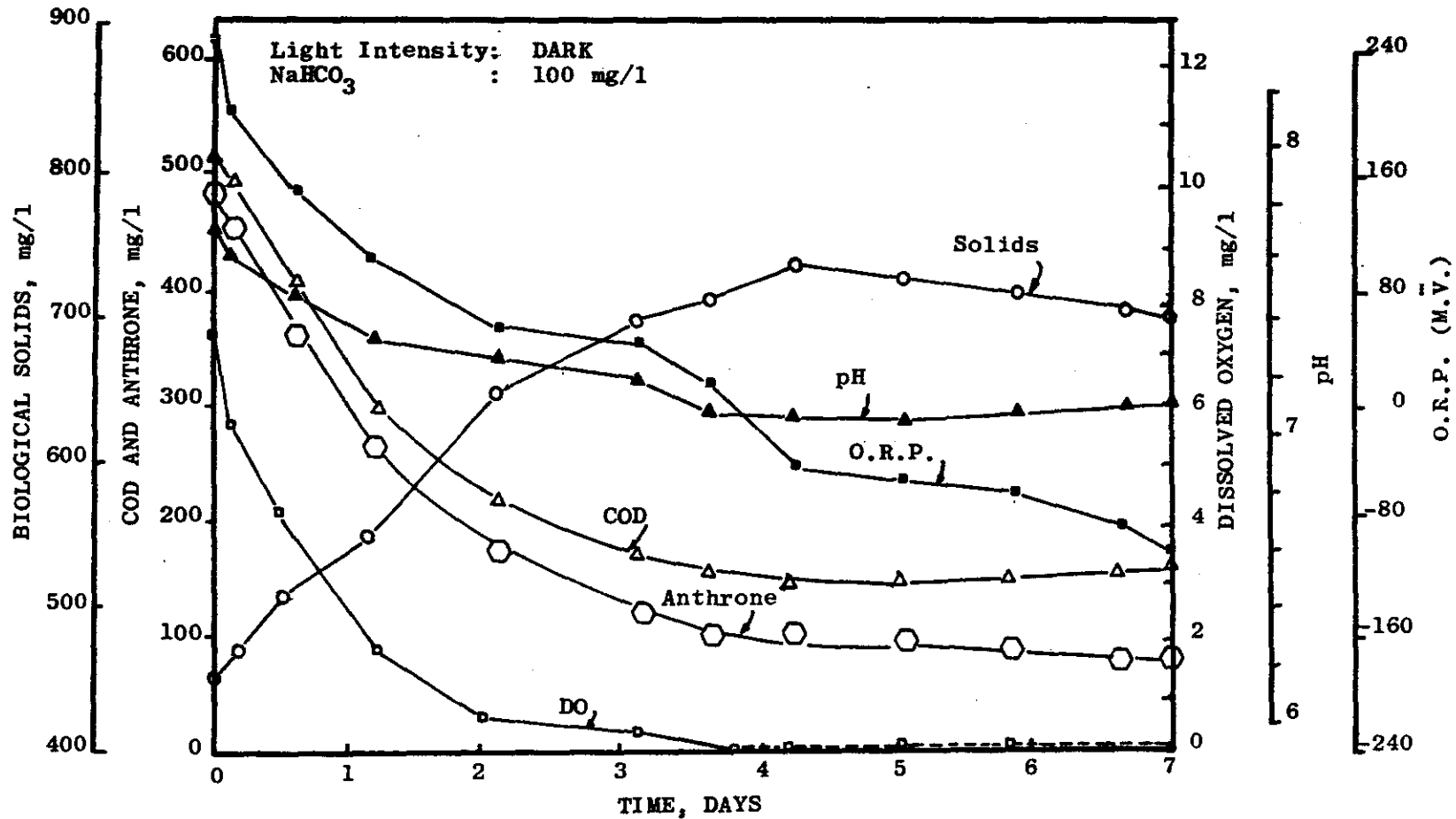


Figure 26. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

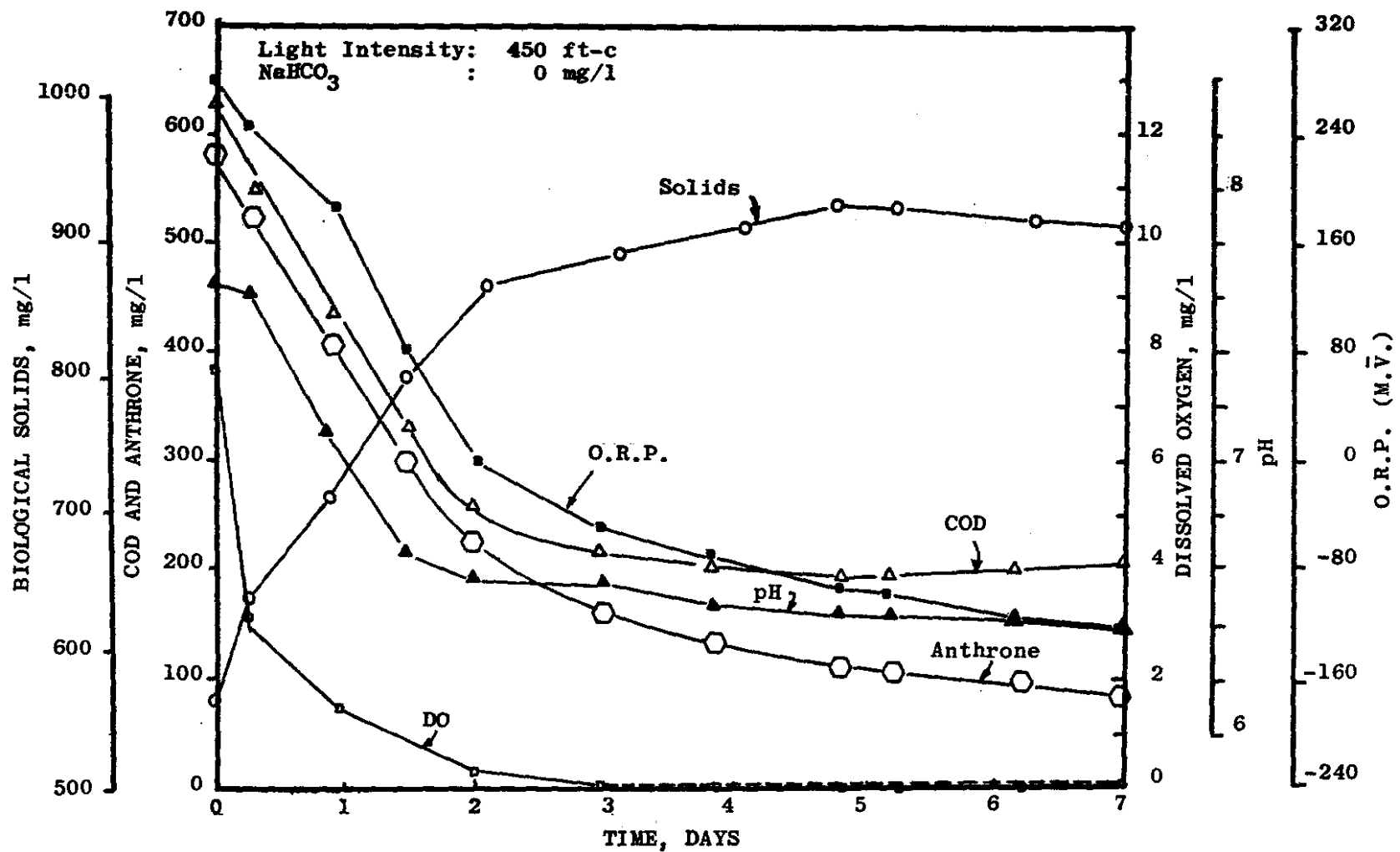


Figure 27. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

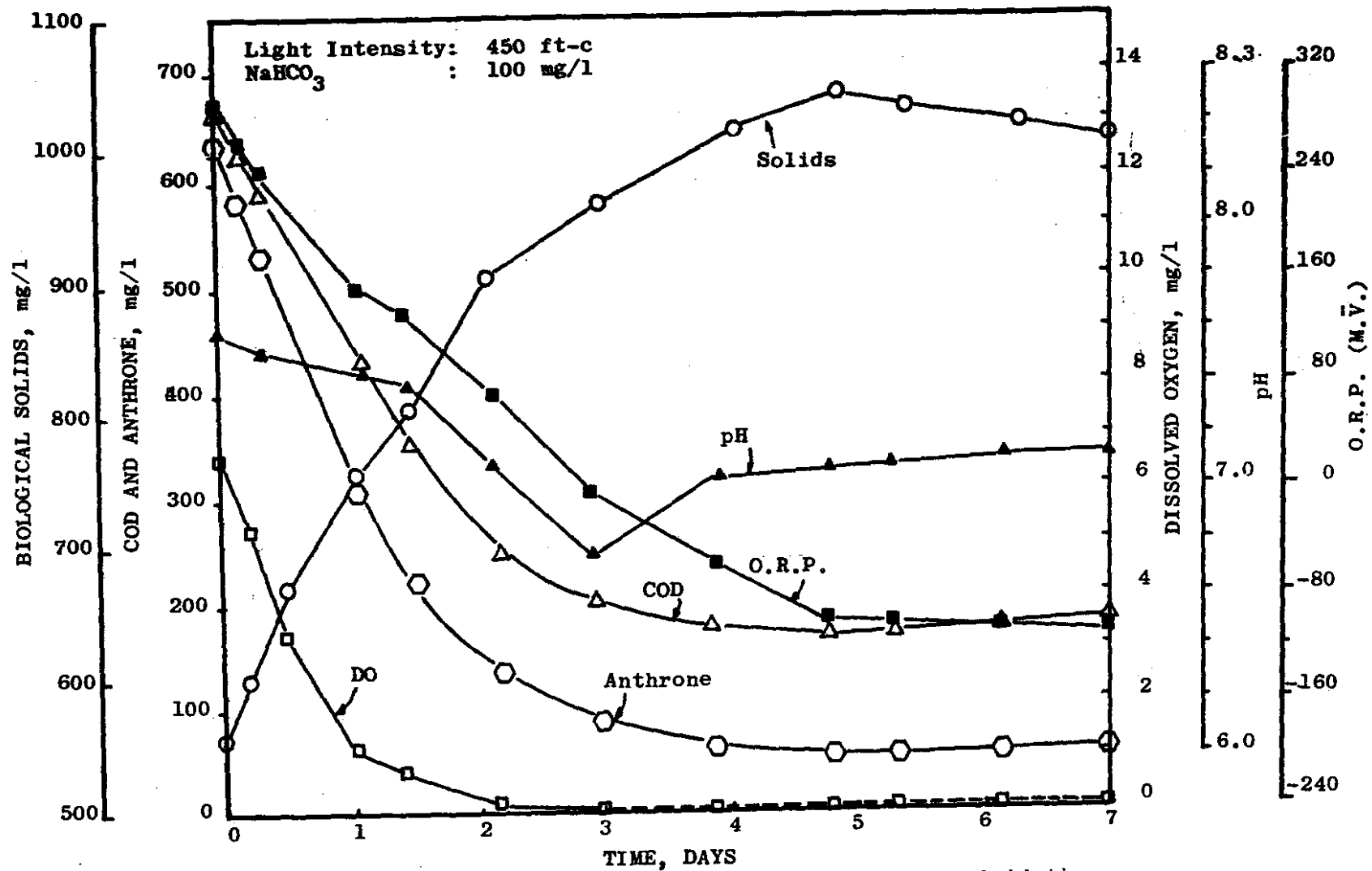


Figure 28. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

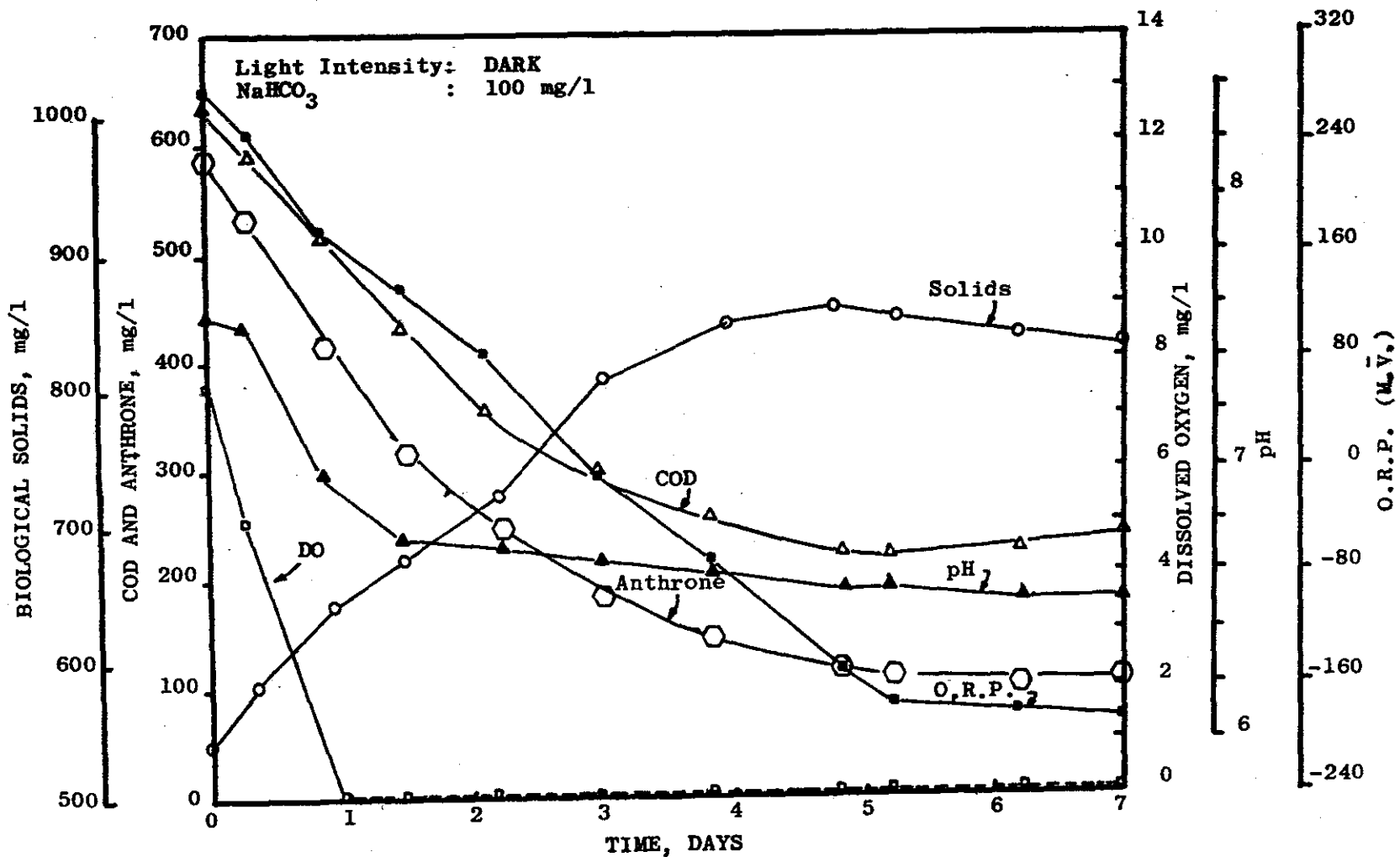


Figure 29. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.



mg/l and 200 mg/l glucose, were employed. All other experimental conditions were the same as those used for the studies reported in the previous subsection.

Figures 30, 31, and 32 show the performance of the three types of systems at the 100 mg/l glucose loading level. It is seen from Figures 30 and 31 that either system which received light performed fairly well with respect to substrate removal, and that in both systems there was a constantly increasing concentration of biological solids. It is also interesting to note that in both of these systems the dissolved oxygen level at the end of each loading cycle after the first cycle was higher than the preceding one. In Figure 32 it is seen that substrate removal efficiency was gradually retarded upon repetitive feeding, and that the dissolved oxygen level was considerably lower than that shown in Figures 30 and 31. The biological solids level increased at a fairly uniform rate in all systems, but the net increase was greater in the lighted ponds.

Figures 33, 34, and 35 show the behavior of the three systems at a loading level of 200 mg/l glucose. It is seen that at the three-day feeding cycle the oxidation pond systems which received light did not remove the carbon source very effectively, nor was there a high concentration of dissolved oxygen present at any time after the initial oxygen had been used. The oxidation reduction potential in Figures 33 and 34 did not exhibit the cyclic

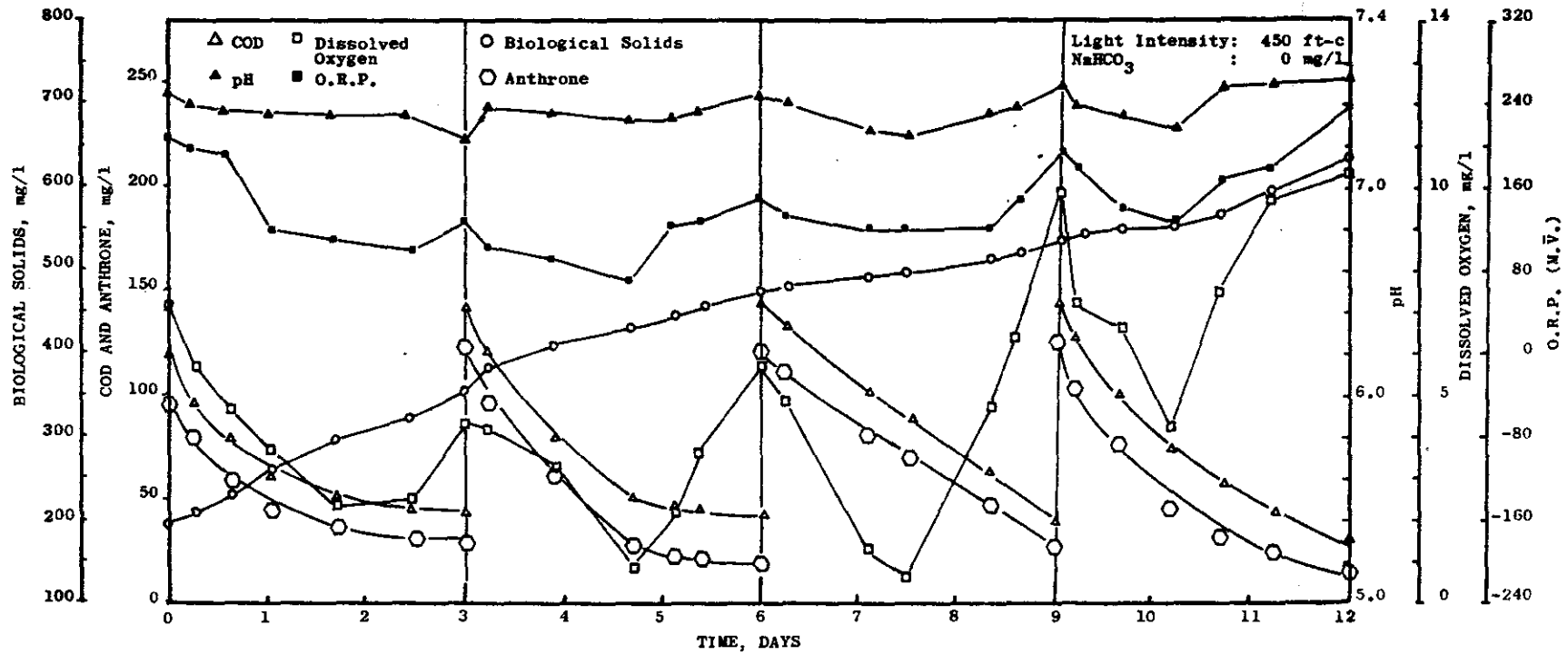


Figure 30. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

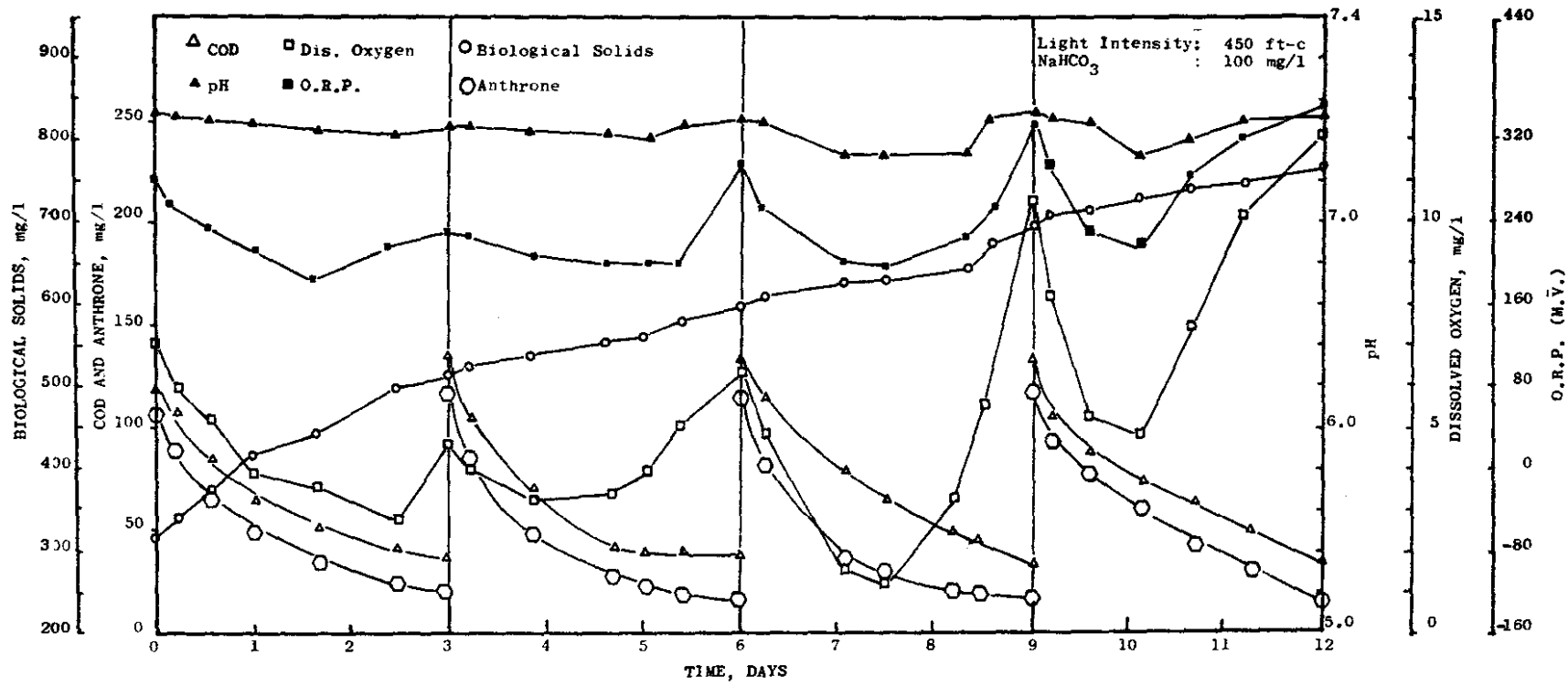


Figure 31. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

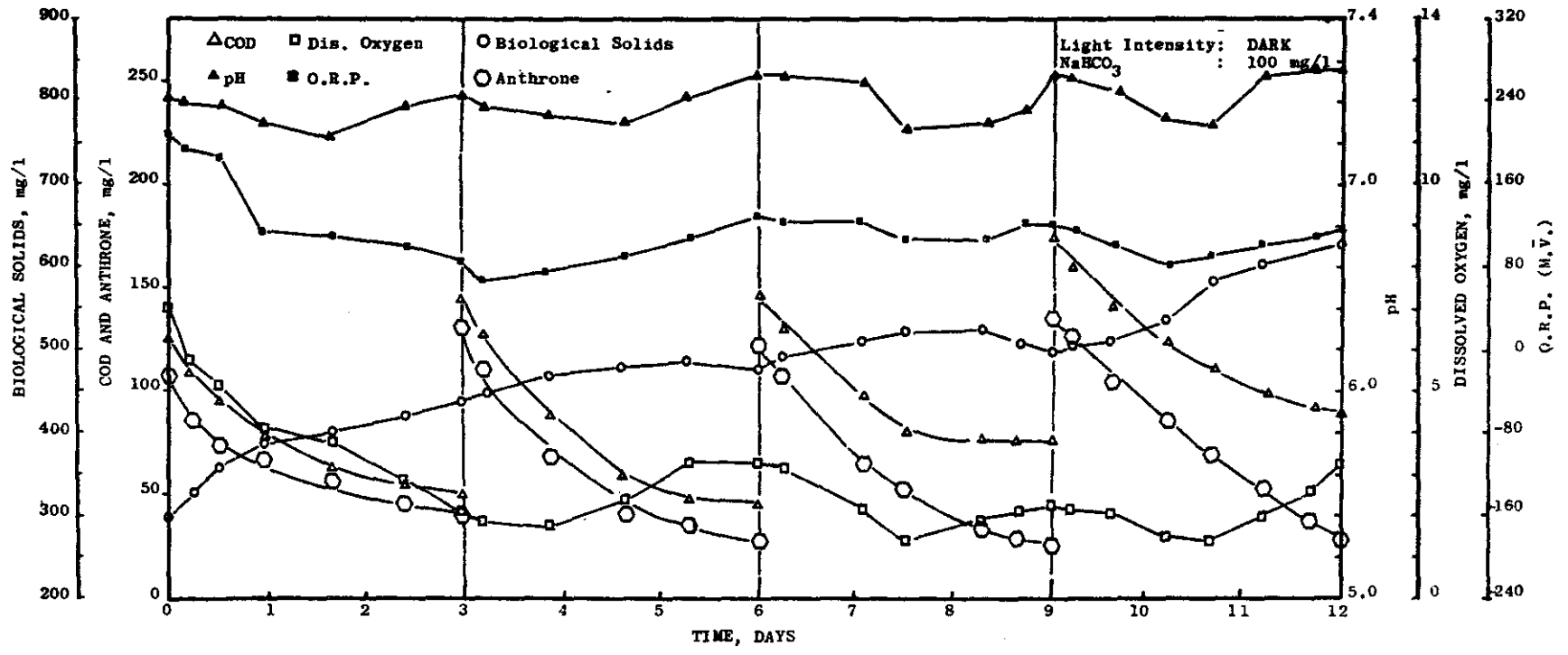


Figure 32. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

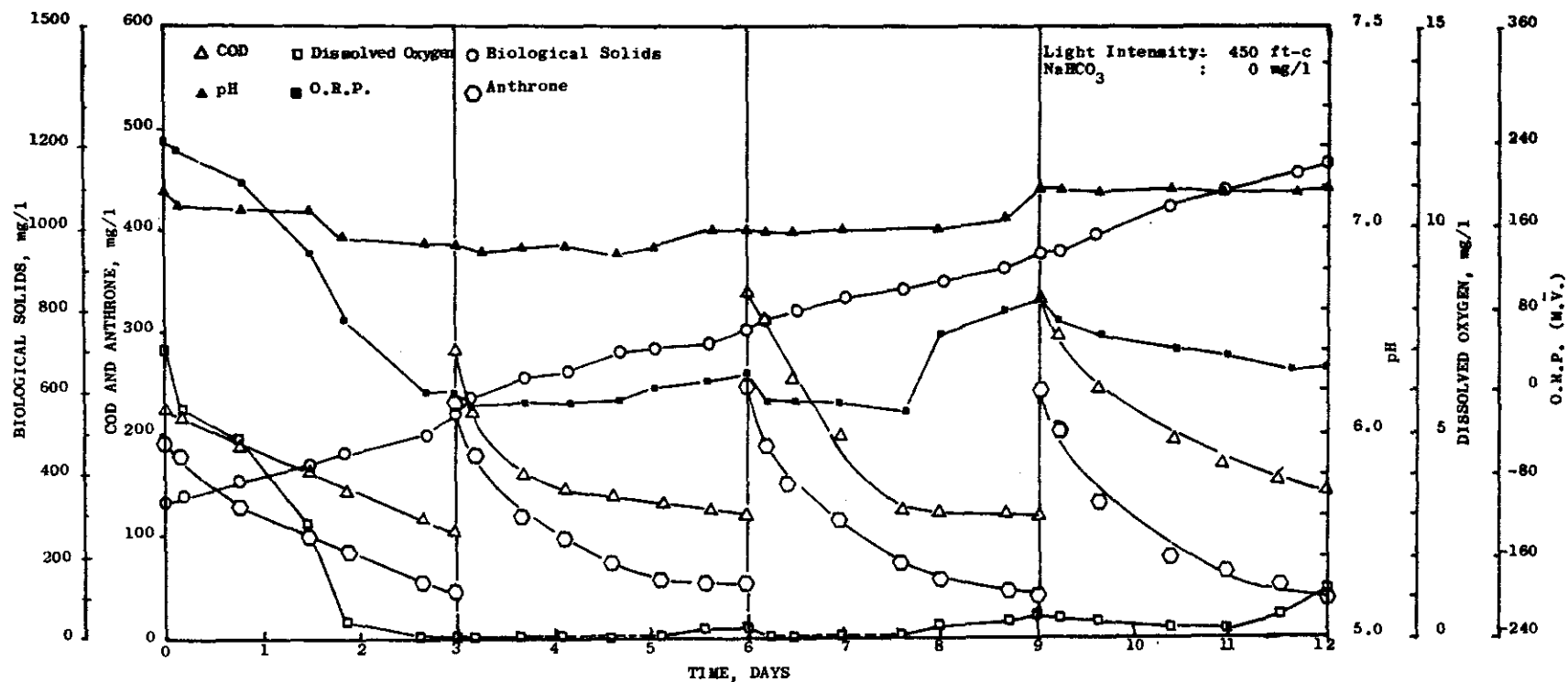


Figure 33. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

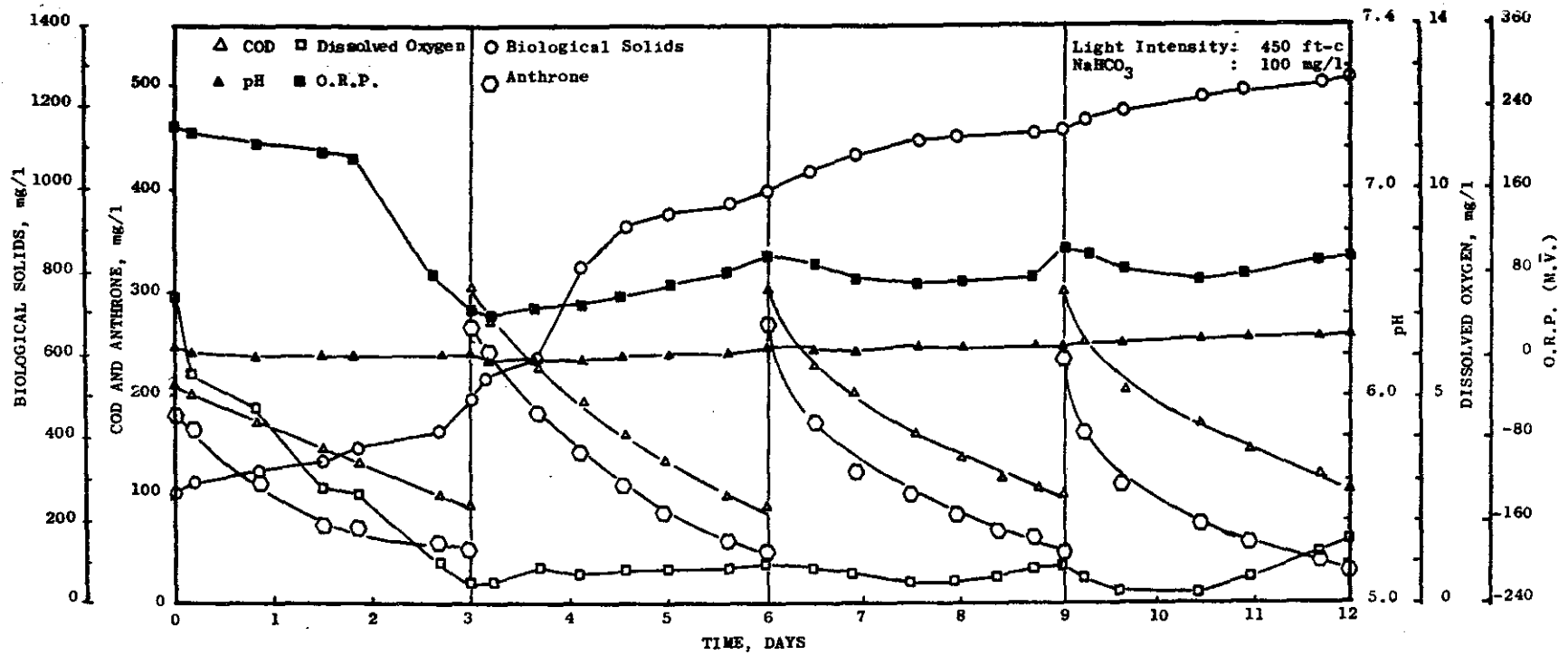


Figure 34. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

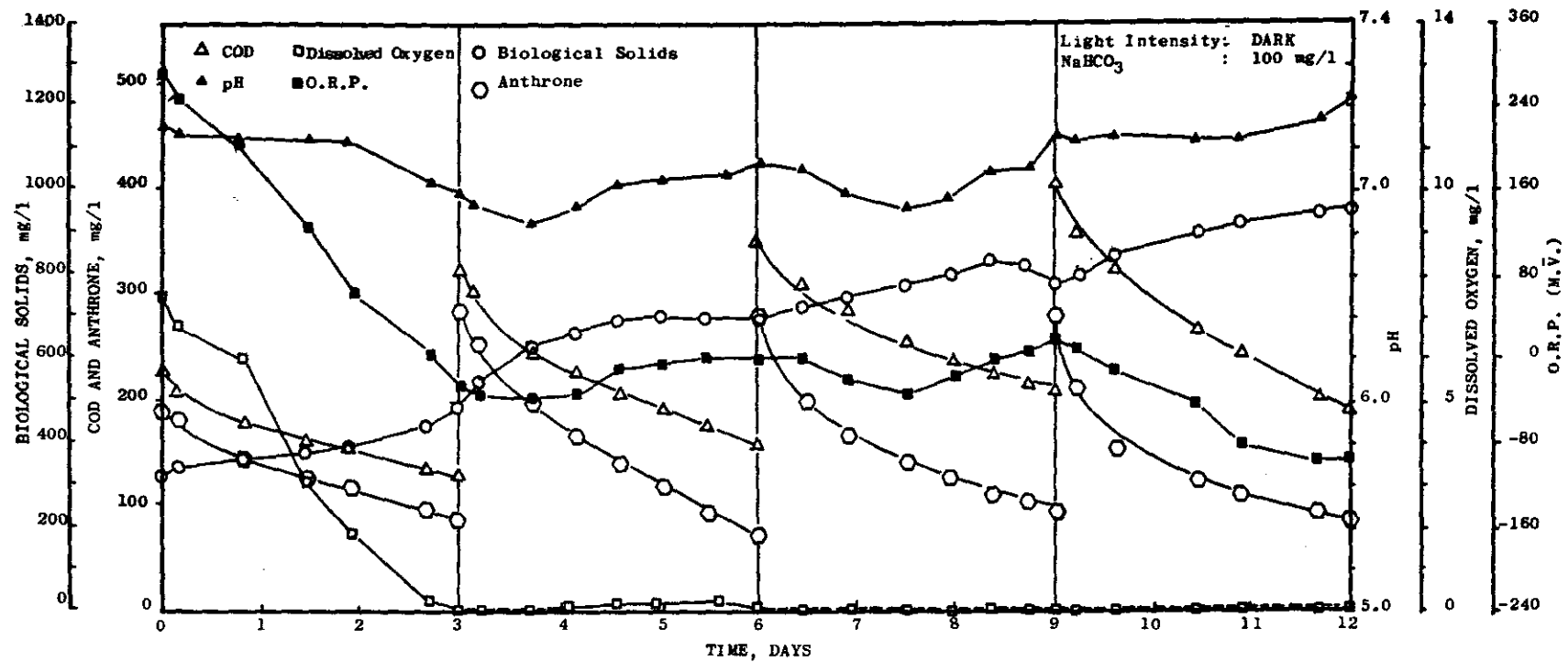


Figure 35. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

pattern shown in Figures 30 and 31 for systems operated at the 100 mg/l glucose loading level. In the system which received no light (Figure 35), it can be seen that anaerobic condition existed after the first feeding cycle. This period of the experiment also marked the onset of other evidence of anaerobiosis, i.e., the typical anaerobic digester smell, hydrogen sulfide. Table VII shows the percent COD removal after the first day and last day of each feeding cycle. The greater efficiency of the lighted systems is apparent from the table.

### 3. Organic Loading Applied Each Day with Systems Observed over Period of Seven Days

Figures 36, 37, and 38 show results for the three types of system studied under loading conditions of 50 mg/l glucose added each day. It is seen that regardless of the presence of light or bicarbonate, the dissolved oxygen concentration in all systems was reduced to zero by the end of the second day of operation. As observed in previous experiments, the color of the units changed from a rather dark green to a light green shortly after the second day of operation when the dissolved oxygen had dropped to zero. In the system which received sodium bicarbonate (Figure 37) there was considerable recovery in the oxidation reduction potential after the fourth day of operation, and it was evident that the dissolved oxygen concentration was increasing during the sixth day of operation. Using oxidation



TABLE VII

PERCENT COD REMOVAL FOR BEGINNING AND ENDING LOADING PERIOD  
FOR THREE-DAY LOADING INTERVALS

(Data taken from Results shown in Figures 30 through 35)

Figure	Substrate Loading, mg/l	COD Removal Efficiency (%)							
		Day 1	Day 3	Day 4	Day 6	Day 7	Day 9	Day 10	Day 12
30	100	46.6	63.3	47.9	71.4	28.2	73.4	43.1	76.2
31	100	44.9	67.3	50	69.7	39.4	75.7	45.6	76.0
32	100	44.4	60.0	46	68.9	33.3	48.3	28.9	56.9
33	200	19.6	50.0	47	56.7	28.4	50	32.6	55.0
34	200	20.4	56.4	32.4	66.2	36.5	66.4	38.6	66.6
35	200	25.4	45.7	28.2	50.2	20.5	39.9	27.4	45.8

System 1: Figures 30, 33 - 12-hr Light, No Bicarbonate

System 2: Figures 31, 34 - 12-hr Light, 100 mg/l Bicarbonate

System 3: Figures 32, 35 - No Light, 100 mg/l Bicarbonate

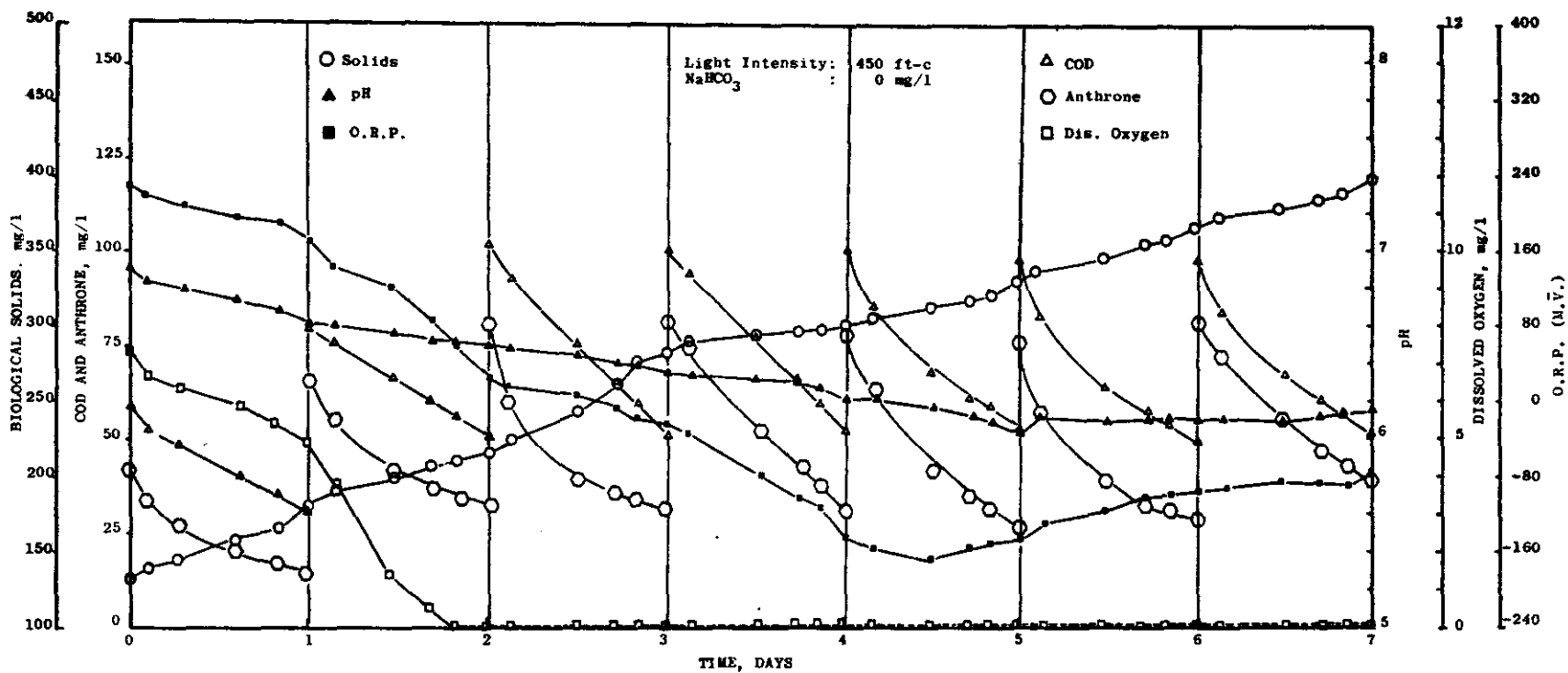


Figure 36. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

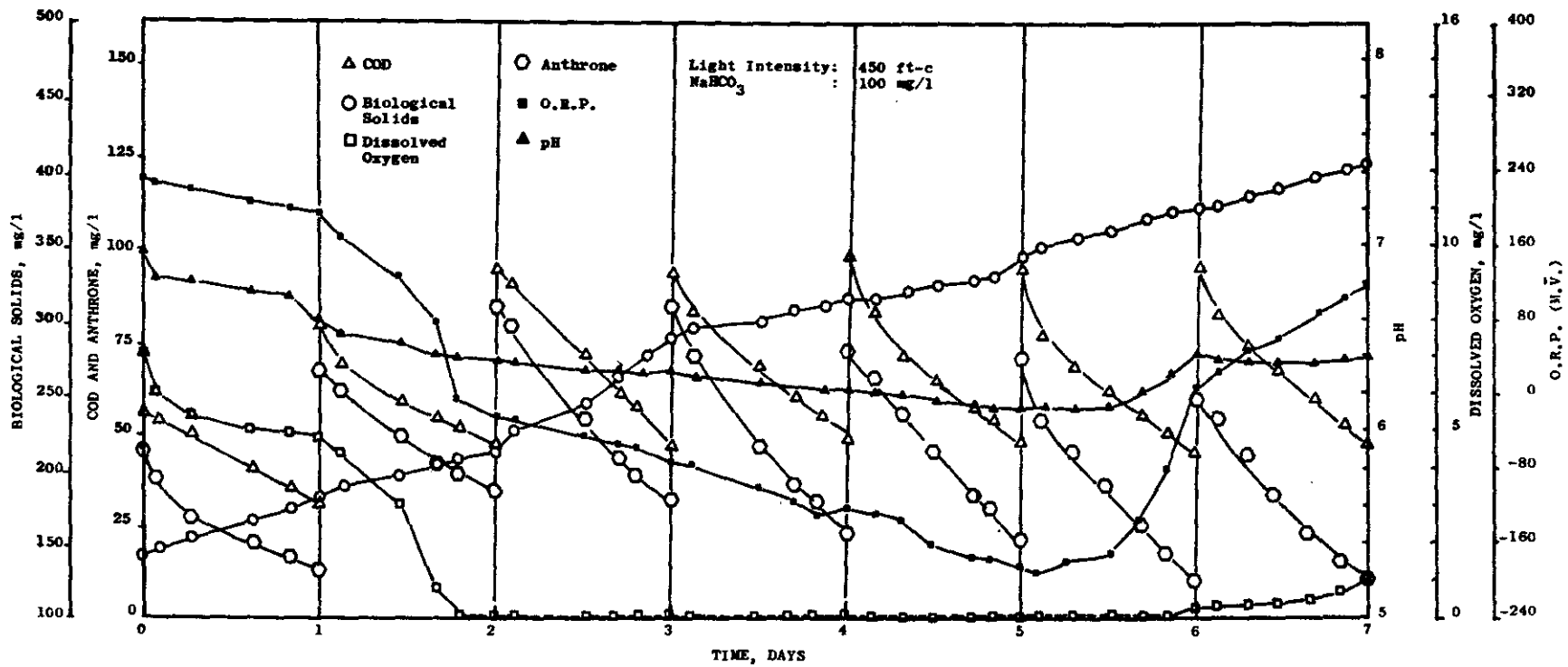


Figure 37. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period = 12 Hrs/Day).

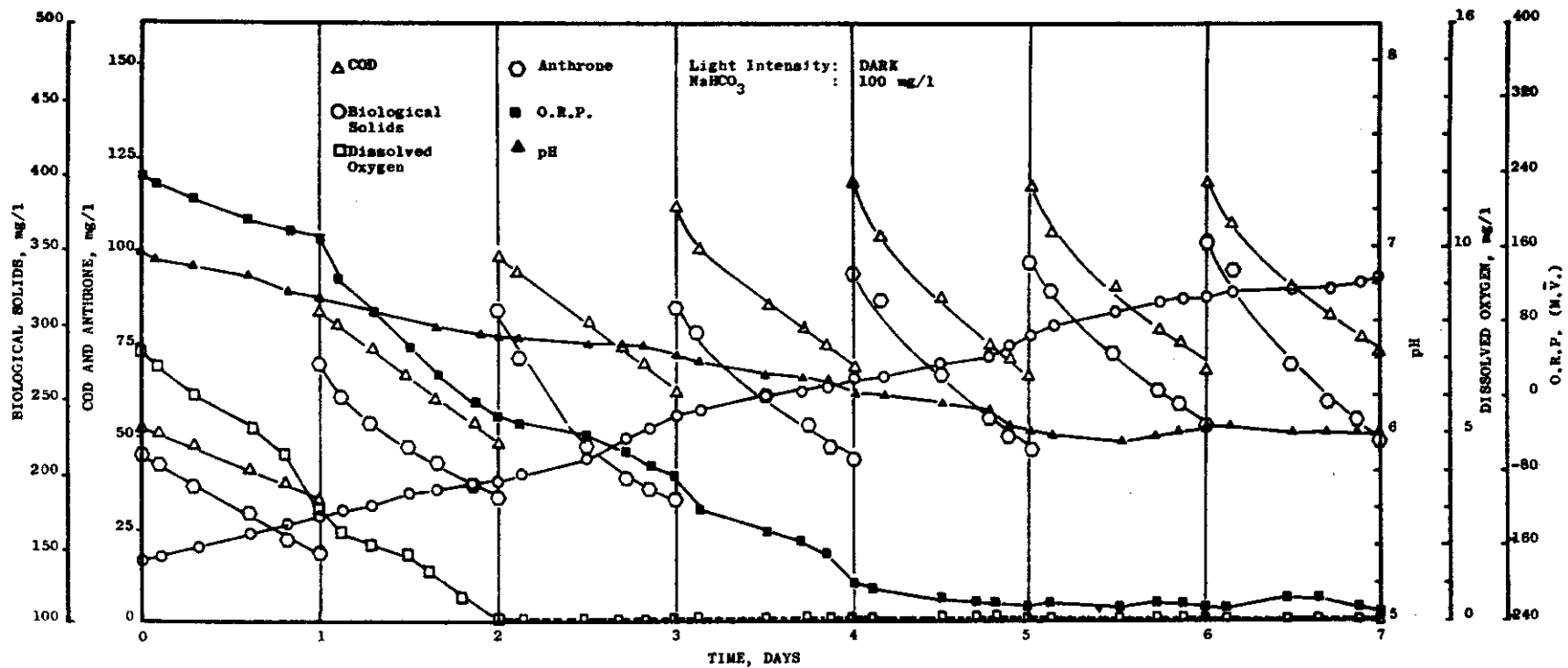


Figure 38. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

reduction potential as an indicator, it seems likely that the system shown in Figure 36 was beginning to recover from a condition of anaerobiosis. It is interesting to note the considerable effect that the addition of sodium bicarbonate had on photosynthetic oxygenation. The beneficial effects of photosynthetic reaeration are rather dramatically shown for the systems by comparison of the oxidation reduction potential curves for the systems which were subjected to light and the system which was not (see Figure 30). Substrate removal was more effective in the lighted systems also.

It was of interest to determine whether the apparent recovery of aerobic conditions exhibited in Figure 37 at the 50 mg/l glucose daily loading would be evidenced at higher loadings during a seven-day period of operation. In order to gain an insight into this possibility, an additional experiment was run for a system which received in addition to 100 mg/l sodium bicarbonate daily, 100 mg/l glucose. The results are shown in Figure 39. It is seen that the dissolved oxygen dropped to zero in about one and one-half days, and exhibited no recovery within the seven-day period of observation. Oxidation reduction potential showed a continual decrease throughout the experiment. The inability of the system to remove substrate effectively is shown by the gradual increase in COD and substrate at the end of each successive feeding cycle.

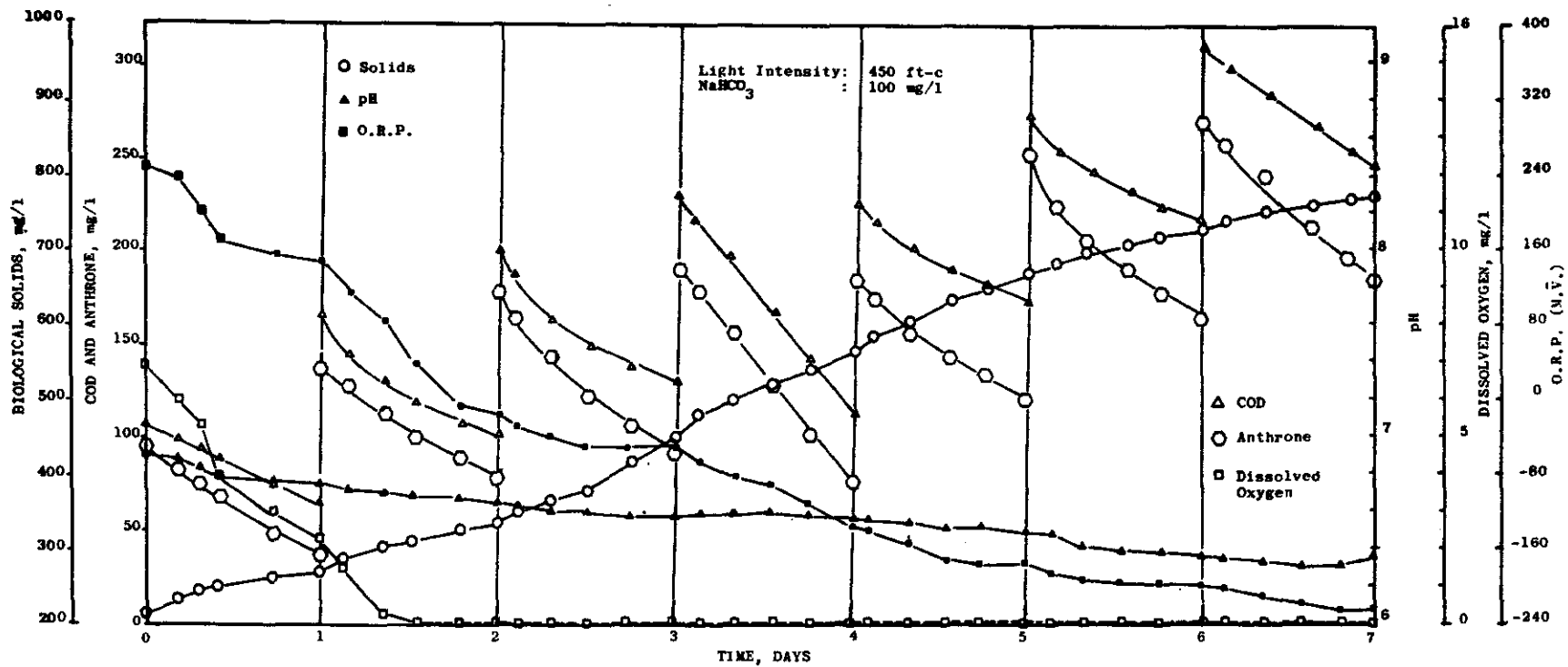


Figure 39. Purification of 100 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

## B. Continuous Flow Studies

The first series of experiments in the continuous flow oxidation ponds were run using a detention time of ten days. The performance of the experimental ponds for loading levels of 100, 200, and 600 mg/l glucose are shown in Figures 40, 41, and 42 under conditions of twelve hours of light and twelve hours of darkness. The performance of the ponds during the three or four-day period of batch operation prior to continuous addition of feed is not given in the figures. At the zero day, the concentration of the glucose in the pond was brought up to the desired feed concentration level, and continuous feeding from the feed reservoir was begun. The period of time required to reach somewhat steady concentration levels varied with the organic loading level. In all cases the plotted values represent the average of the top, middle, and bottom samples. In general the values of the levels were very close, except that the biological solids concentration of the bottom sample was always slightly higher than that found at the other two levels. Samples were always taken within one-half hour after turning on the light. In Figure 40 it is seen that in the system fed 100 mg/l glucose the oxidation reduction potential and dissolved oxygen concentration achieved relatively high values after the initial sag. At the 300 mg/l glucose feeding level (Figure 41) the dissolved oxygen and O.R.P. were noticeably lower than at the 100 mg/l glucose feeding

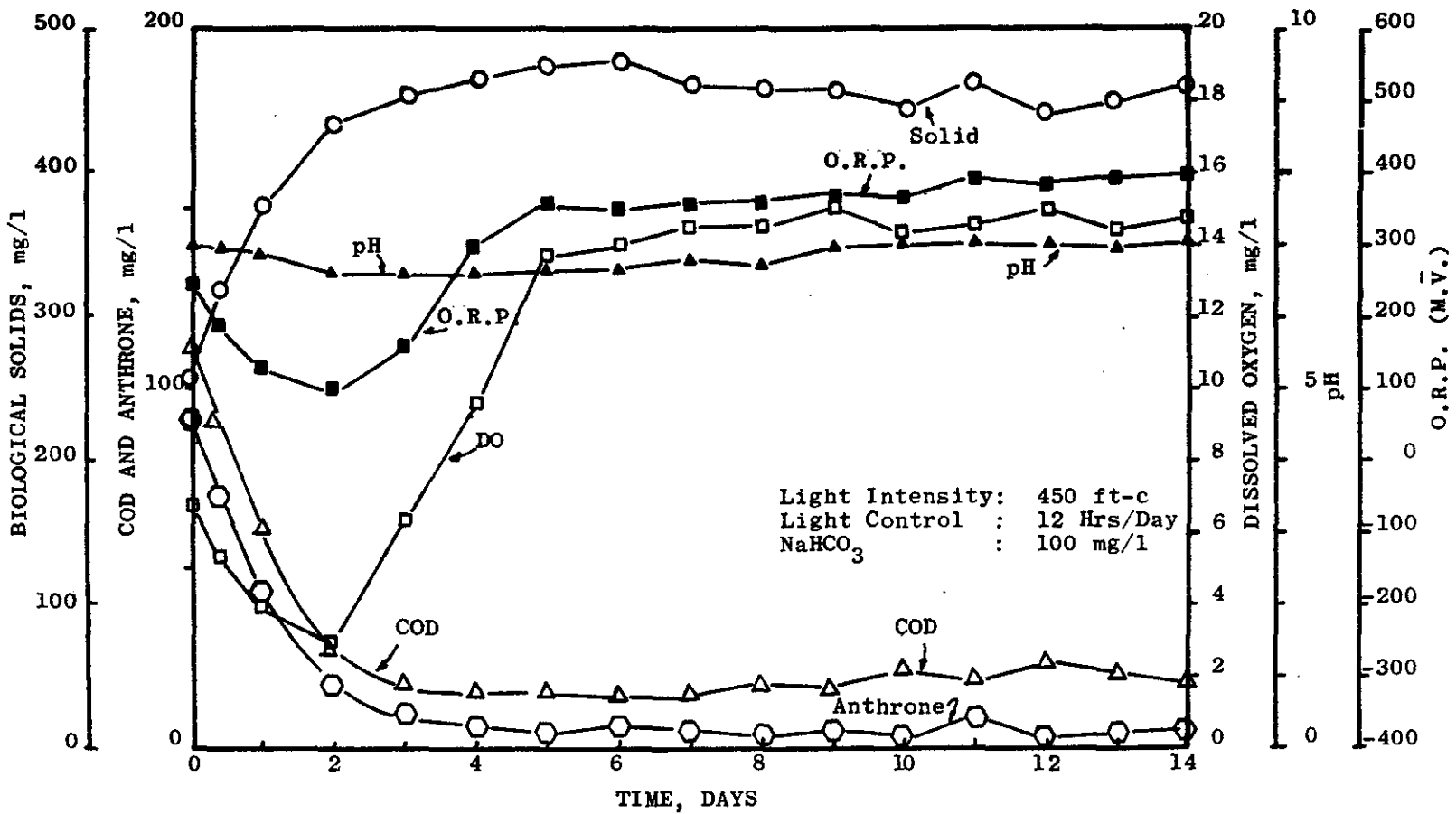


Figure 40. Purification of 100 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.



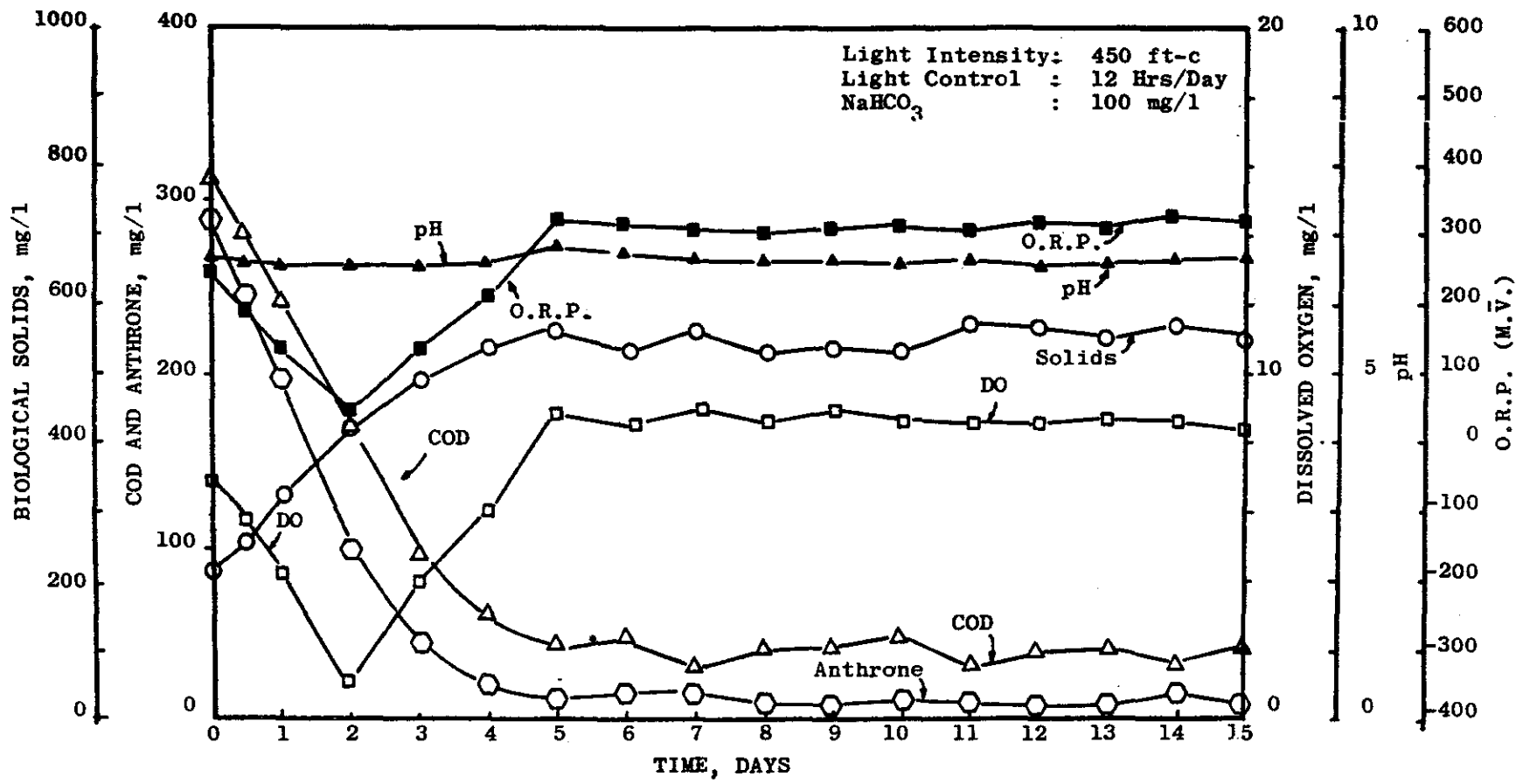


Figure 41. Purification of 300 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.

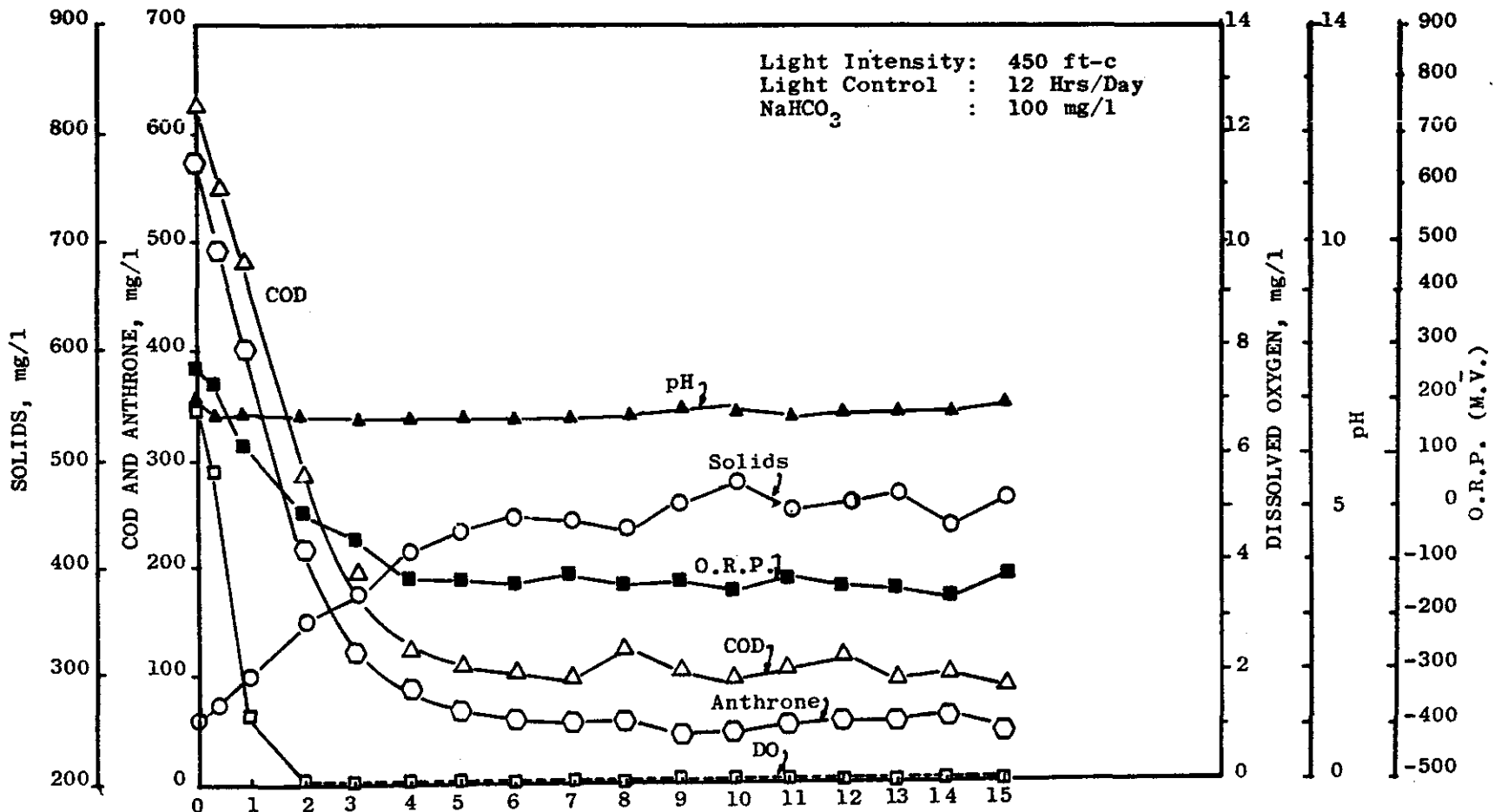


Figure 42. Purification of 600 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.

level. In Figure 42 it is seen that at the 600 mg/l feeding level the system was anaerobic, whether judged by the negative O.R.P. or the absence of dissolved oxygen. The unit also exhibited typical odors of anaerobiosis. Also, it is interesting to note that again, when zero dissolved oxygen and negative O.R.P.'s were recorded, the color of the pond content changed from a bright green to greenish-yellow and thence to a yellow color. It is interesting to note in Figure 43 that even though anaerobic conditions existed, the substrate removal efficiency was fairly good.

It will be recalled that the samples were taken within the first half hour after initiating a light period. Since the light was on for twelve hours and off for twelve hours, it would be expected that there might be some fluctuation in the dissolved oxygen concentration, and indeed, some fluctuation in some other parameters as well. For this reason although it is believed that the unit approached fairly good mixing conditions, it cannot be claimed that these continuous flow ponds operated under conditions of complete mixing. It was of interest for the pond operated at a loading level of 600 mg/l glucose to determine whether the dissolved oxygen recorded at the time of sampling was representative of the dissolved oxygen throughout a 24-hour day. Therefore, after the completion of the fifteen days of operation shown in Figure 42, the pond was sampled on a round-the-clock basis for the next twenty-

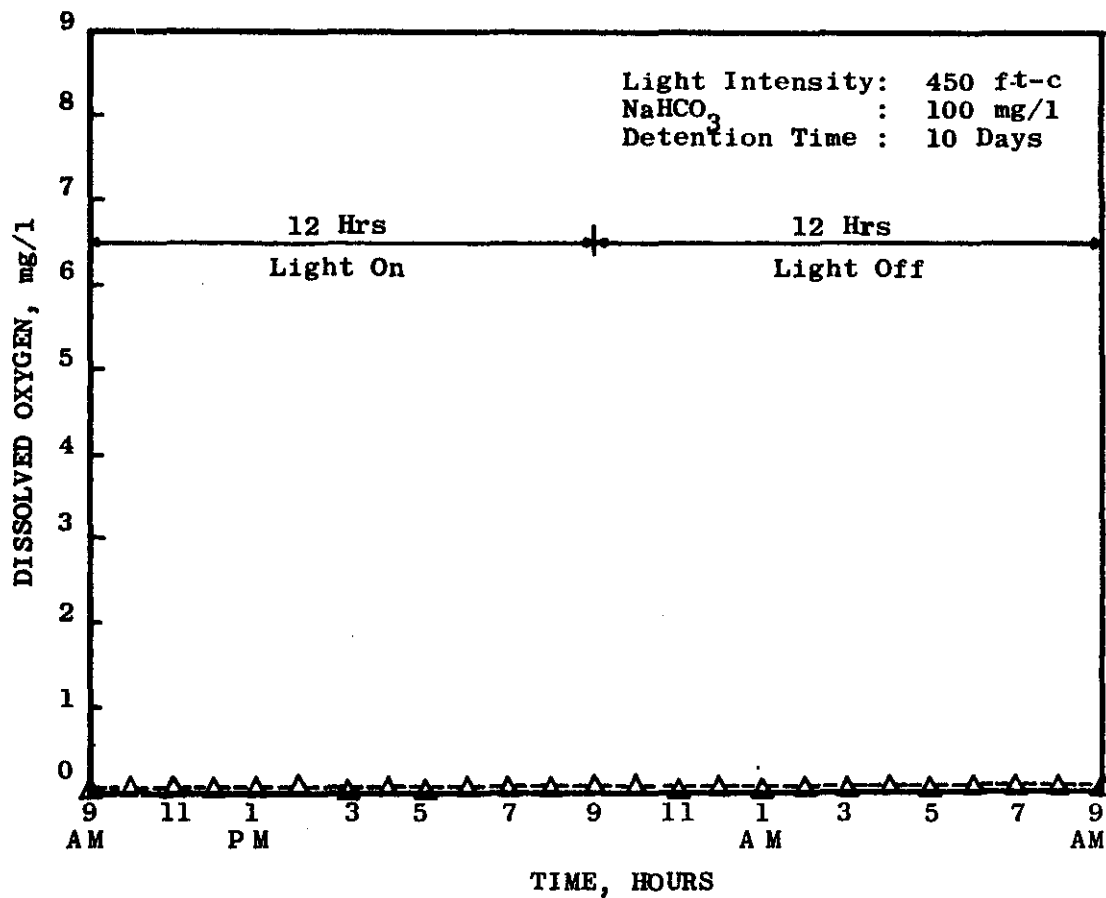


Figure 43. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose (Light Period - 12 Hrs/Day).

four hours, and dissolved oxygen determinations were made. The results are shown in Figure 43. It is seen that at this level of loading there were no cyclic tendencies observed in the dissolved oxygen concentration. On the next day the light was turned on for twenty-four hours, and dissolved oxygen determinations were made during this period. The results are shown in Figure 44, where it is seen that the dissolved oxygen remained at zero.

It was of interest to determine whether a loading of 600 mg/l glucose could be applied at the ten-day detention time if the system was subjected to continuous lighting. The results are shown in Figure 45. Comparison of Figure 46 with Figure 42 shows that the major difference made by operating with the light source on for twenty-four hours was the time taken to reach a concentration of zero dissolved oxygen. Also, the oxidation reduction potential level in Figure 45 is somewhat higher than that in Figure 42. It is seen from Figure 45 that even under conditions of total lighting the oxidation pond experienced anaerobic conditions, as adjudged by the oxidation reduction potential and the dissolved oxygen concentration. The pond also exhibited the typical odors of anaerobiosis.

At the twenty-day detention time experiments were run at glucose loading levels of 100, 300, 600, and 1000 mg/l. It is seen from Figures 46 and 47 that the systems operated extremely well at the 100 and 300 mg/l loading levels.

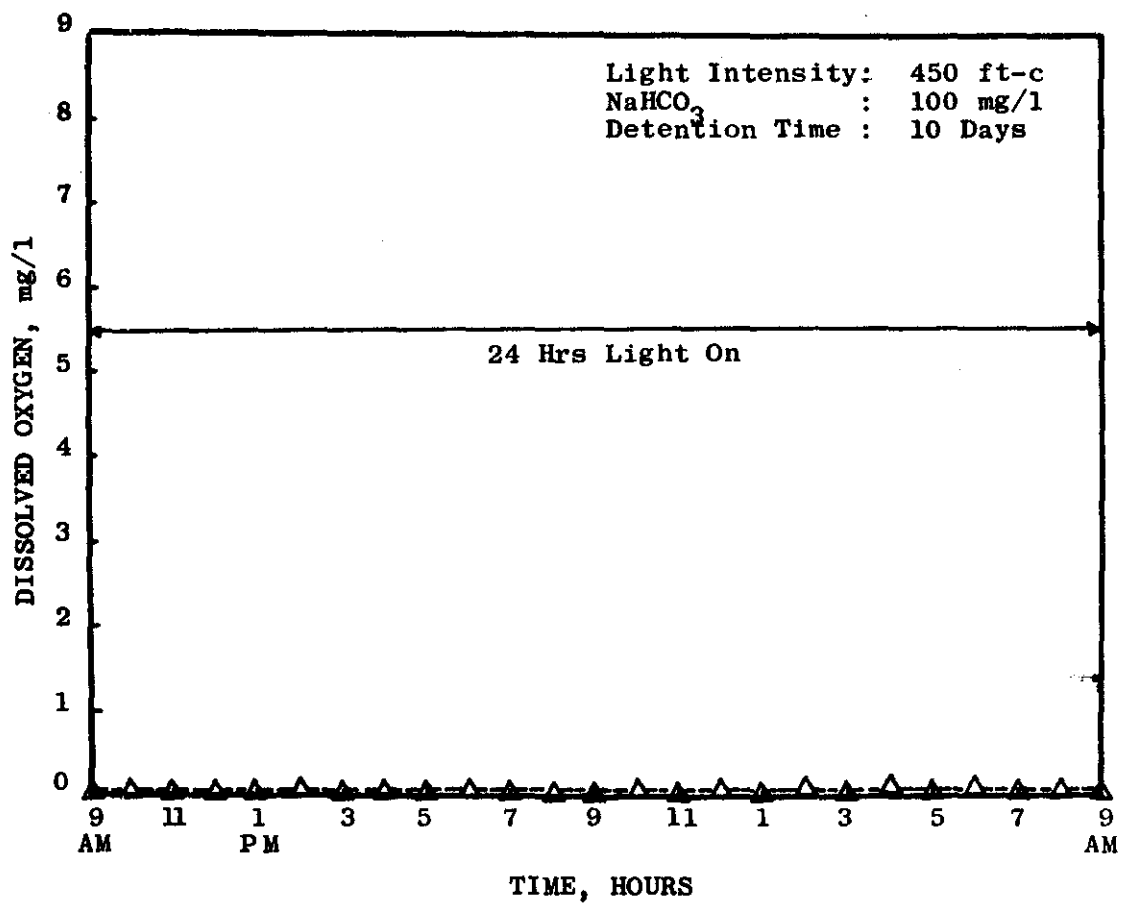


Figure 44. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose (Light Period - 24 Hrs/Day).

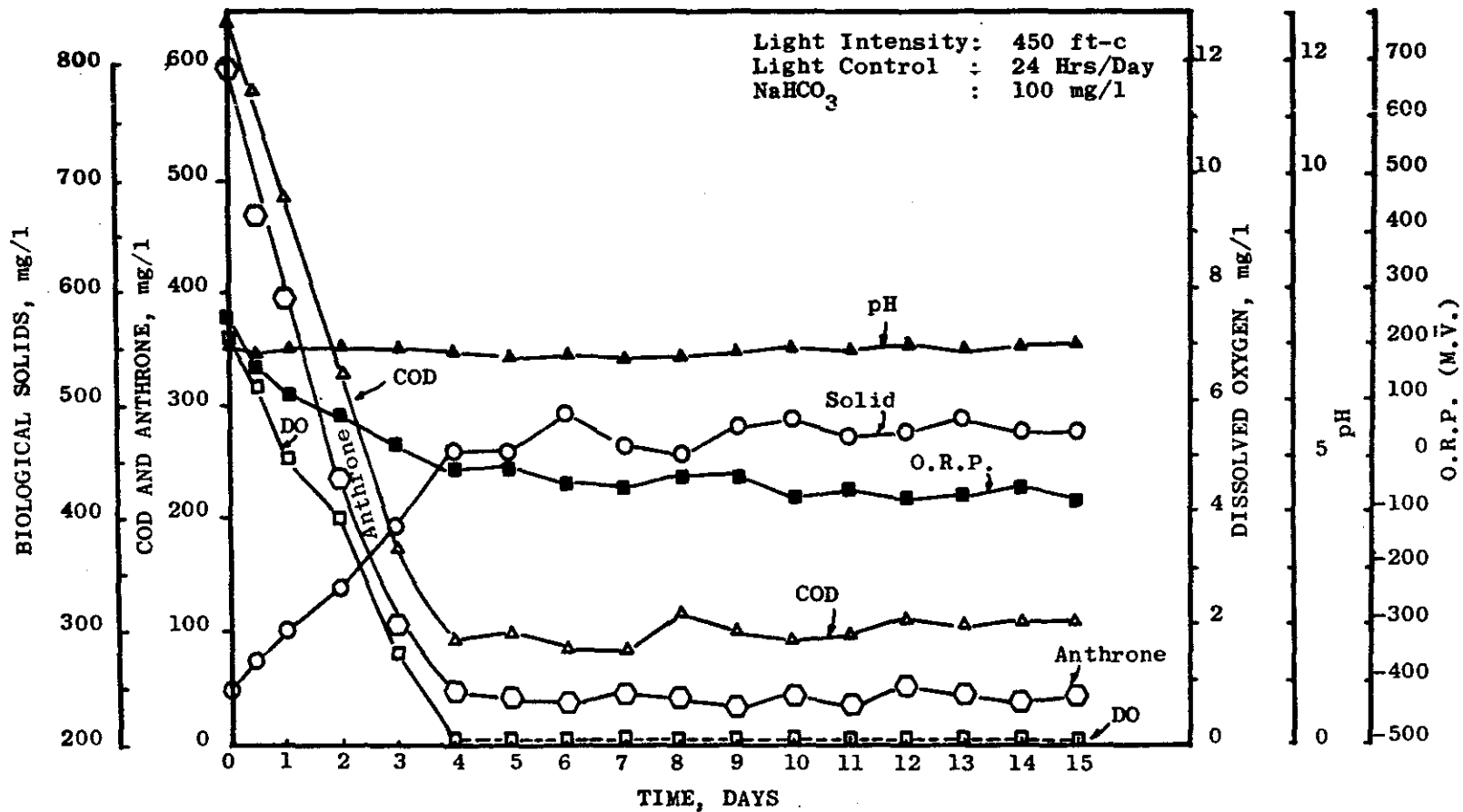


Figure 45. Purification of 600 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at Detention Time of 10 Days.

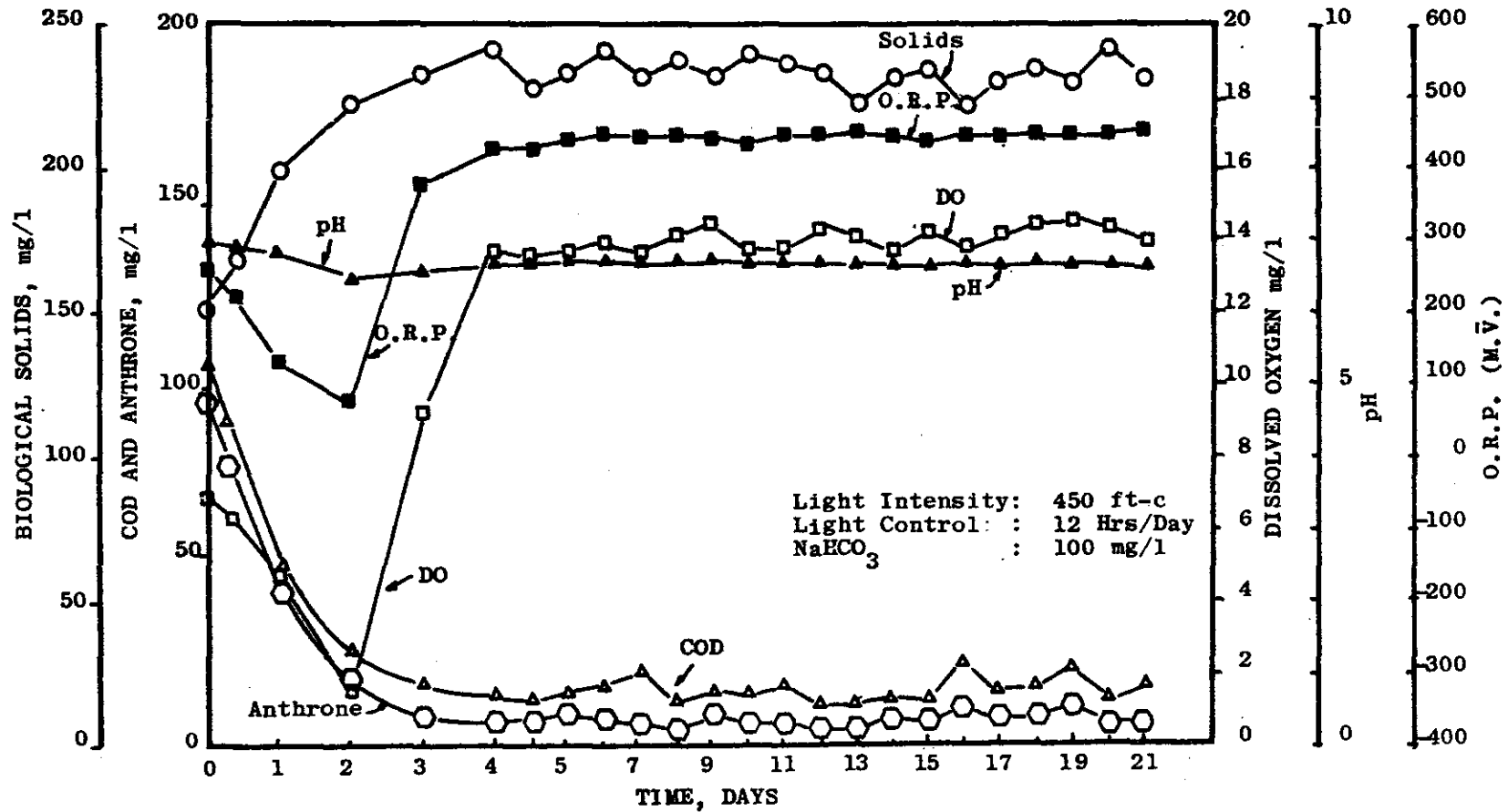


Figure 46. Purification of 100 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.



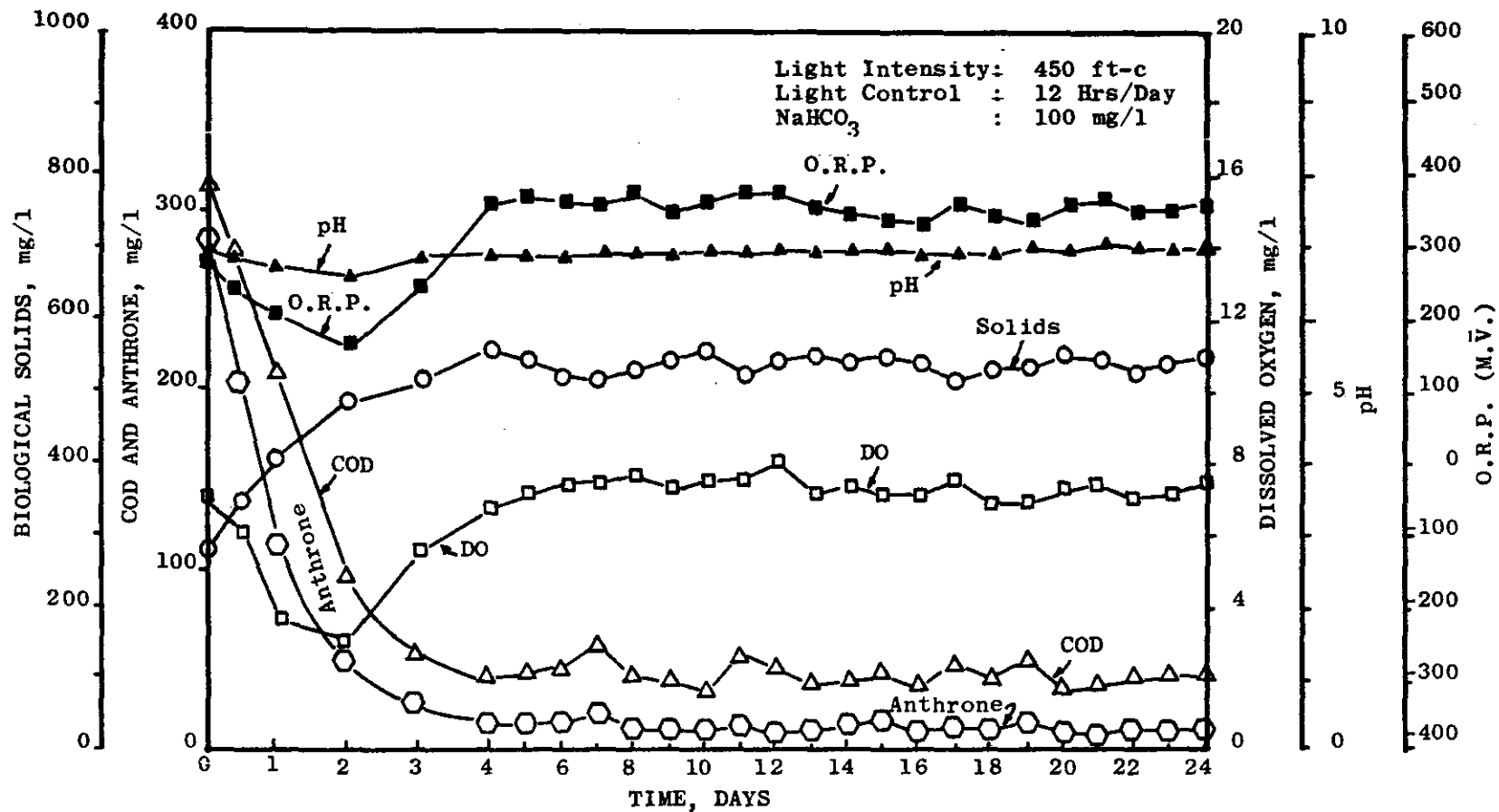


Figure 47. Purification of 300 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

It will be recalled that at the ten-day detention time the system underwent anaerobiosis at the 600 mg/l level (Figure 42). However, at the same loading level and a detention time of twenty days (Figure 48) the system remained aerobic and gave fairly good substrate removal efficiency. On the twenty-first day of operation, dissolved oxygen concentration was run on an around-the-clock basis; the results are shown in Figure 49. It is seen that at this detention time DO concentration underwent an increasing and decreasing cycle during the on and off period. On the twenty-second day of operation the light was left on, and dissolved oxygen was again determined at frequent intervals throughout the twenty-four hour period. The results are shown in Figure 50, where it is seen that the dissolved oxygen concentration exhibited an increasing trend. There is no immediately apparent explanation for the slight dip in oxygen concentration which occurred in the early morning hours toward the end of the period. There was no significant variation in the temperature during this period. Therefore the depression in the dissolved oxygen concentration cannot be attributed to retarded metabolic activity of algae due to a lowered temperature during the early morning hours.

Experiments of twenty-day detention time were also run using 1000 mg/l glucose feed. The results are shown in Figure 51; it is seen that anaerobic conditions devel-

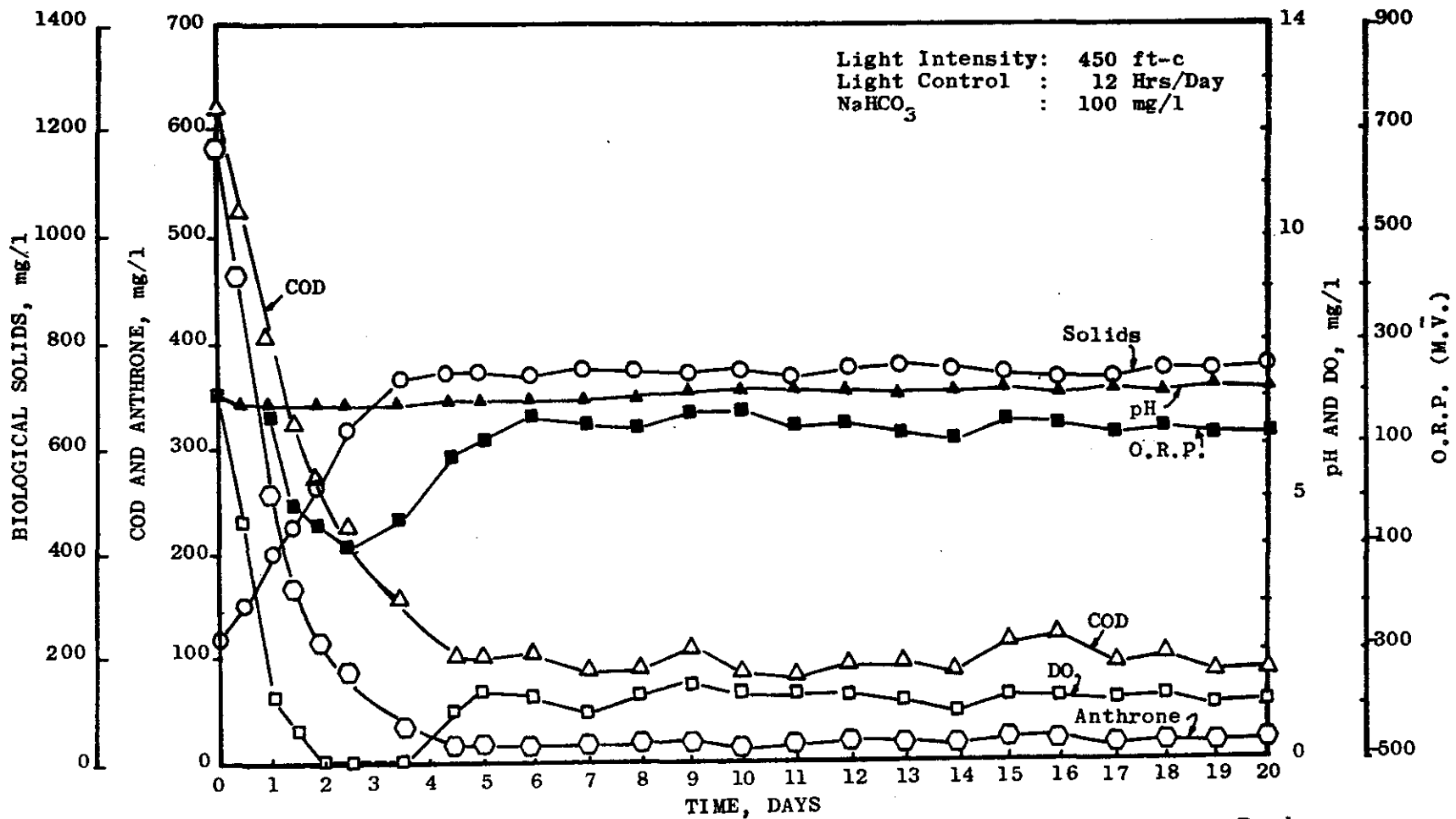


Figure 48. Purification of 600 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

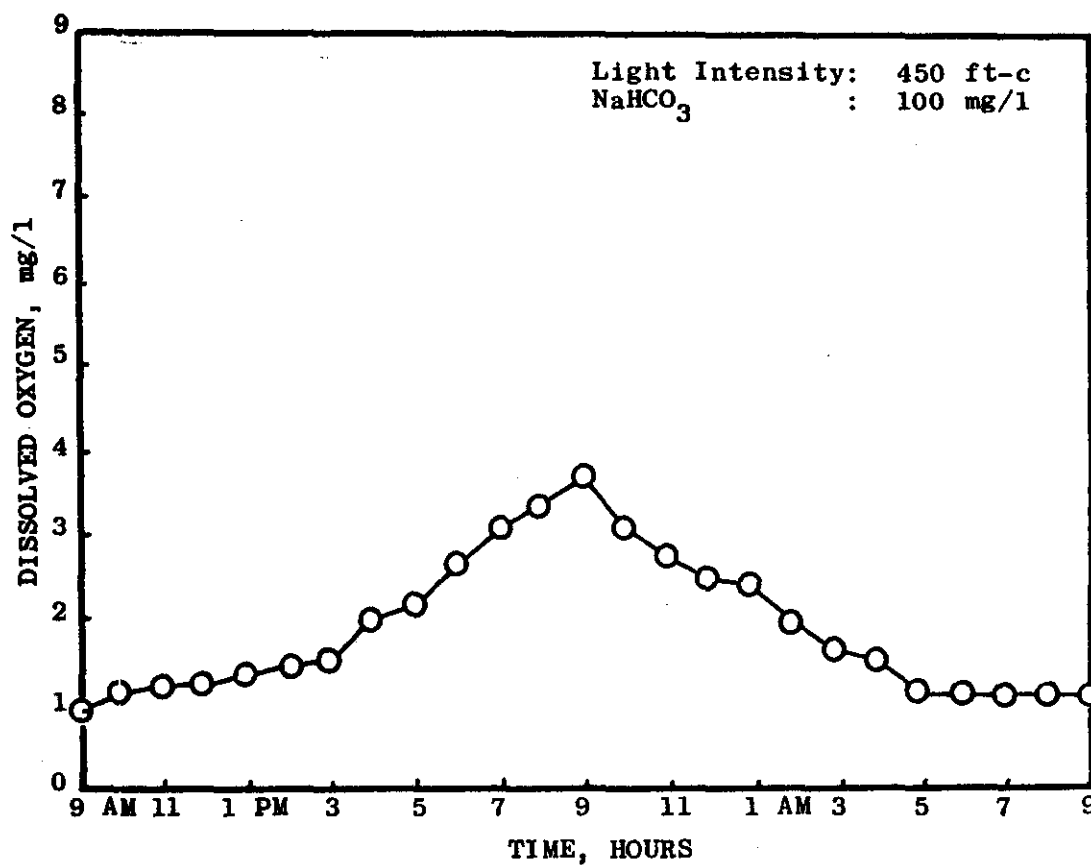


Figure 49. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose Operated at a Detention Time of 20 Days (Light Period - 12 Hrs/Day).

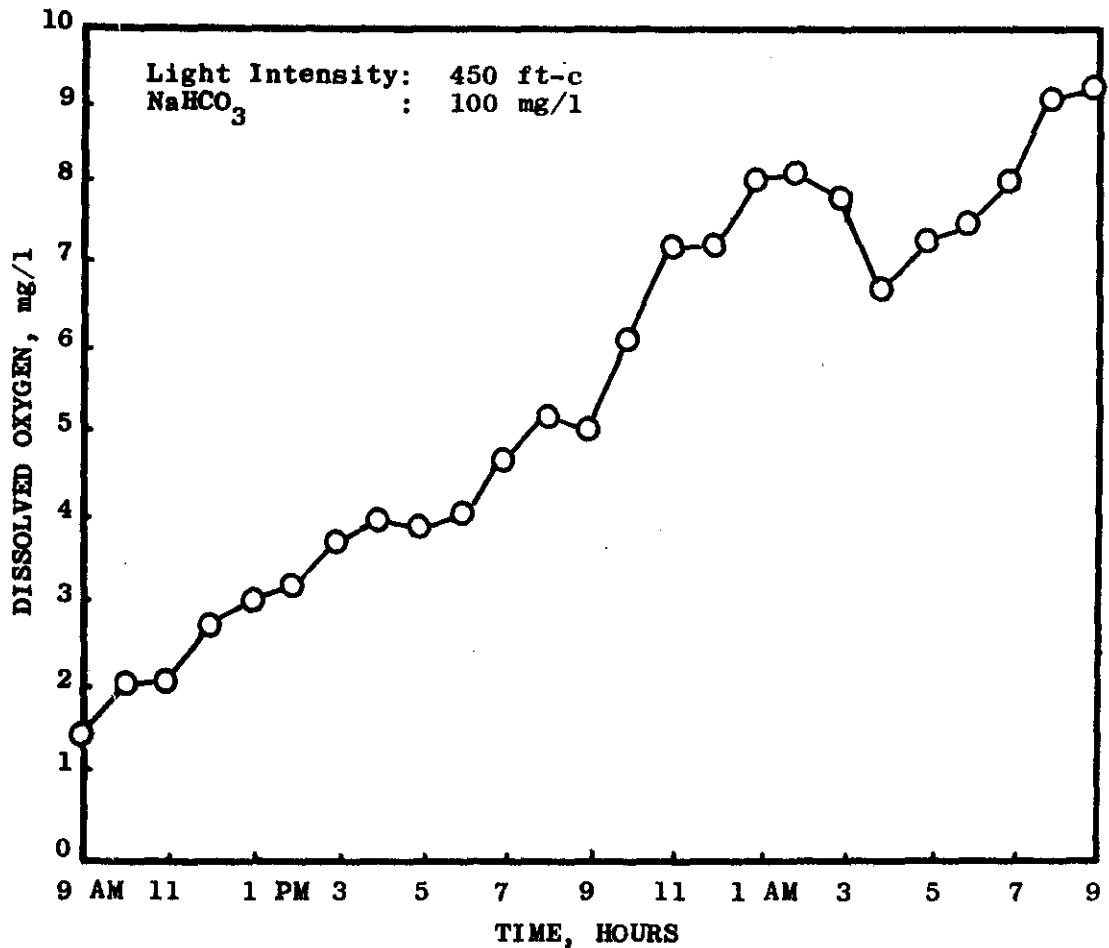


Figure 50. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose Operated at a Detention Time of 20 Days (Light Period - 24 Hrs/Day).

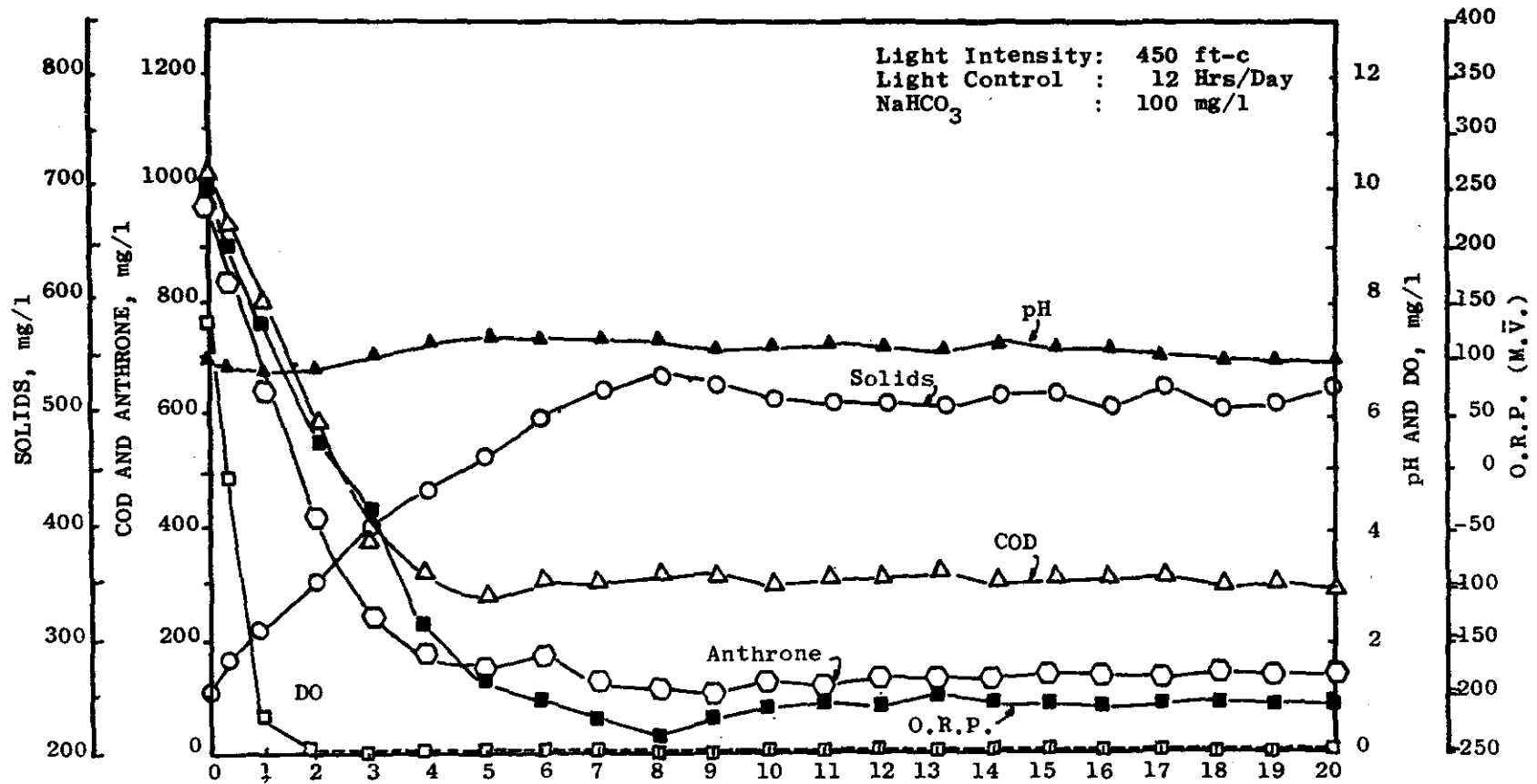


Figure 51. Purification of 1000 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

oped at this loading level. Also, there was a decrease in COD removal efficiency although removal still averaged approximately 70% even at this loading. Another continuous flow experiment at the same loading level and detention period was run under conditions of twenty-four hours of light per day. The results are shown in Figure 52. Even under conditions of continual light the system was anaerobic, and the major difference between the results shown in Figures 51 and 52 is the time required to reach the anaerobic condition. The steady state solids level was slightly higher for the system which was continually lighted, and it attained a steady state condition more rapidly than the system which was subjected to alternate light and dark periods.

It is often observed in the field that oxidation ponds provide excellent breeding places for mosquitoes. It is interesting to note that the same may be said for laboratory oxidation ponds. Toward the end of the experiments conducted at the 1000 mg/l glucose loading level (Figures 51 and 52), the surface of the ponds literally became covered with mosquitoes in the larval stage. The experiments were terminated before they matured.

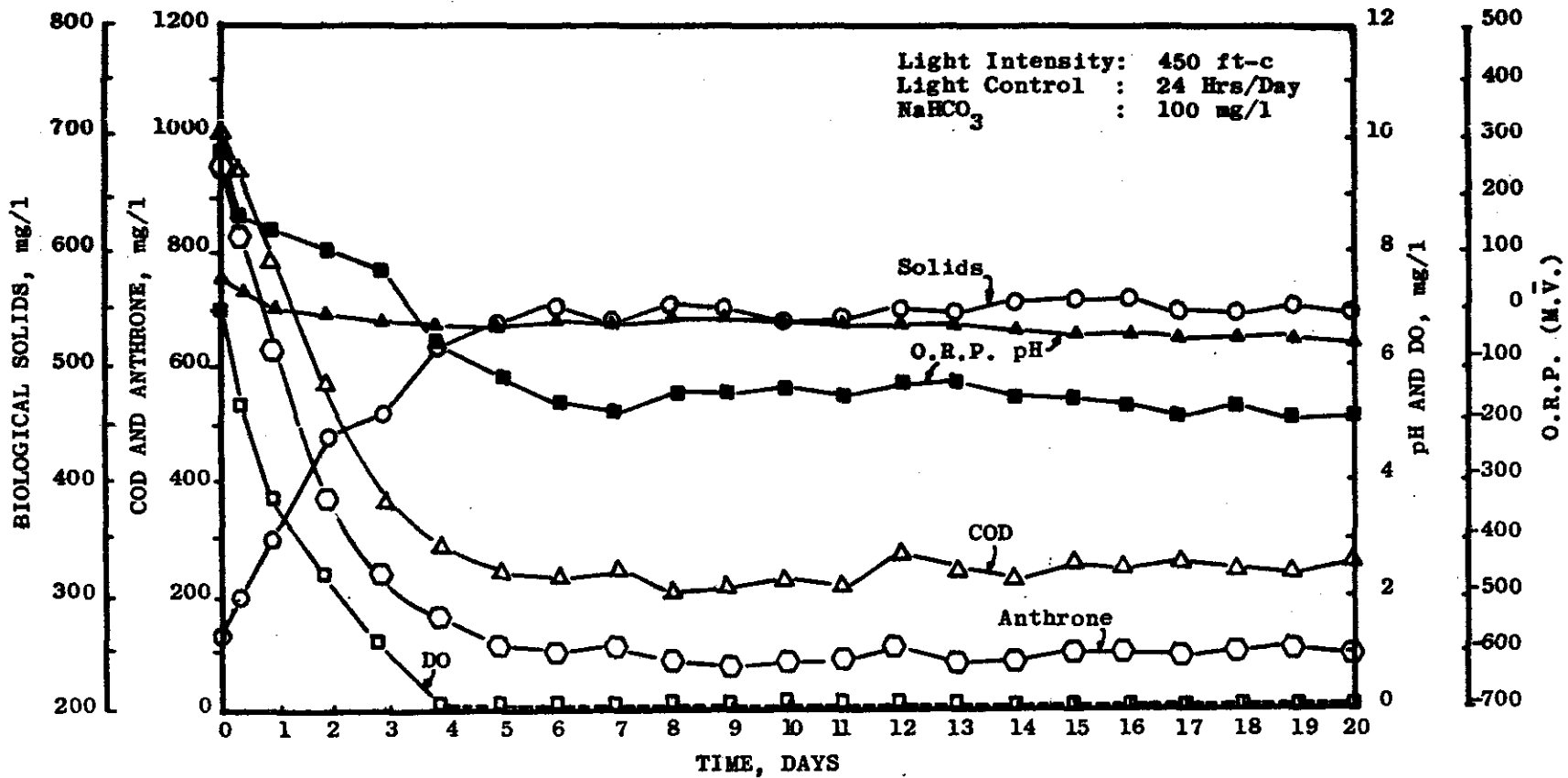


Figure 52. Purification of 1000 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.



## CHAPTER V

### DISCUSSION

The studies on physical reaeration in the laboratory oxidation pond and the early studies on reaeration due to photosynthesis conducted in the BOD bottle and in the laboratory ponds essentially are of a preliminary nature and were intended to provide insights for subsequent experimental design.

The results of the physical reaeration studies (Figure 5) indicated, as would be expected, that the course of physical reaeration did follow first order kinetics over a wide range of DO values. In the late hours of the experiment when the change in DO concentration was rather small, there appeared to be some divergence from the first order kinetics; however, it should be noted that in these experiments the "saturation value" from which the deficits were computed were not true saturation values.

The results shown in Figure 7 show the enhanced rate of reaeration due to algal growth; the level of dissolved oxygen in the pond which received no sodium bicarbonate was considerably lower than in those to which bicarbonate was added. It is also interesting to note that the dissolved

oxygen level obtained for the system which received no added bicarbonate in Figure 7 were considerably lower than the dissolved oxygen values attained solely by physical reaeration (compare Figures 5 and 7). This result may be partially due to the fact that for the results shown in Figure 5 distilled water was employed, whereas in Figure 7 the salts medium was used. This could affect the rate of reaeration and the amount of dissolved oxygen which could be held in the system.

It is also interesting to note in Figure 7 that the oxygen production using 100 mg/l sodium bicarbonate was slightly greater than that using 300 mg/l. The same effect was noted for the results shown in Figure 8 which were conducted in closed systems (BOD bottles). In this figure it is clearly seen that at 300 and 500 mg/l sodium bicarbonate there was a definite suppressing effect upon the rate of reoxygenation and the total amount of oxygen produced. The beneficial effect of adding sodium bicarbonate is manifested to a greater degree in Figure 8 than in Figure 7, most probably because for the system shown in Figure 7 carbon dioxide could enter the system from the atmosphere. Algal solids production for these systems (shown in Figure 9) reflect the same trend as the oxygen production data shown in Figure 8, thus indicating that either oxygen production or solids production can be used as a useful parameter to measure the activities of the algae. The additional exper-

iment run using various levels of sodium bicarbonate in which the medium was first deoxygenated (Figure 10) yielded the same trend as the previous experiments. As a result of these preliminary experiments, the 100 mg/l level of sodium bicarbonate was selected for inclusion in the synthetic medium used in subsequent loading studies.

The results shown in Figure 11 are particularly interesting, since they show very prominently the considerable oxygenating capacity of the algae. This is especially evident in the experiment for which 15 mg/l glucose was added. It is also interesting to note that in these experiments a plateau in oxygen production was noted. This is of particular interest, since many studies in the Bioengineering Laboratories of Oklahoma State University have shown that plateaus exist in oxygen utilization, as well (43, 44, 45).

The loading studies in the laboratory oxidation ponds comprised the major research effort. The first set of such studies was conducted in batch, using a detention time of seven days with a single initial loading varying from 100 to 600 mg/l. Comparison of the overall results shown in Figures 12 through 29 allows the following generalizations to be drawn: Concerning biological solids production, the system which received light and which contained sodium bicarbonate (system 2) produced more biological solids than did the system which received light but contained no added sodium bicarbonate (system 1). However, system 1

produced much more solids than did system 3, which contained sodium bicarbonate but received no light. Concerning the prevailing dissolved oxygen concentration, there were no large differences between systems 1 and 2; however, much more oxygen was produced in systems 1 and 2 than in system 3, which received no light. Considering the oxidation reduction potential, system 2, to which sodium bicarbonate was added, recovered to high values more effectively than did system 1. However, in system 1 the O.R.P. values recovered more rapidly than in system 3. The trends given above held true in general over the entire range of loadings which were studied. In systems which received light, anaerobiosis, as measured by dissolved oxygen concentration, existed until the end of the seven-day period for systems loaded above 400 mg/l. In the systems which did not receive light, there was only a slight recovery in dissolved oxygen concentration at a loading level of 200 mg/l glucose.

Pertaining to one of the most important parameters assessed, that is, COD removal rate, there were no great differences between systems 1 and 2; however, the removal rates in both of these systems were much greater than those observed in system 3, which received no light. This result would, of course, be expected at the higher loadings, where dissolved oxygen or oxygen tension might be expected to play a rate-limiting role in COD or substrate removal. However, it may be seen in Figures 12 through 17 that the same

trend, i.e., greater removal rate in systems receiving light than in the dark system, were observed for systems in which the dissolved oxygen concentration did not decrease enough to limit the rate of COD removal. This result could be construed as an indication that the algae may have metabolized some of the glucose, thus adding to its rate of removal. The utilization of organic matter by algae has been reported in the literature, both in light and dark environments. Chlorella has been grown in the dark in a medium containing 1% glucose (46). Harris, et al. (47) found that Chlorella used exogenous carbohydrate preferentially for synthesis of lipids even in the presence of light and CO<sub>2</sub>. Other algal species have been shown to be capable of metabolizing organic carbon sources (48, 49).

In the experiment shown in Figures 30-35, two different loading levels were employed. The load was applied at three-day intervals, and the response of the systems were followed for a total of twelve days. These results again show the increased efficiency due to algal oxygen production, but in these experiments it is not possible to assess the role of the algae in direct removal of COD. In the tanks which were loaded at the rate of 100 mg/l glucose per three days, the addition of bicarbonate to the lighted tanks had little effect on dissolved oxygen concentration, but both solids concentration and oxidation reduction potential were greater when bicarbonate was added. The

O.R.P. measurement was also considerably different in the three systems at the higher loading. While both the lighted tank without bicarbonate and the dark tank were completely devoid of DO after the third day, the oxidation reduction potential was considerably lower in the dark tank. From these results and those previously obtained in batch studies, it would appear that O.R.P. is a much more sensitive measure of the degree of anaerobiosis than is dissolved oxygen. The provision of bicarbonate at the 200 mg/l loading level had a marked effect upon both solids production and dissolved oxygen. The increased algal growth in the tank to which bicarbonate was added was just sufficient to prevent anaerobiosis.

COD removal efficiency for the three systems is shown in Table VII. At the lower loading level, both lighted tanks showed approximately equal efficiency. Considering the per cent COD removed at the end of each three-day period, all systems were approximately equivalent for the first two loading periods, but the lighted systems increased slightly in efficiency throughout the study, whereas the efficiency of the dark system was seriously impaired during the latter part of the experiment as total COD increased. At the higher loading level the effect of algal production of oxygen and its enhancement by the addition of bicarbonate is more pronounced. At all loading periods, the lighted tank containing bicarbonate exhibited the

greatest efficiency, while the poorest COD removal occurred in the dark tank. From these results it can be concluded that the ponds used herein could maintain reasonable COD removal efficiency at the loading rate of 200 mg/l per three days only if algal growth was optimal. The pond shown in Figure 34 would seem to be operating very near the allowable loading limit.

A change in the loading schedule to 50 mg/l applied daily (Figures 36-38) resulted in overloading and anaerobiosis in all three systems. Dissolved oxygen was completely exhausted in all systems after the second day; however, the beneficial effect of algae and its enhancement by addition of bicarbonate was again evident in the differences in O.R.P. and in substrate removal efficiency. Dissolved oxygen also showed a slight recovery in the final period of operation in the lighted tank to which bicarbonate was added. Although all ponds were overloaded, the condition of the dark pond was more serious than that of the lighted ponds, and the pond containing bicarbonate might possibly have functioned fairly efficiently if the experiment had been extended to allow further recovery. When the loading level was doubled, even though bicarbonate was added and light was provided for twenty-four hours, complete anaerobiosis and breakdown of substrate removal efficiency occurred, and there was no evidence of potential recovery after seven days.

In the continuous flow studies, conditions found to be optimal in the batch studies were employed, i.e., bicarbonate was added at a concentration of 100 mg/l and the tanks were exposed to light for twelve-hour periods each day. At all three loading levels, 100, 300, and 600 mg/l, steady operation with regard to all parameters measured was reached after approximately four days of operation. At both 100 and 300 mg/l loadings, the ponds operated quite efficiently, removing approximately 85% of the applied COD and maintaining high dissolved oxygen and O.R.P. levels. At 600 mg/l, aerobic conditions could not be maintained, and solids production dropped, but COD removal efficiency was only slightly affected, remaining at approximately 80%. Continuous lighting at the same loading level increased COD removal efficiency only slightly.

The completely anaerobic condition experienced by the overloaded ponds in these experiments probably resulted in the death of a large portion of the algal population. As noted previously, the dark green color characteristic of aerobic ponds was replaced by a yellow color. Franck and Gaffron (50) have reported that while many algae, such as Scenedesmus, Ankistrodesmus, and Rhathidium may survive under anaerobic conditions for days, Chlorella is much more sensitive to anaerobiosis, and is permanently injured by long anaerobic periods. Another possible explanation of the color change and the inability of the algae to



overcome the oxygen deficit may be found in the work of Ludwig and Oswald with Euglena gracilis (51). They reported that the color of Euglena changes with age, as does its oxygen productivity. The young cells were reported to be dark green, and to produce more oxygen than they respired, while old cells were yellow and used more oxygen than they produced. The latter explanation is probably not applicable to the present work, since elimination of the dark (oxygen utilization period) did little to improve the condition of the pond. Also, the characteristics reported for Euglena may not be applicable to Chlorella, and indeed, are undoubtedly not general for algae, since detention periods of considerably longer than ten days are commonly used in ponds without resulting in loss of green color. Therefore, it appears most probable that if complete anaerobiosis is established in a pond and persists for a period of several days, Chlorella, at least, will be eliminated from the algal population. It is possible that a mixed population containing algae more resistant to anaerobiosis might have eventually overcome the anaerobiosis exhibited in the overloaded pond. In comparing these continuous flow experiments with the previous batch experiments, it can be seen that, although dissolved oxygen depletion occurred in the batch studies also, recovery was possible. However, the O.R.P. levels in the batch experiments were never as low as those reached in the continuous flow experiments; thus

O.R.P. may be the determining factor in the survival of Chlorella.

When the detention time in the continuous flow studies was increased to twenty days (i.e., when the daily loading rate was decreased by half for any given substrate concentration), the COD removal efficiency was increased slightly at the 100 mg/l level but was approximately the same at 300 and 600 mg/l levels. The pond which received a substrate concentration of 600 mg/l did not become anaerobic at this detention time, but substrate removal efficiency was slightly lower than that for the equivalent daily loading with a ten-day detention time (300 mg/l). Since the algae in these ponds should have had an average age twice that of the algal population at a ten-day detention time, these results support the foregoing conclusion that for Chlorella, at least, anaerobic conditions have a much more deleterious effect upon cell survival than does culture age. At the longer detention time the pond remained aerobic, and the color change to yellow was not observed. The detention time (and therefore, possibly, cell age) does, however, appear to affect net oxygen production rather severely. For equivalent loading factors, 300 mg/l at ten-day detention and 600 mg/l at twenty-day detention times, the dissolved oxygen levels were 4 mg/l and 1 mg/l, respectively. The level of 4 mg/l DO was reached in the tank with longer detention time only at the end of the light period, as

shown in Figure 49. When the substrate concentration was increased to 1000 mg/l at the twenty-day detention time, the results were similar to those obtained at 600 mg/l with a ten-day detention time. The pond became anaerobic, but substrate removal efficiency remained fairly high (approximately 70%). Some improvement in both COD removal (77%) and O.R.P. were obtained by lighting the pond continuously, but again there was no evidence of recovery from anaerobiosis. In Table VIII are shown the loading factors for all experiments calculated on the basis of pounds COD or glucose/acre/day. Based upon the maintenance of a measurable level of dissolved oxygen and absence of objectionable indications of anaerobiosis, the maximum loadings tolerated under each loading schedule were as follows:

1. Batch studies, single loading, seven-day detention, 117.6 lb/COD/acre/day
2. Batch studies, loading applied at three-day intervals, 137.4 lb/COD/acre/day
3. Batch studies, loading applied daily, less than 103 lb/COD/acre/day
4. Continuous flow studies, 61.7 lb/COD/acre/day.

In these comparisons only the batch systems which received both light and bicarbonate are considered. Based on a COD removal efficiency of greater than 70%, the optimum loading for either batch or continuous flow systems would appear to lie in the vicinity of 60-70 lb/COD/acre/

TABLE VIII  
 SUMMARY LOADING FACTORS FOR PONDS IN ALL LOADING  
 STATIONS

<u>Batch Studies, Single Loading, 7-Day Detention</u>		
Glucose, mg/l	Glucose, lb/acre/day	COD, lb/acre/day
100	27.6	29.4
200	55.2	58.8
300	82.8	88.2
400	110.4	117.6
500	138.0	147.0
600	165.6	176.4
<u>Batch Studies, Loading at 3-Day Intervals</u>		
100	64.4	68.7
200	128.8	137.4
<u>Batch Studies, Loading Applied Daily</u>		
50	96.6	103.1
100	193.2	206.2
<u>Continuous Flow Studies, 10-Day Detention</u>		
100	19.3	20.6
300	57.9	61.7
600	115.8	123.3
<u>Continuous Flow Studies, 20-Day Detention</u>		
100	9.7	10.3
300	29.0	31.0
600	57.9	61.7
1000	96.6	103.0

day. It may be surmised from the results of these loading studies which were conducted under closely controlled conditions and somewhat ideal operational conditions that there is considerable justification for the low loading factors which are recommended for oxidation pond design by many state regulatory agencies. It must be remembered that the ponds employed in these studies were considerably more shallow than would be used in the field, and that even under the ideal conditions employed in the laboratory, loading factors of 60-70 lb/COD/acre/day could not be exceeded if the ponds were to be operated with the absence of anaerobic odors, and reasonably good removal efficiencies.

## CHAPTER VI

### CONCLUSIONS

Based upon the experimental results, the following conclusions may be drawn:

1. If oxidation ponds are to be operated with reasonably good (approximately 70% or better) COD removal efficiency under strictly aerobic conditions, relatively low loadings must be employed. In the present study these loadings were in the range of 60-70 lbs/COD/acre/day for ponds operated under relatively ideal conditions in the laboratory. While the results should not be directly translated to the field, it is expected that actual design loadings would most probably be 25-30% of those herein observed.

2. There is based upon the experimental results of this study, some indication that algae cannot be subjected to severe anaerobic conditions without impairing their oxygenating capability. This would indicate that the organic loading to oxidation ponds should be such that an oxygen surplus is built up during the light period which is sufficient to prevent the dissolved oxygen from dropping to zero during the dark or night period.

3. As it affects the survival of algae or the reoxygenating ability of the algae, oxidation reduction potential appears to be a better barometer for measuring the degree of anaerobiosis than is dissolved oxygen, since the ability of the algae to recover from DO depletion appeared to depend upon the minimum O.R.P. reached during the anaerobic period.

4. The optimum concentration of sodium bicarbonate for promotion of algal growth was, under the conditions of the present study, approximately 100 mg/l. Concentrations higher than this appeared to have a somewhat inhibiting effect.

5. There was some indication in these studies that the algae may metabolize organic matter even in the light; thus, they may play an active but probably minor role in assimilation of the organic carbon sources in a waste.

## CHAPTER VII

### SUGGESTIONS FOR FUTURE WORK

There is a great need, from an engineering standpoint, to devise ways and means of making accurate materials balances for oxygen supply and depletion in oxidation ponds, and there is an equally great need for devising kinetic models of predictive value concerning the oxygen balance. However, the results of the present study indicate that there is an equal and perhaps greater need for more basic study concerning the basic metabolic behavior of the algae, since they comprise the primary mechanism of deoxygenation in the oxidation pond method of waste water treatment. In this respect it is felt that the present line of investigation should be extended to include the following studies:

1. Loading studies similar to those herein presented should be performed using algal strains which are more resistant to anaerobiosis.

2. The effect of dissolved oxygen concentration and oxidation reduction potential on the survival of Chlorella should prove to be an interesting and useful study.



3. Studies should be made on a variety of algae and in mixed populations of algae to determine the extent of their ability to remove or metabolize organic carbon sources under daylight conditions.

4. An interesting and useful study could be designed to determine the type and extent to which organic materials may be released by algae, especially under conditions of low oxidation reduction potential.

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VITA

Yeun Cheng Wu

Candidate for the Degree of  
Master of Science

Thesis: EFFECT OF ORGANIC LOADING ON REAERATION IN  
SEMI-QUIESCENT WATERS

Major Field: Engineering

Biographical:

Personal Data: Born in Tainan, Taiwan, Republic of  
China, November 25, 1940, the son of Yun Tai Wu.

Education: Attended Nan Kung Middle School in  
Tainan, Taiwan, Republic of China; received the  
Bachelor of Science Degree from Taiwan Provin-  
cial Cheng Kung University, Tainan, Taiwan,  
Republic of China, in June, 1963.

Profession Experience: Served in China Army as a  
Civil Engineering officer from July, 1963, to  
July, 1964.





John T. Solook  
Master's Thesis

SEMI-QUIESCENT WATERS  
LIGHT AND DARK MICROBIAL RESPONSE IN

APPENDIX II

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  
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for the degree of  
MASTER OF SCIENCE  
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Name: John Thomas Solook                      Date of Degree: May, 1968

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: LIGHT AND DARK MICROBIAL RESPONSE IN  
SEMI-QUIESCENT WATERS

Pages in Study: 66

Candidate for Degree of Master of Science

Major Field: Sanitary and Public Health Engineering

Scope and Method of Study: In this study, algal response to an organic substrate in both a light-dark cycle and complete darkness was observed. Controlled laboratory experiments were conducted in batch systems with both heterogeneous microbial populations and pure cultures of algae; the relationships among dissolved oxygen, substrate removal, oxygen uptake, oxidation-reduction potential, and pH were investigated.

Findings and Conclusions: The relative rates of substrate removal were slower in the dark systems than in the systems receiving light. During substrate removal, which was due primarily to the bacteria present in the systems, the oxidation-reduction potential exhibited dynamic values. Oxygen consumption by a pure algal culture in the dark was negligible when compared to that of a mixed microbial population under similar conditions. 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.

ADVISER'S APPROVAL \_\_\_\_\_

LIGHT AND DARK MICROBIAL RESPONSE IN  
SEMI-QUIESCENT WATERS

Thesis Approved:

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Thesis Adviser

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Dean of the Graduate College

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## 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 Kuttyurin 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 percentage 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.

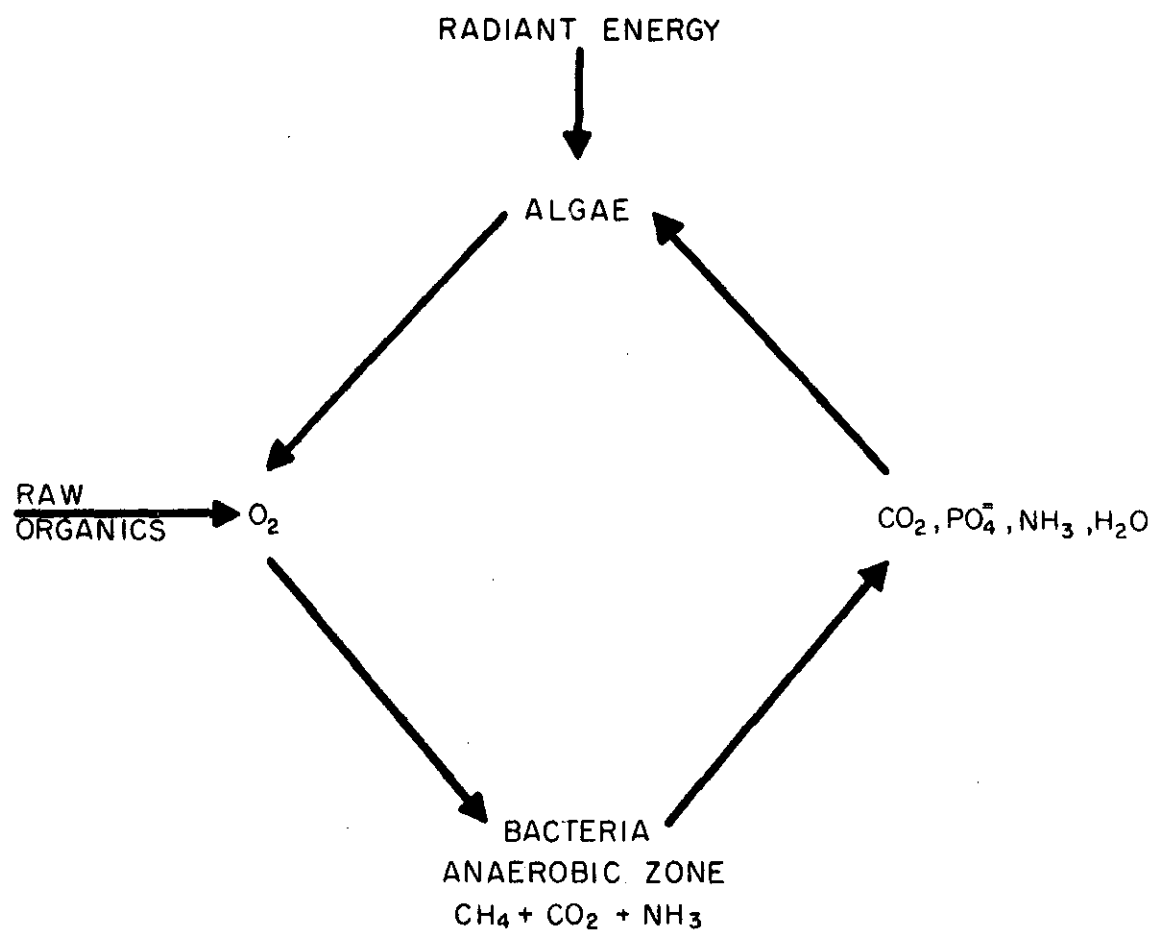


Figure 1. The general symbiotic relationship between the two existing biological phases in an oxidation pond.

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 <sup>2</sup>
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
$\text{NH}_4\text{Cl}$	1000 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	200 mg/l
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	50 mg/l
$\text{CaCl}_2$	20 mg/l
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	2 mg/l
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	1 mg/l
$\text{K}_2\text{HPO}_4$	1000 mg/l
$\text{NaHCO}_3$	100 mg/l

TABLE II  
COMPOSITION OF MINIMAL MEDIUM

Constituent	Concentration
*Glucose	200 mg/l
$(\text{NH}_4)_2\text{SO}_4$	250 mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	100 mg/l
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	10 mg/l
$\text{CaCl}_2$	7.5 mg/l
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	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 m $\mu$ ) 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°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  $24^{\circ}\text{C} \pm 1^{\circ}$ . 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.

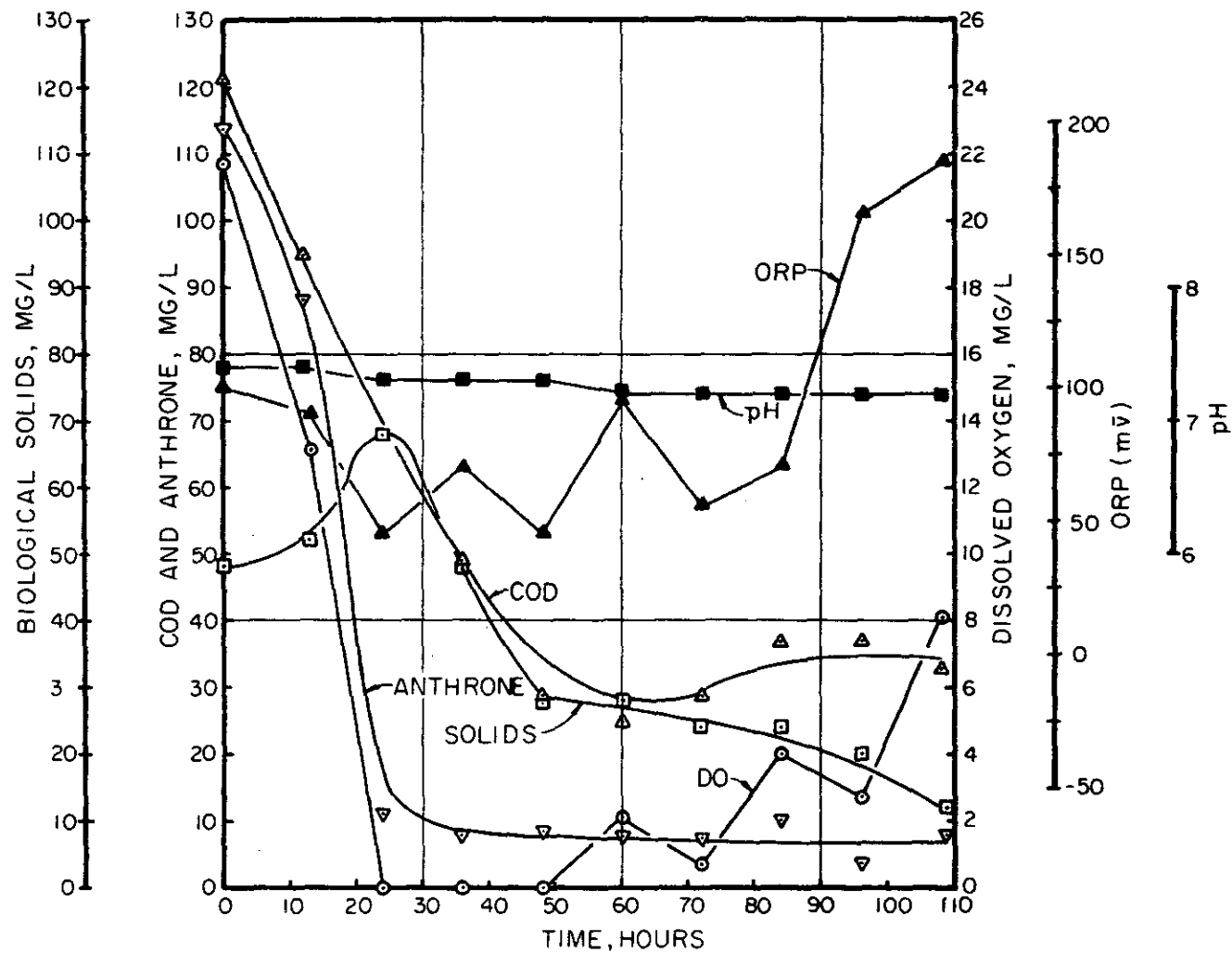


Figure 2. Biological response in semi-quiescent waters to glucose loading of 100 mg/l; lighting conditions, 12 hours on, 12 hours off.

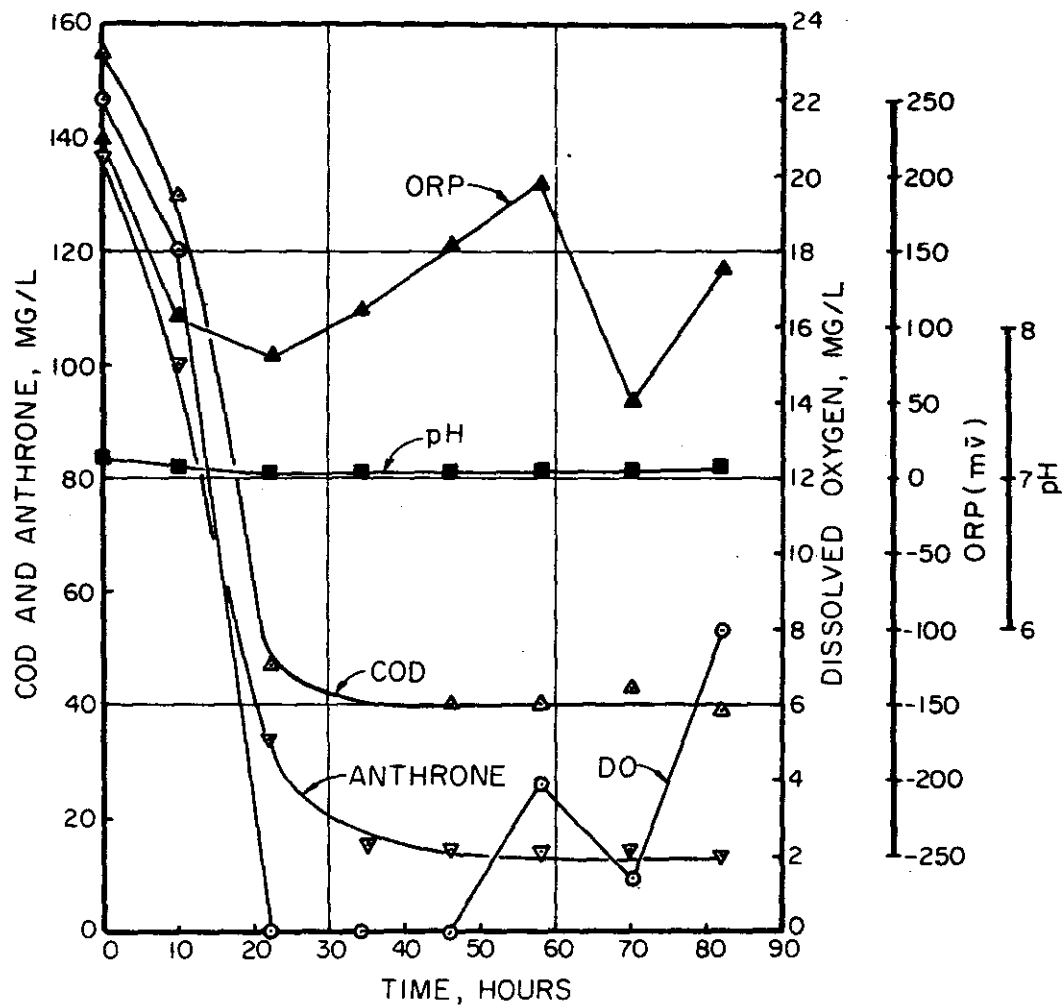


Figure 3. Biological response in semi-quiescent waters to glucose loading of 150 mg/l; lighting conditions, 12 hours on, 12 hours off.



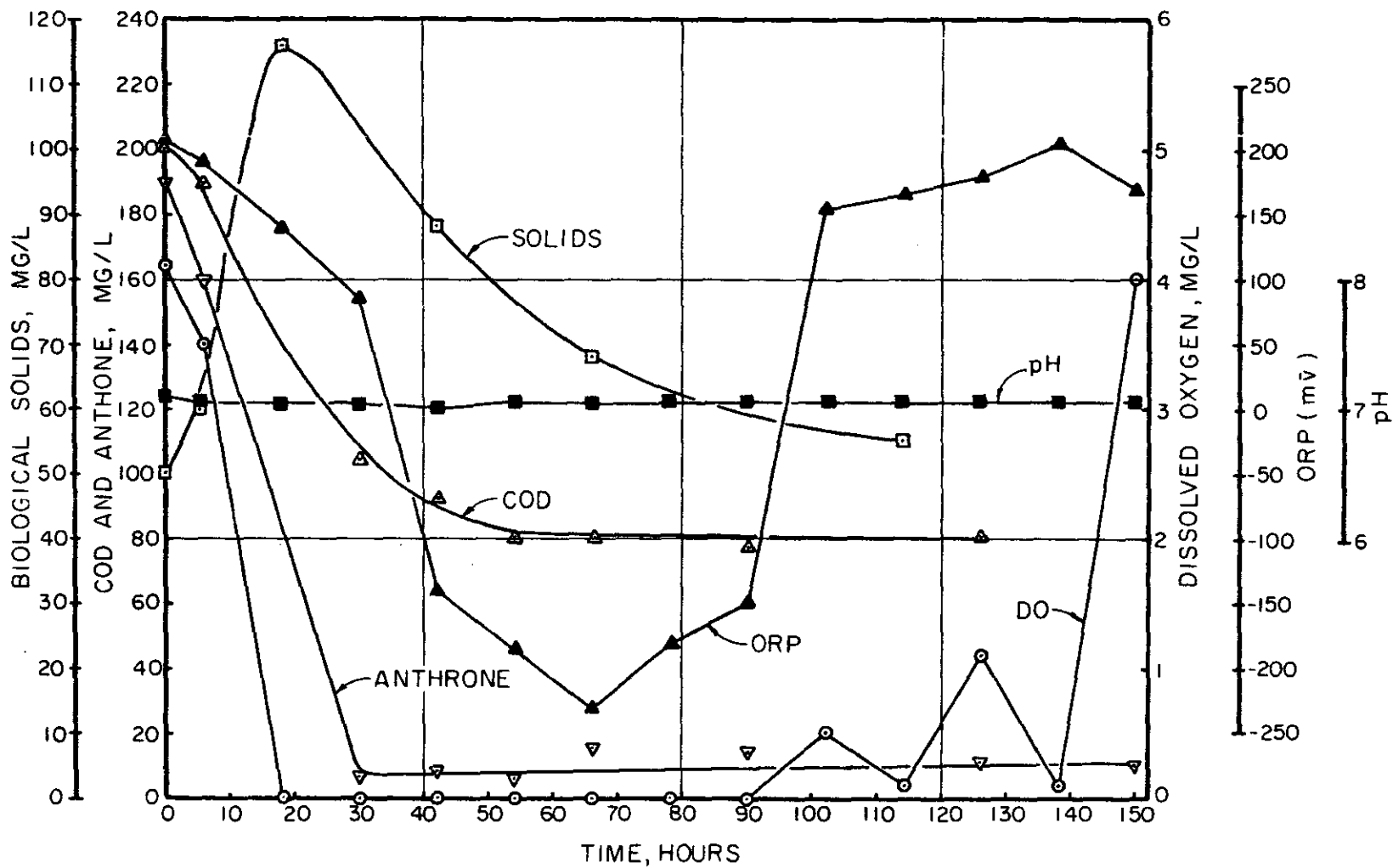


Figure 4. Biological response in semi-quiescent waters to glucose loading of 200 mg/l; lighting conditions, 12 hours on, 12 hours off.

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,

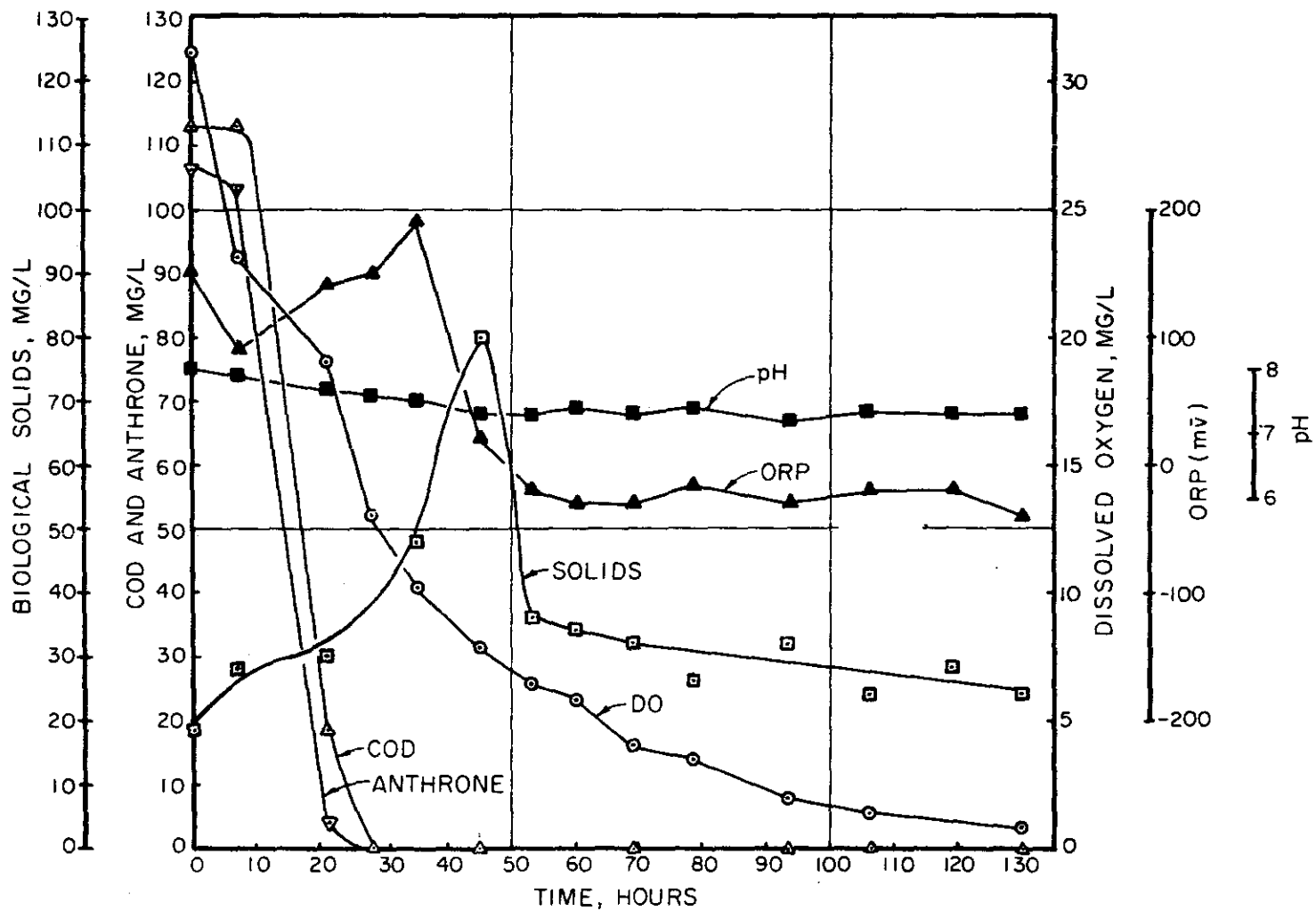


Figure 5. Biological response in semi-quiescent waters to glucose loading of 100 mg/l; lighting conditions, 24 hours darkness.

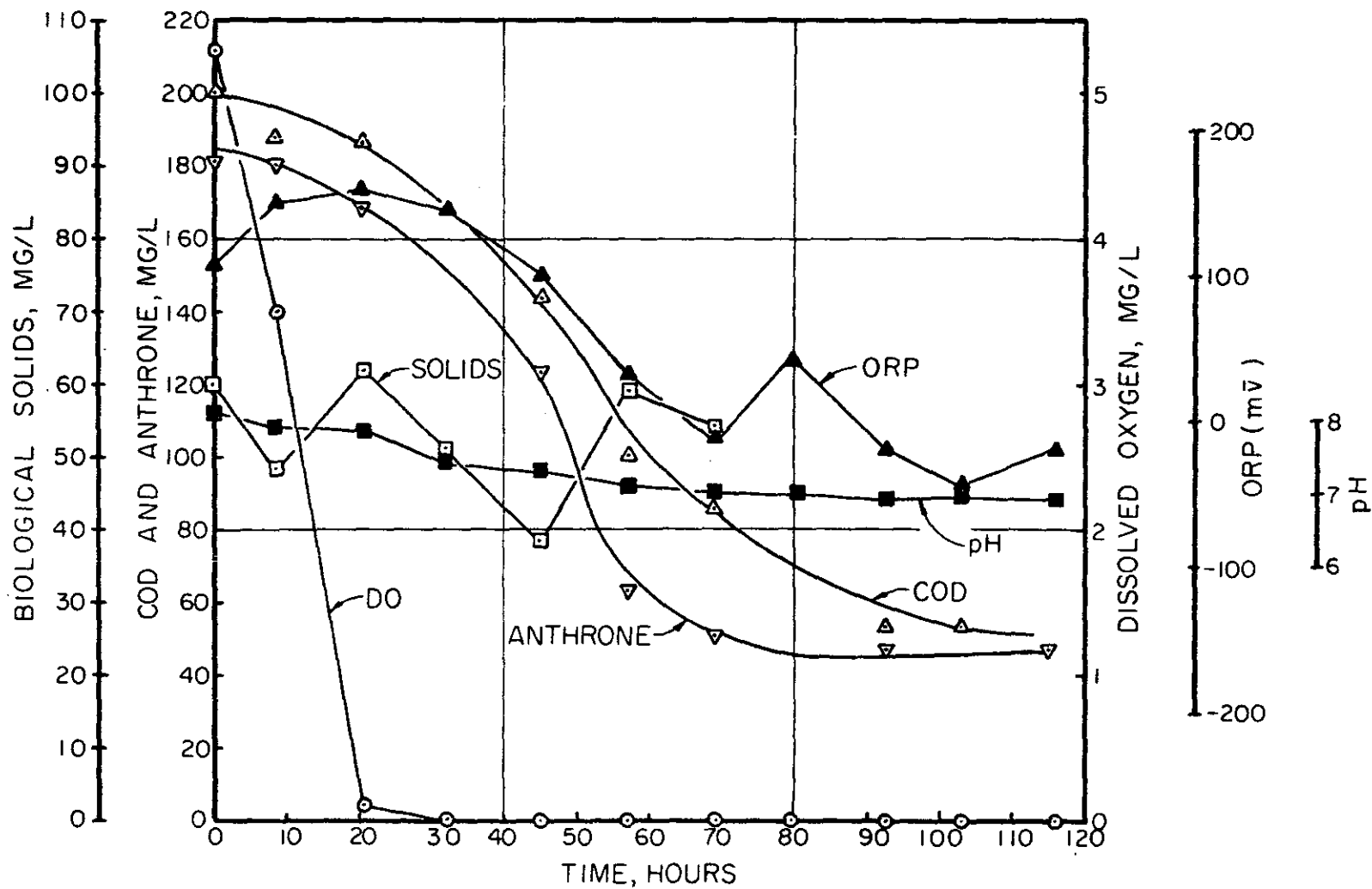


Figure 6. Biological response in semi-quiescent waters to glucose loading of 200 mg/l; lighting conditions, 24 hours darkness.

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

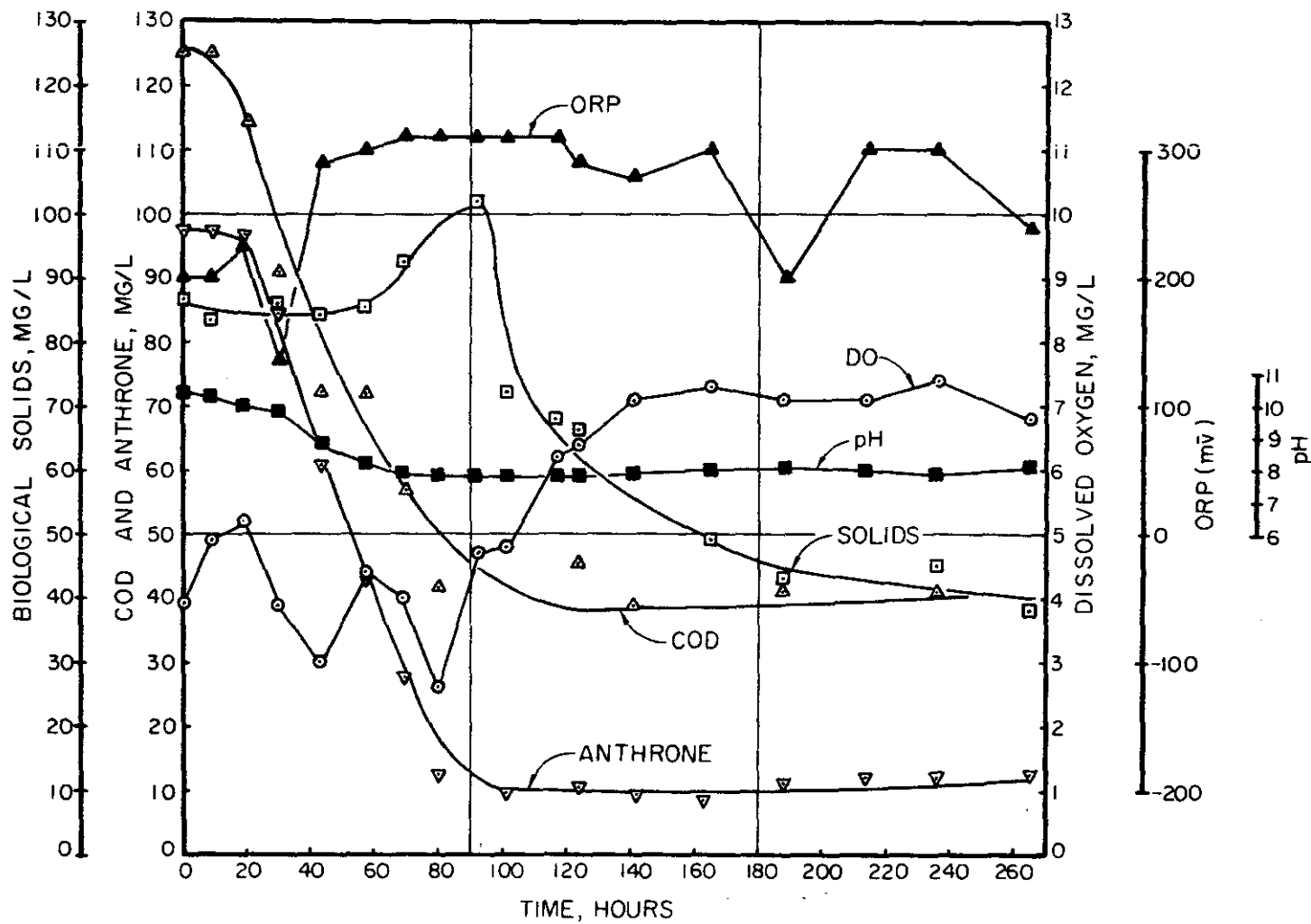


Figure 7. Biological response in semi-quiet waters to glucose loading of 100 mg/l, with mixing; lighting conditions, 24 hours darkness.



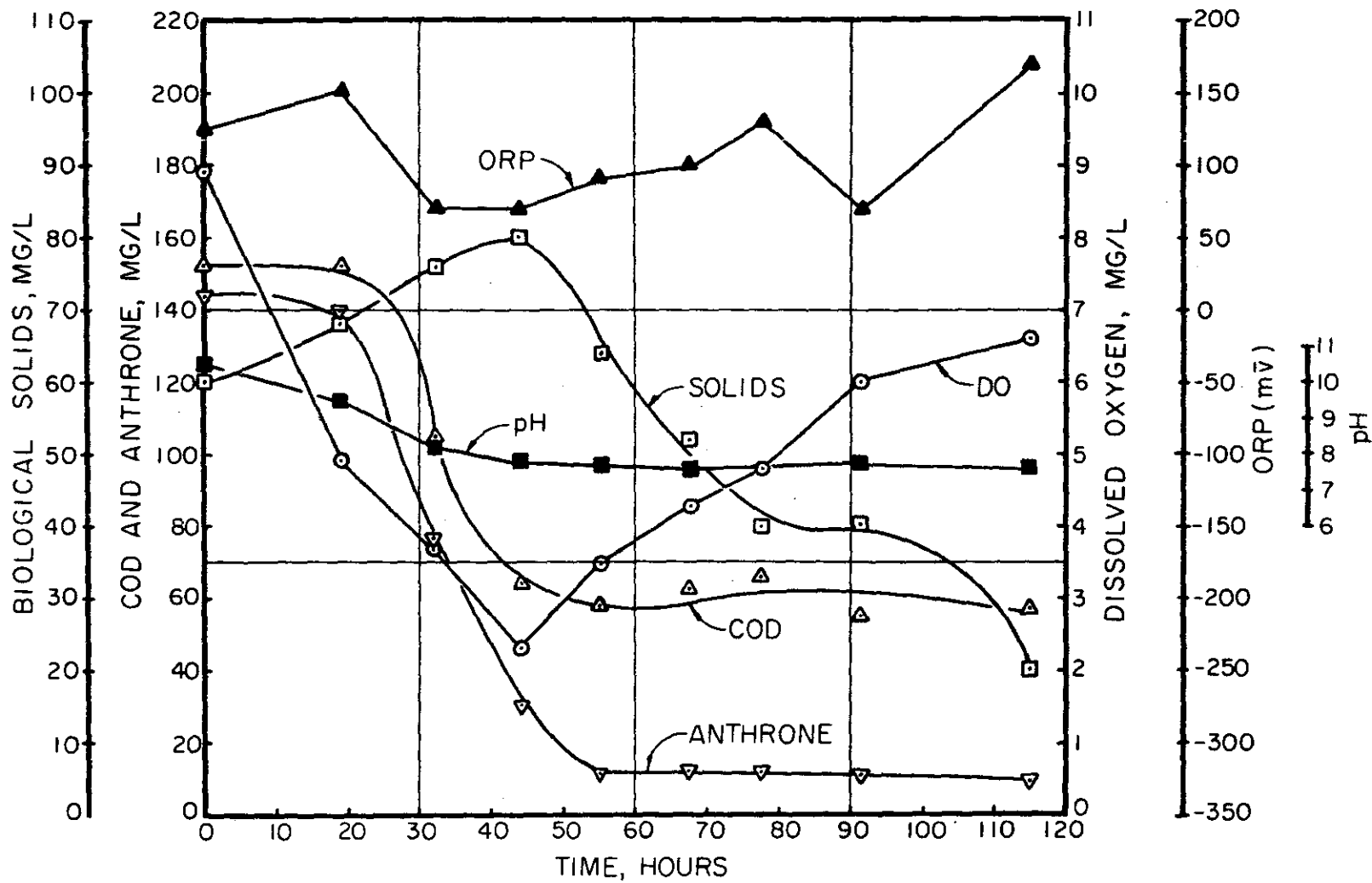


Figure 8. Biological response in semi-quiescent water to glucose loading of 150 mg/l, with mixing; lighting conditions, 24 hours darkness.

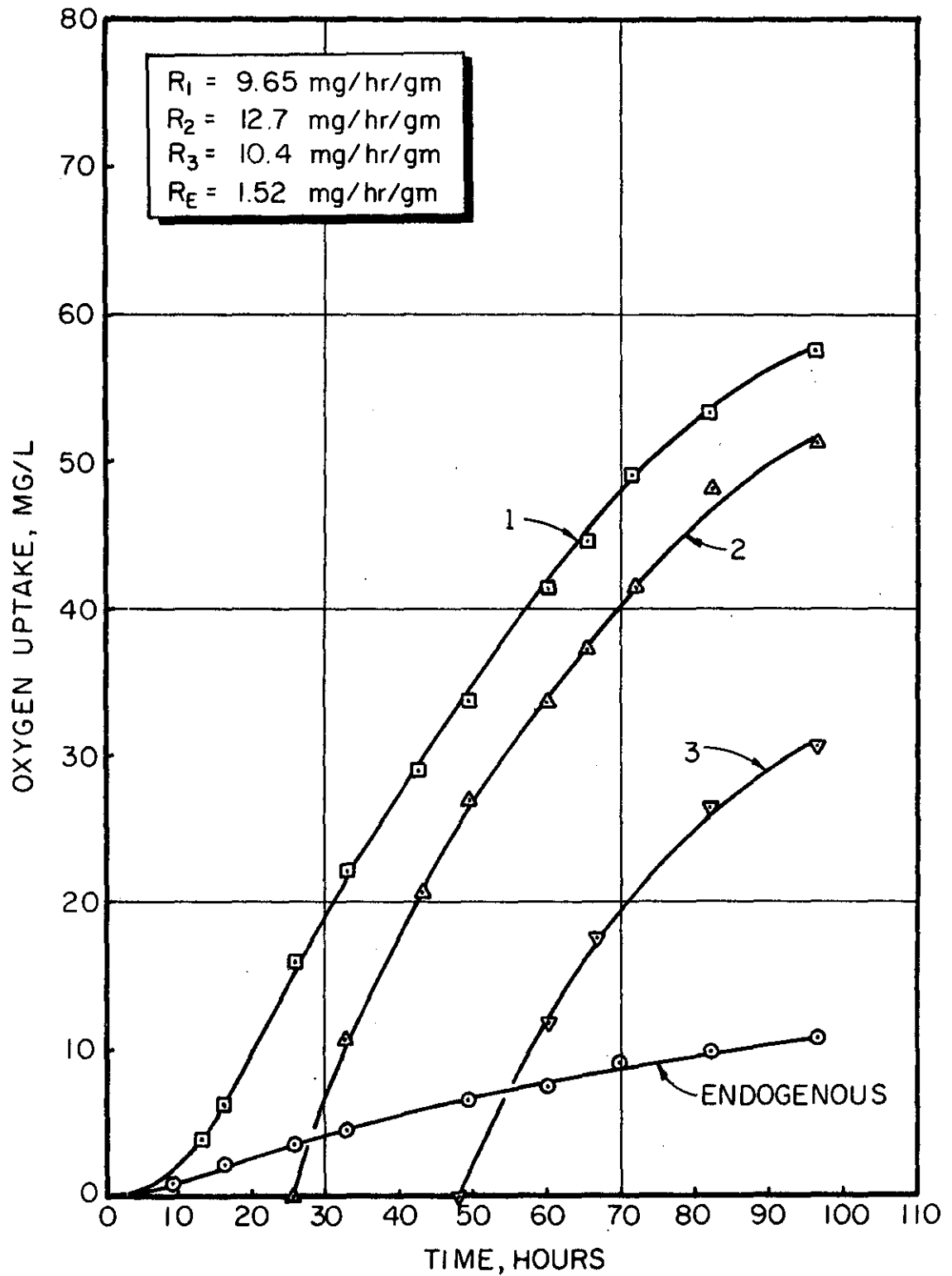


Figure 9. Cumulative oxygen uptake for 100 mg/l system.

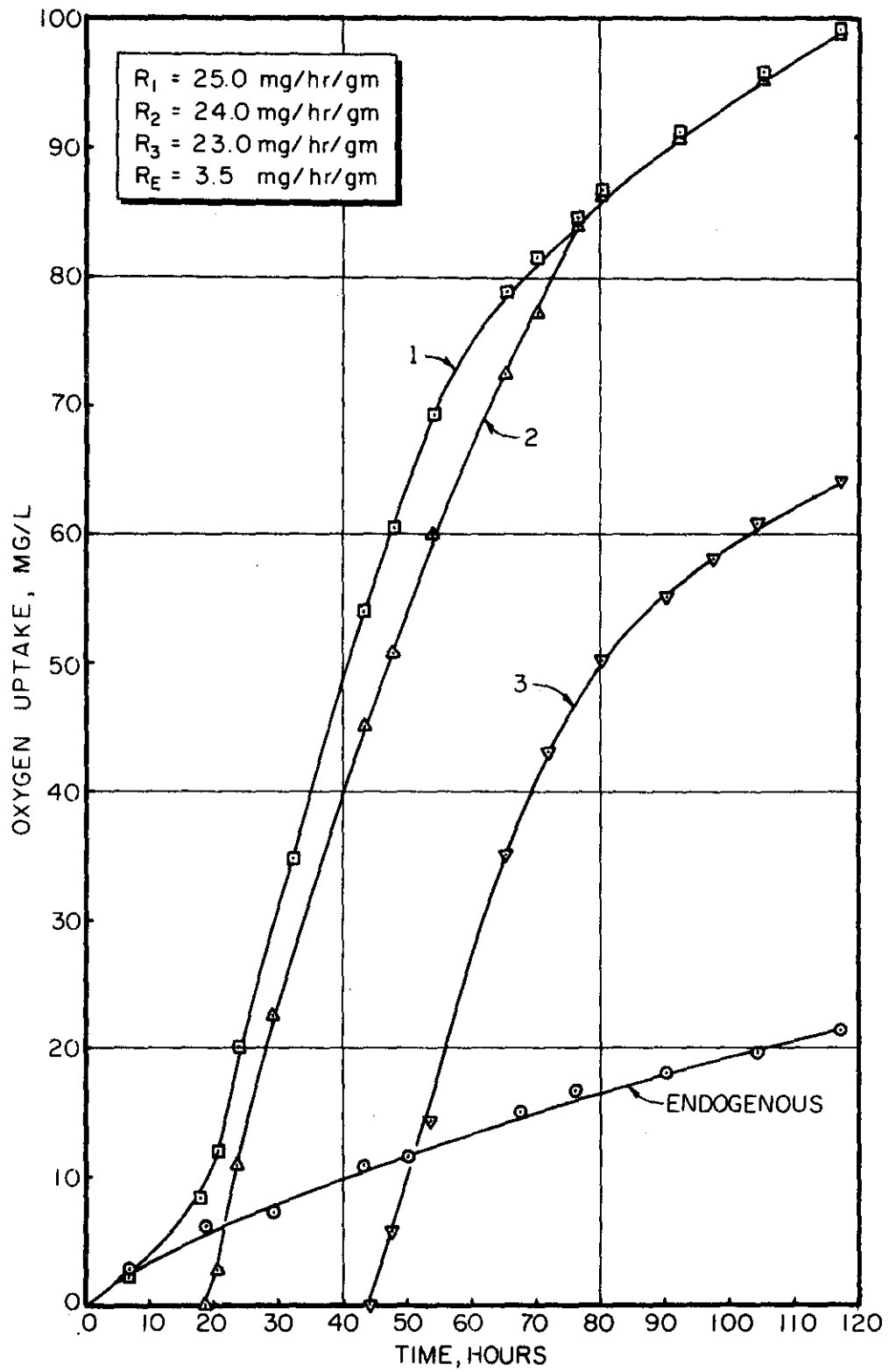


Figure 10. Cumulative oxygen uptake for 150 mg/l system.

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.

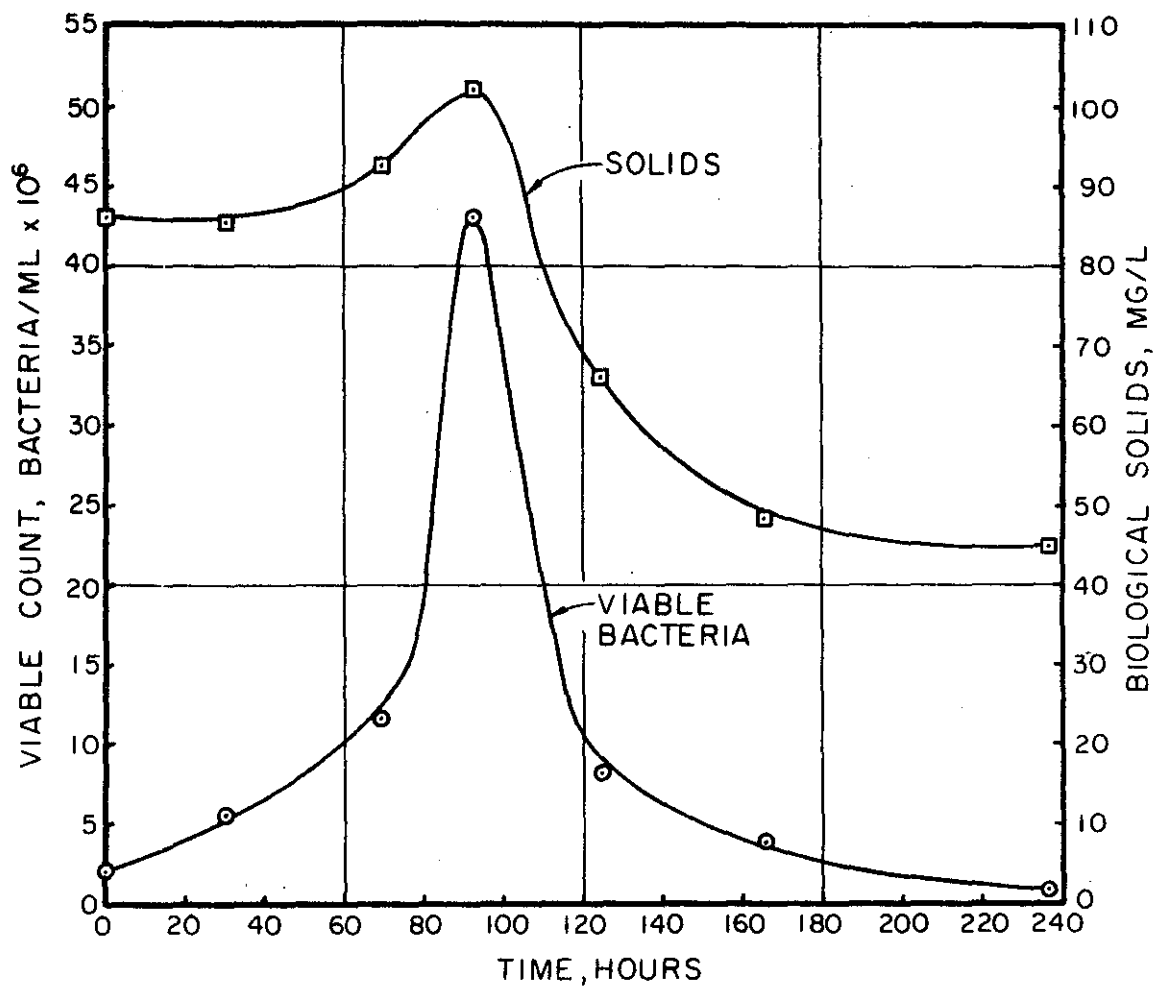


Figure 11. Relationship between biological solids and viable bacteria for glucose loading of 100 mg/l.

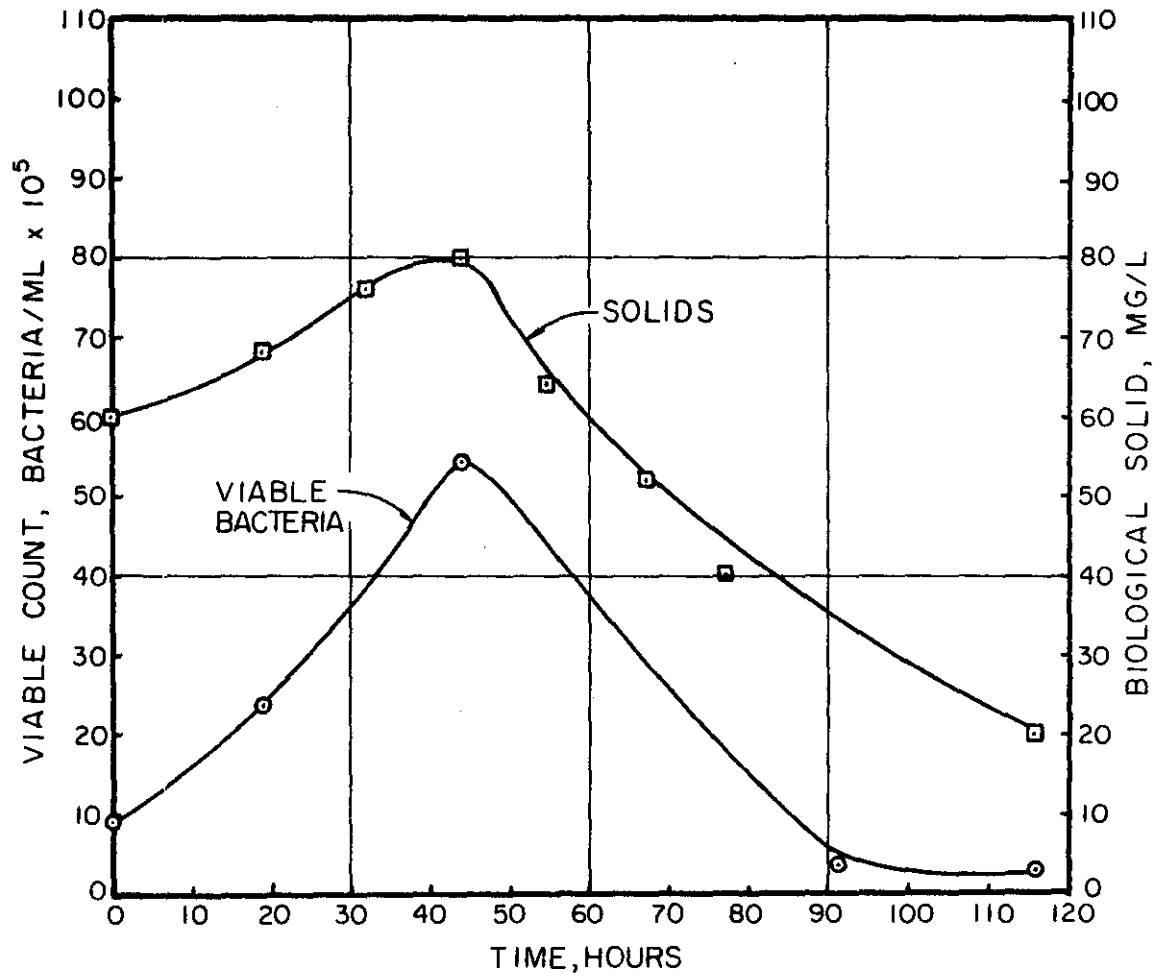


Figure 12. Relationship between biological solids and viable bacteria for glucose loading of 150 mg/l.

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 ( $\text{Fe}^{+++}$ ) 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

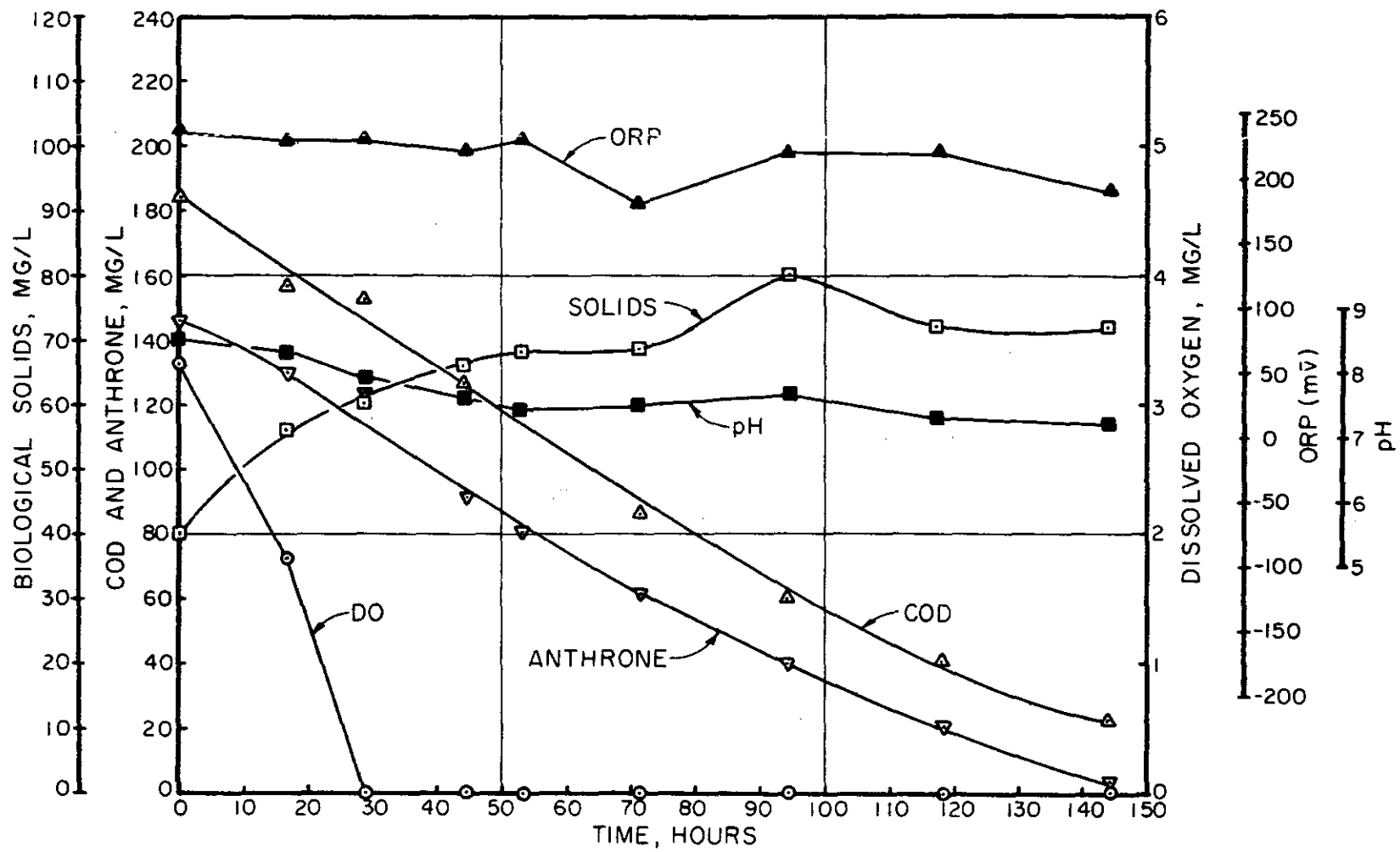


Figure 13. Biological response of closed, non-buffered system to glucose loading of 150 mg/l; lighting conditions, 24 hours darkness.



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

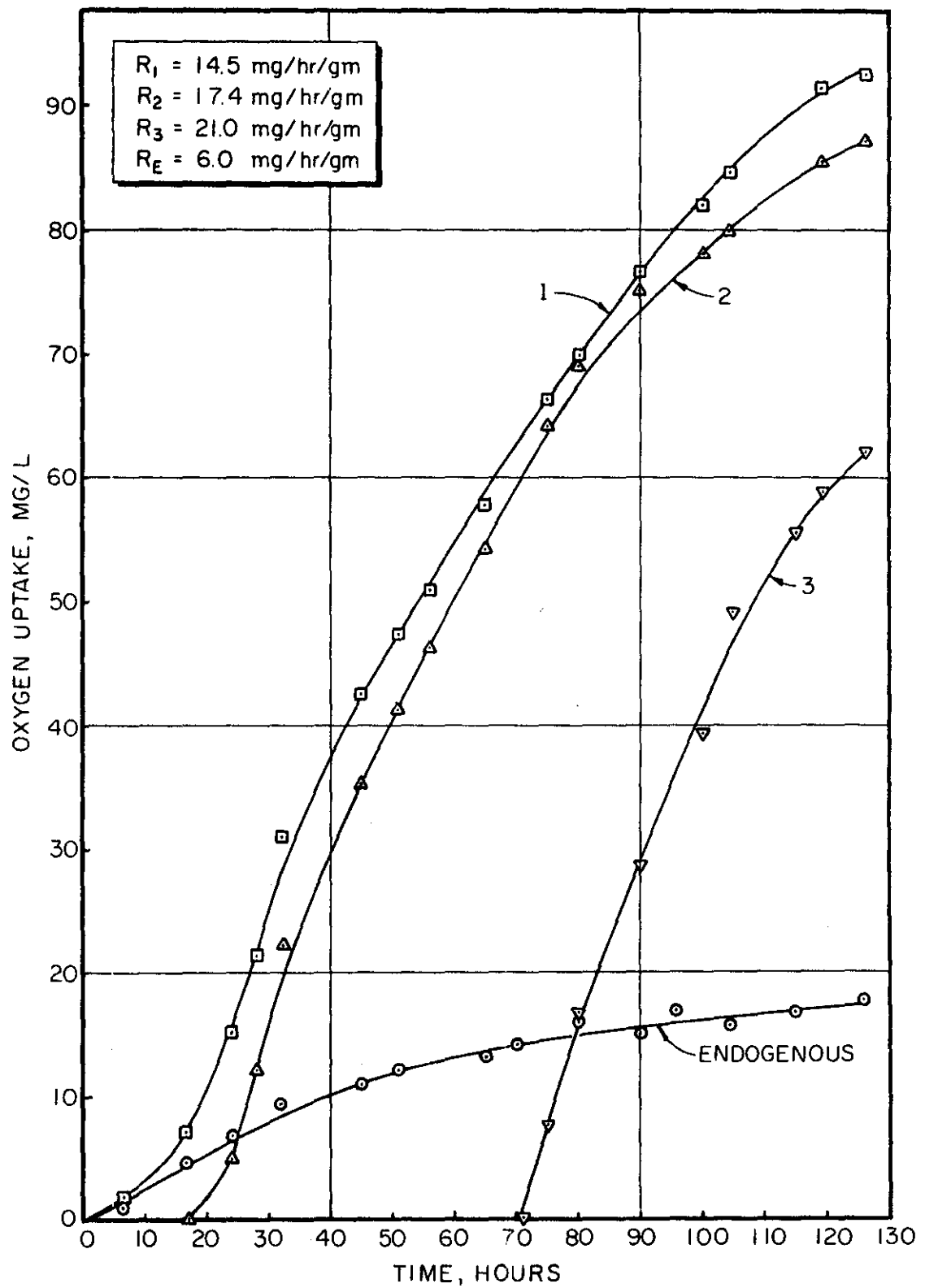


Figure 14. Cumulative oxygen uptake for closed, non-buffered system loaded with 150 mg/l glucose.

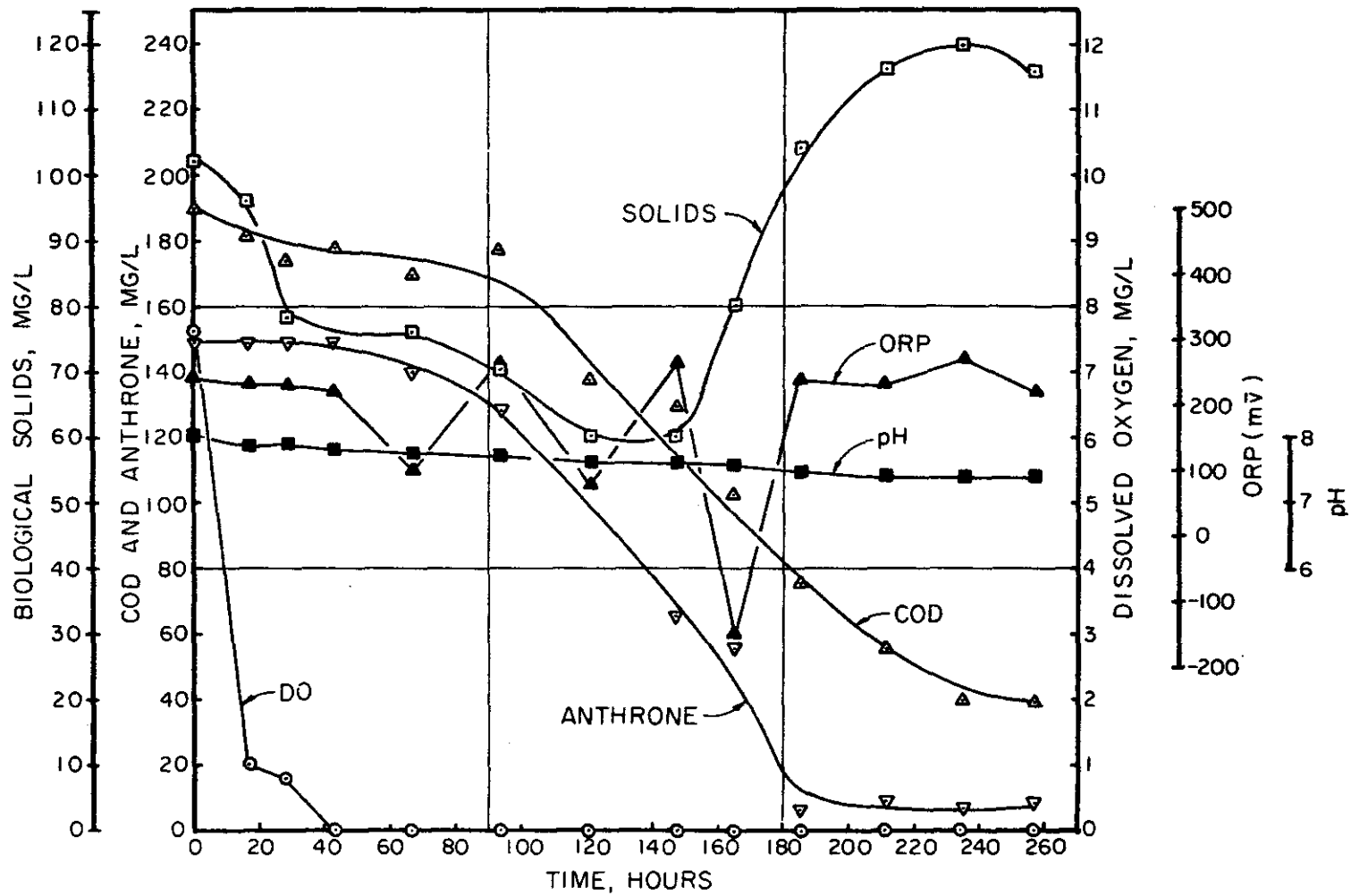


Figure 15. Biological response of closed, buffered system to glucose loading of 150 mg/l; lighting conditions, 24 hours darkness.

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

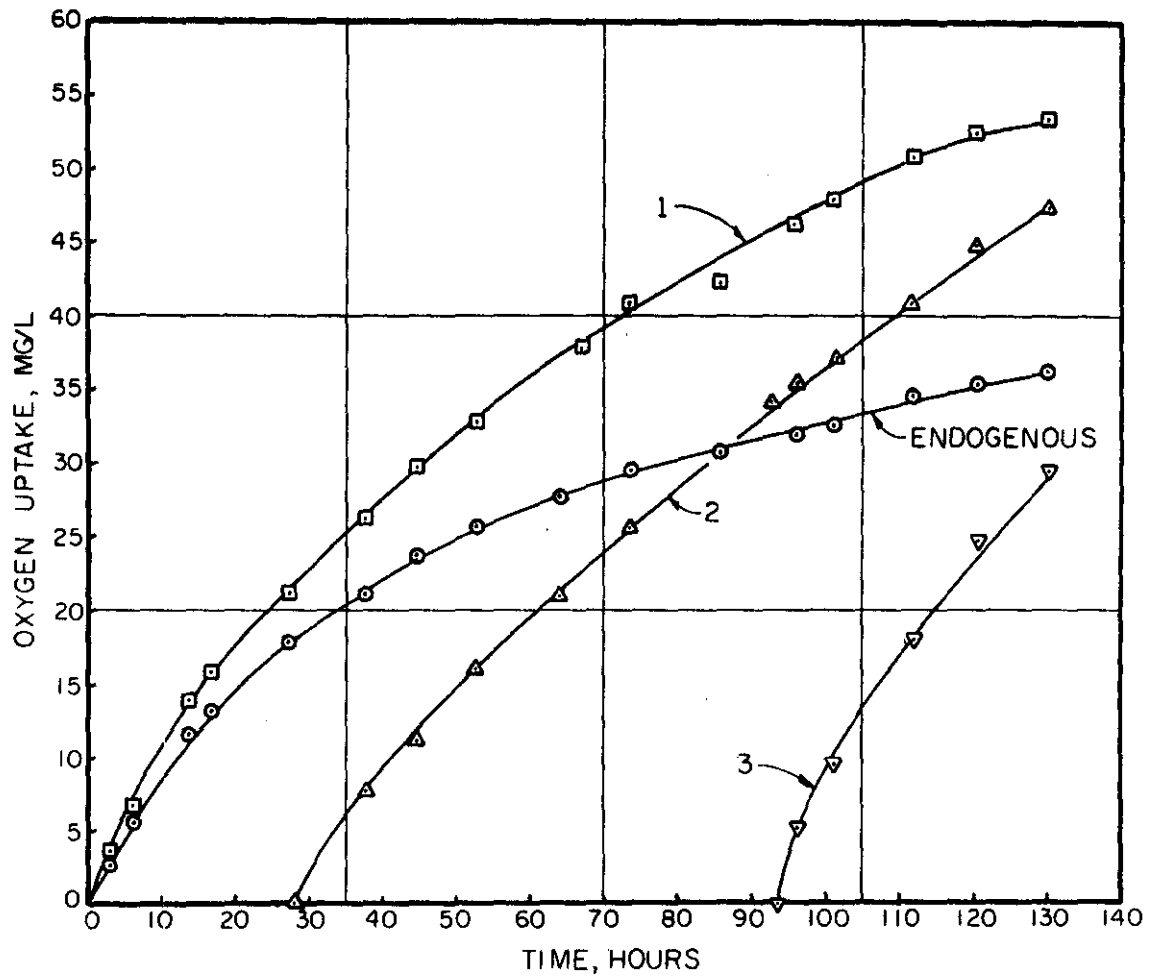


Figure 16. Cumulative oxygen uptake for closed, buffered system loaded with 150 mg/l glucose.

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 viable 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,

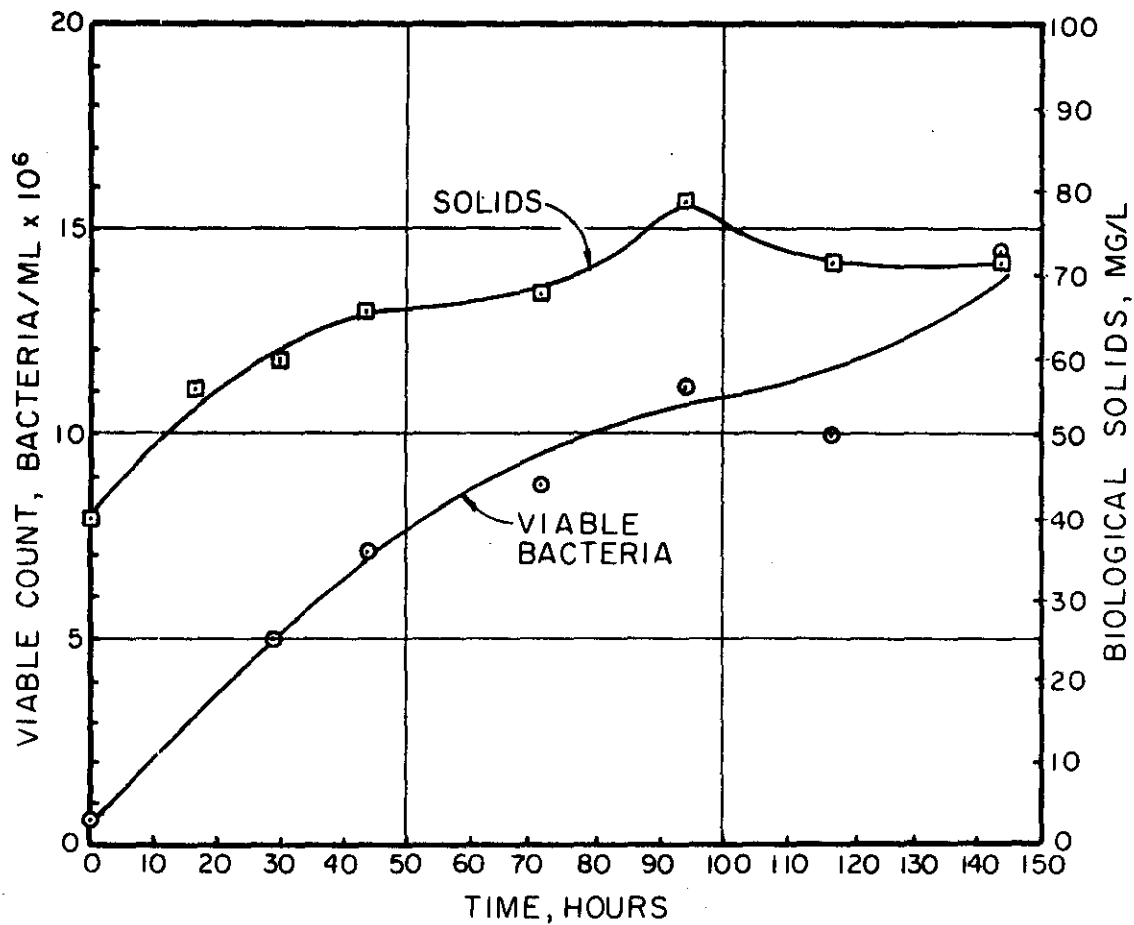


Figure 17. Relationship between biological solids and viable bacteria for the closed, non-buffered system.

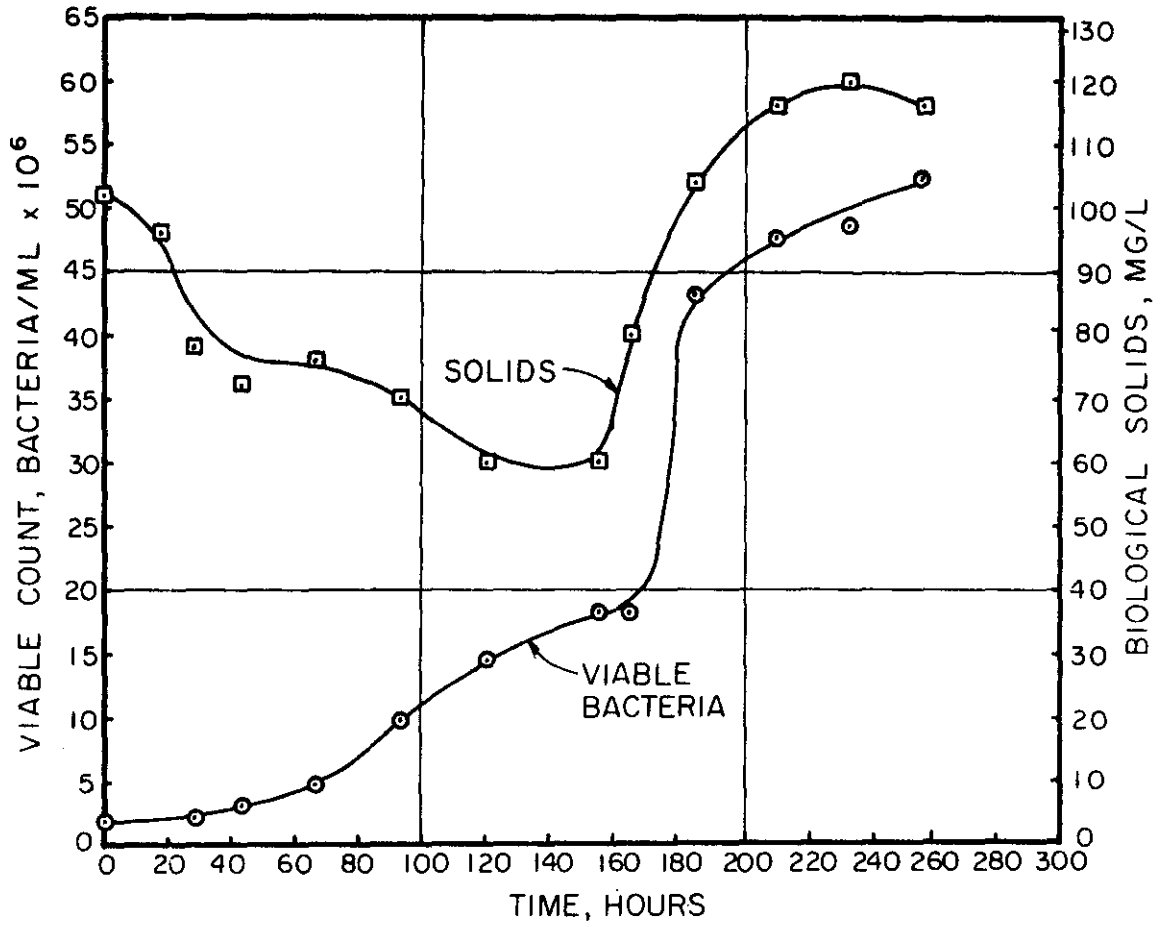


Figure 18. Relationship between biological solids and viable bacteria for closed, buffered system.



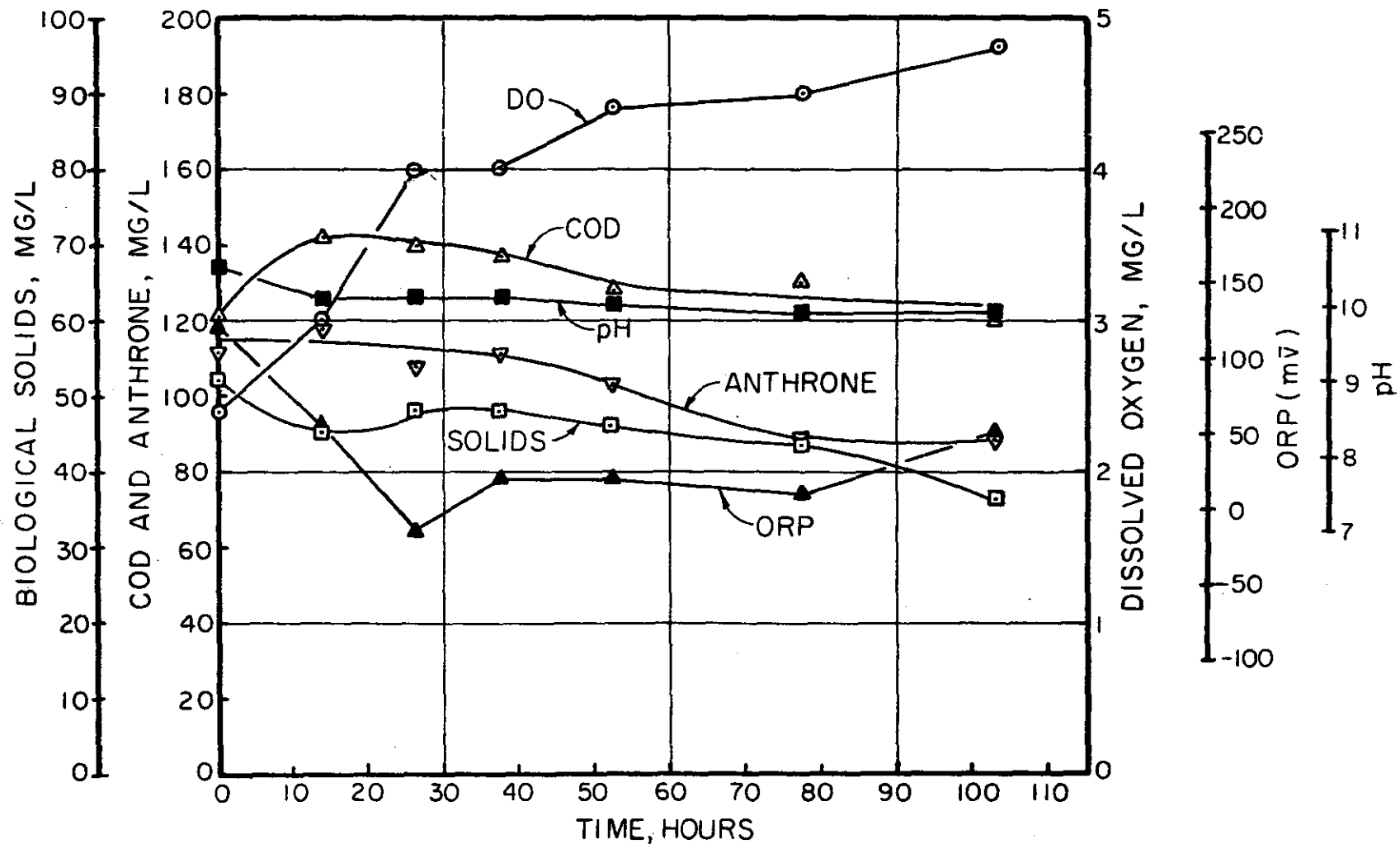


Figure 19. Open, non-buffered system with negligible response to a glucose loading of 100 mg/l; lighting conditions, 24 hours darkness.

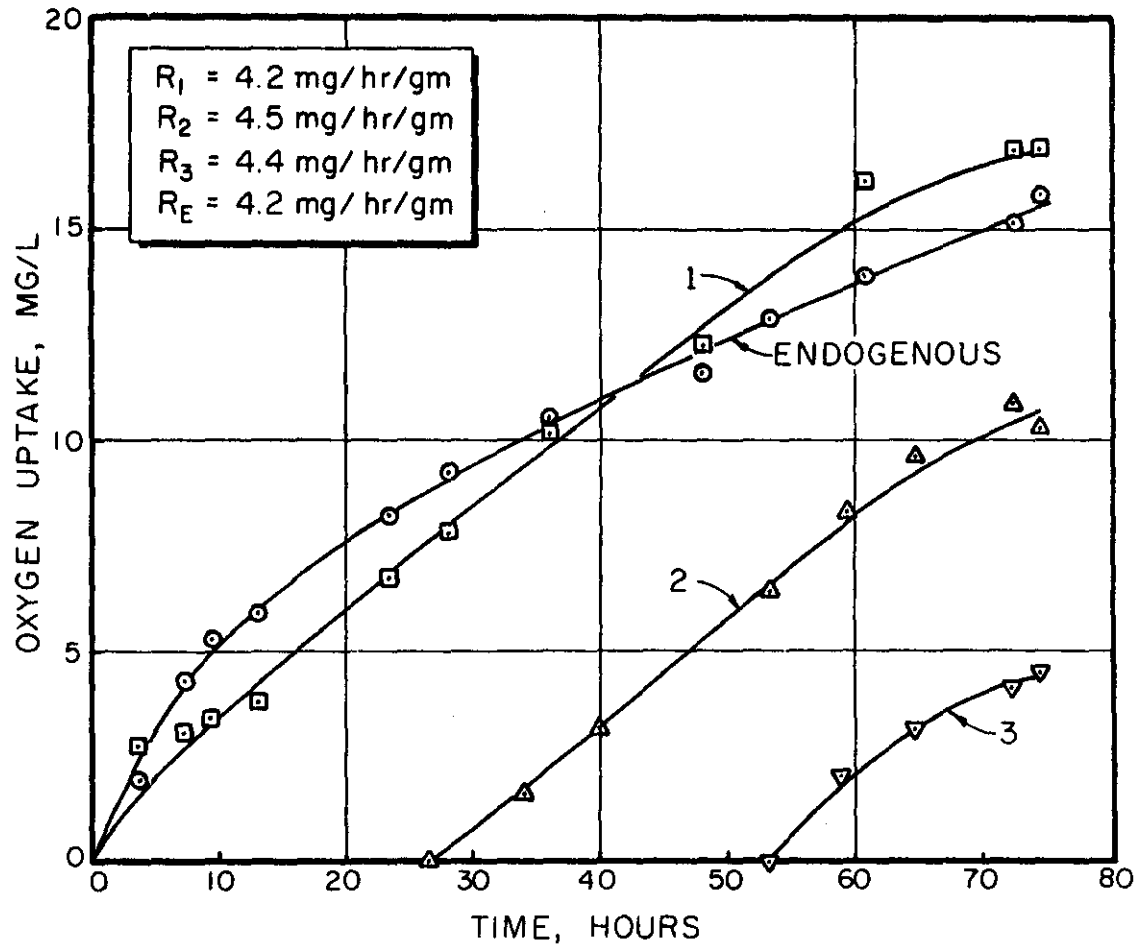


Figure 20. Cumulative oxygen uptake for the open, non-buffered system with negligible response to glucose loading of 100 mg/l.

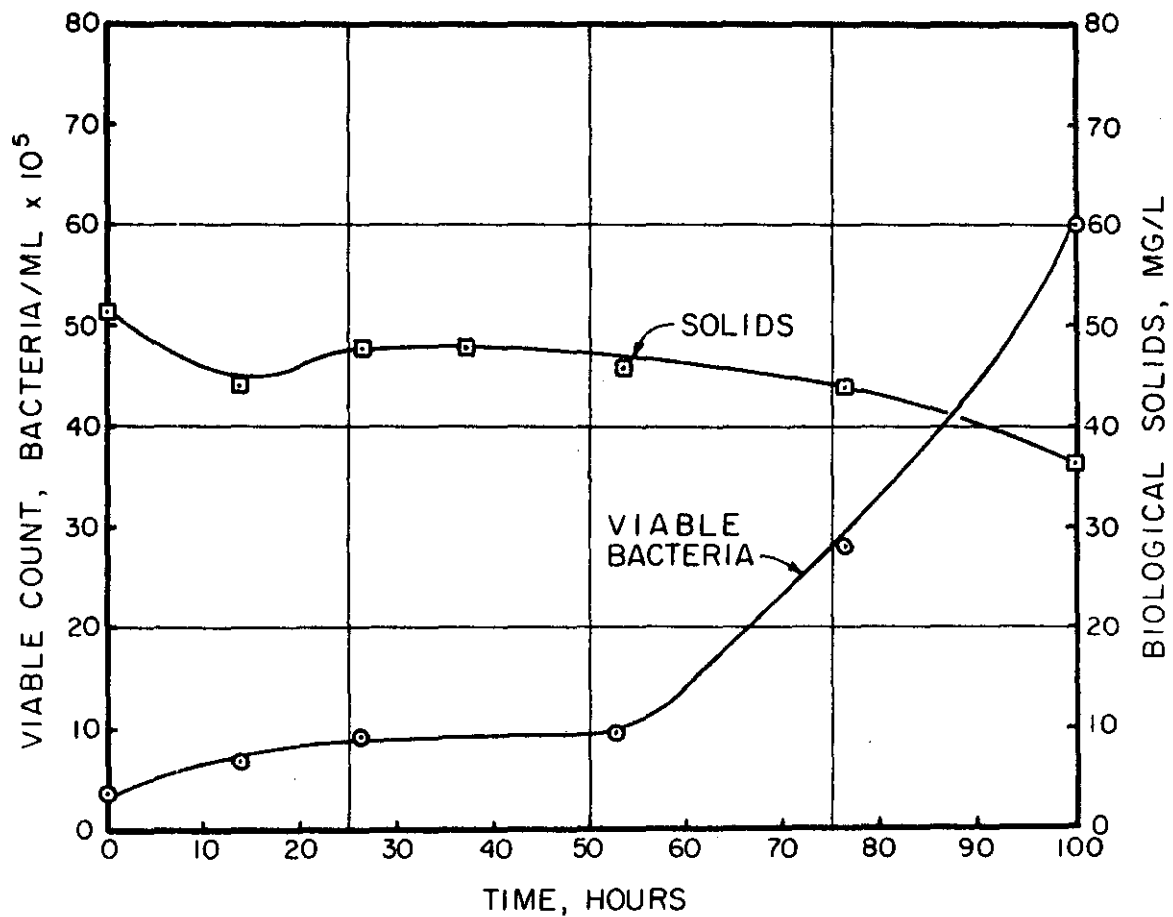


Figure 21. Relationship between biological solids and viable bacteria for the open, non-buffered system with negligible response to glucose loading of 100 mg/l.

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<sup>+</sup> 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 <sub>i</sub> ) mg/l	pH <sub>i</sub>	pH <sub>F</sub>	Time Hours	RO <sub>2</sub> mg O <sub>2</sub> /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.

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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 Engi-  
neering at Oklahoma State University in May, 1968.

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

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

O. V. Natarajan

Ph.D. Thesis

OXIDATION PONDS

OXYGEN CONCENTRATIONS IN AEROBIC

STUDIES ON FACTORS AFFECTING DISSOLVED

APPENDIX III

**STUDIES ON FACTORS AFFECTING DISSOLVED  
OXYGEN CONCENTRATIONS IN AEROBIC  
OXIDATION PONDS**

By

**O. V. NATARAJAN**

**Bachelor of Engineering  
University of Madras, India  
1957**

**Master of Science  
Public Health Engineering  
Annamalai University, India  
1963**

Submitted to the  
faculty of the Graduate College of the  
Oklahoma State University in partial  
fulfillment of the requirements  
for the degree of  
**DOCTOR OF PHILOSOPHY**  
May, 1969

Name: O. V. Natarajan

Date of Degree: May, 1969

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: STUDIES ON FACTORS AFFECTING DISSOLVED  
OXYGEN CONCENTRATIONS IN AEROBIC OXIDATION  
PONDS

Pages in Study: 176

Candidate for the Degree of Doctor of Philosophy

Major Field: Engineering

Scope and Method of Study: Widely acceptable rational criteria for the design of oxidation ponds is not available, since many intricate factors have not been taken into consideration and the functions of various interdependent processes have not been fully delineated. The aim of the present research was to study separately and in combination the significant processes causing a change in the dissolved oxygen concentration in oxidation ponds. A laboratory scale oxidation pond was designed and used to study the various combinations of physical and biological processes governing the oxygen balance. Making the unit airtight, the effect of separating physical gas transfer from the composite process was measured. The capacity of the experimental unit to treat the organic waste in the absence of photosynthetic oxygenation was also explored. The rates and modes of oxygen exchange due to the physical gas transfer process alone were determined. Biological deoxygenation by organotrophs was estimated in the experimental unit as well as in the BOD bottles. Oxygen uptake by photo-autotrophs was determined in the Warburg respirometer. The parameters measured were dissolved oxygen, oxygen uptake, COD, biological solids, pH, ORP, and carbohydrate concentration.

Findings and Conclusions: It was found that in oxidation ponds operated at low loadings with a detention period of ten days, bacteria did not supply all of the required carbon dioxide for the algae, forcing the algae to fix more  $\text{CO}_2$  from the atmosphere than the  $\text{CO}_2$  lost to the atmosphere, and hence the effluent contained more carbon than the influent. At times, the physical aeration process was a liability rather than an asset to the DO resources of the pond. Closing the pond with a transparent material increased the effectiveness of the pond, especially at lower loadings. For satisfactory performance under aerobic conditions, open ponds should not be loaded above 63 lb COD/acre/



day. The contribution made by algal oxygenation to the DO resources of the pond was approximately twice that from atmospheric oxygenation by physical gas transfer process. Though the causes were not found, enough data were obtained to indicate that the rates of deoxygenation were different in experimental ponds and in standard BOD bottles. As previously found for bacteria, the initial algal concentration also exerts an effect on the kinetic mode of oxygen utilization under conditions of heterotropic metabolism.

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## CHAPTER I

### INTRODUCTION

Reuse of water resources is recognized as the only method which, in the future, will satisfy the ever-increasing demand for the indispensable necessity, water. Therefore, it is natural that the removal of organic matter, soluble and colloidal, will receive increasing attention. The oxidation pond is one of the widely employed secondary treatment processes. It is also often employed as the sole treatment process in some places, and as a tertiary process for polishing the secondary effluent in other cases. Unlike other secondary processes, there are not many modifications of the basic process. The study of factors governing the purification process in such ponds provides challenging opportunities to improve upon the existing process and, possibly, to develop new modifications.

Even though the impoundment of waste water was practiced long ago, the purification powers of oxidation ponds were accidentally discovered at Santa Rosa, California, in 1924 (1). In 1929, an oxidation pond was designed and constructed for the town of Sonoma, California. In 1933, Texas A. & M. College constructed a 14-acre lake. It gave satisfactory results in spite of the high BOD loading of

475 ppm (2). The city of Imperial switched to this system in 1941, followed by the city of Modesto, and many others. At present this process is used throughout the world. In a survey conducted in 1959 it was found that 652 cities in the United States were employing this process (3). Porges reported in 1963 that 827 ponds were used for treating industrial wastes from twenty different industries (4).

A brief analysis of stabilization pond literature (which included seventy-nine references) was made by Fitzgerald and Rohlich in 1958 (5). Yeoh evaluated the oxidation pond as a waste treatment process in 1965 (6). His report contained eighty-three references. Both reports concluded that within certain limitations this process will yield good results.

Despite the fact that this process is widely employed, procedures for design of oxidation ponds are far from satisfactory and, for the most part, they are designed either on an empirical basis or on a "similar type" or precedent design basis. A few significant attempts have been made (7) (8) (9) (10) (11) to rationalize the design procedures. In a recent publication (11) an attempt was made to develop design criteria based upon reactor design in the chemical engineering field. In the author's opinion, none of the above has solved the problem completely. One of the reasons for the failure to arrive at widely acceptable and practicable rational design criteria is oversimplification of the various interdependent processes. Many intricate factors

are not taken into consideration, and are sidestepped. Under these circumstances any attempt to rationalize the design criteria on a scientific basis is bound to fail. Understanding the functions of the various interconnected reactions and measurement of the magnitude and the rate of the constituent processes are prerequisites for the development of any sound formulae for oxidation pond design.

An engineer is one who "measures and controls." In the preceding paragraph the importance of "measurement" was indicated. Once the effect of a particular factor on the composite system can be measured satisfactorily, steps should be taken to control that factor with the ultimate objective of improving the efficiency of the composite system. Two such factors which are important in oxidation ponds are light intensity and carbon-dioxide concentration.

Within certain limits, the greater the light intensity, the faster will be the algal growth rate, with concomitant production of more oxygen (12) (13). The excessive oxygen produced will help to keep the oxidation pond aerobic. Even in places where light intensity is excessive during a normal day (e.g., Oklahoma), light may be growth-limiting on cloudy days. To offset this and to utilize the excessive light energy more effectively, Mayer, et al. (14) attempted to introduce light into the depths of mass cultures. They used inverted transparent pyramids, thereby essentially increasing the lighted surface. Such "controls" are not yet practiced in waste water purification.

Carbon dioxide, regardless of its origin, is the carbon source for algal growth under photo-autotrophic conditions. Growth increases as the  $\text{CO}_2$  concentration in the medium increases. Since the partial pressure of  $\text{CO}_2$  in the atmosphere is very low, the  $\text{CO}_2$  concentration in any body of water equilibrating with the atmosphere (e.g., an oxidation pond) will also be low. When the  $\text{CO}_2$  produced by bacterial metabolism exceeds the saturation concentration in the medium, it tends to be stripped to the atmosphere. If stripping could be prevented by means of an impermeable sheet, an "accelerated symbiosis" might be forced. The increased  $\text{CO}_2$  concentration would be of help in accelerating algal growth which, in turn, might lead to increased purification efficiency. Innovations such as this have not been reported to date, and such study could lead to improvements upon the existing oxidation pond process.

The objective of the present research was to study the significant processes causing a change in the dissolved oxygen (DO) concentration in oxidation ponds. To accomplish this objective, biological deoxygenation, by both bacteria and algae, was investigated separately. The physical reaeration and deaeration characteristics of the experimental ponds were studied. Furthermore, studies in open ponds and corresponding studies in ponds closed to the atmosphere were undertaken in order to aid in the assessment of the effect of free gas transfer on changes in DO concentration when biological deoxygenation and physical aeration were

occurring simultaneously under various organic loadings.

It was felt that separate investigation of each major process affecting DO concentration could provide useful information pertaining to the critical factors for maintenance of aerobic conditions in oxidation ponds. For example, it was envisioned that the work would allow definite conclusions to be drawn concerning the relative contributions to the DO resource of the system due to algae and to physical transfer of  $O_2$  across the air-liquid interface of the pond.

## CHAPTER II

### LITERATURE REVIEW

#### General Principles

Figure 1 is a simplified schematic diagram of the various inherent constituent processes in, and components of, an aerobic oxidation pond. The organic wastes are degraded by bacterial action. Dissolved oxygen (DO) in the medium is utilized for this oxidative process. The significant end products of this process are carbon dioxide and water. When light energy along with inorganic nutrients are present, and in the absence of inhibitory substances, the carbon dioxide is used by algae as a carbon source. Oxygen is evolved as a byproduct in this process of photosynthesis. This oxygen replenishes the DO level depleted by bacterial metabolism, and thus the cycle is completed.

Some portion of the organic wastes may be metabolized (heterotrophically) by algae to  $\text{CO}_2$  at the expense of the DO resource. Alkalinity present in the waste may also contribute to the  $\text{CO}_2$  pool. The  $\text{CO}_2$  and  $\text{O}_2$  in the medium are always subject to transfer to the atmosphere, dependent upon their concentration in the medium. From the above considerations it can be seen that (1) organic wastes,

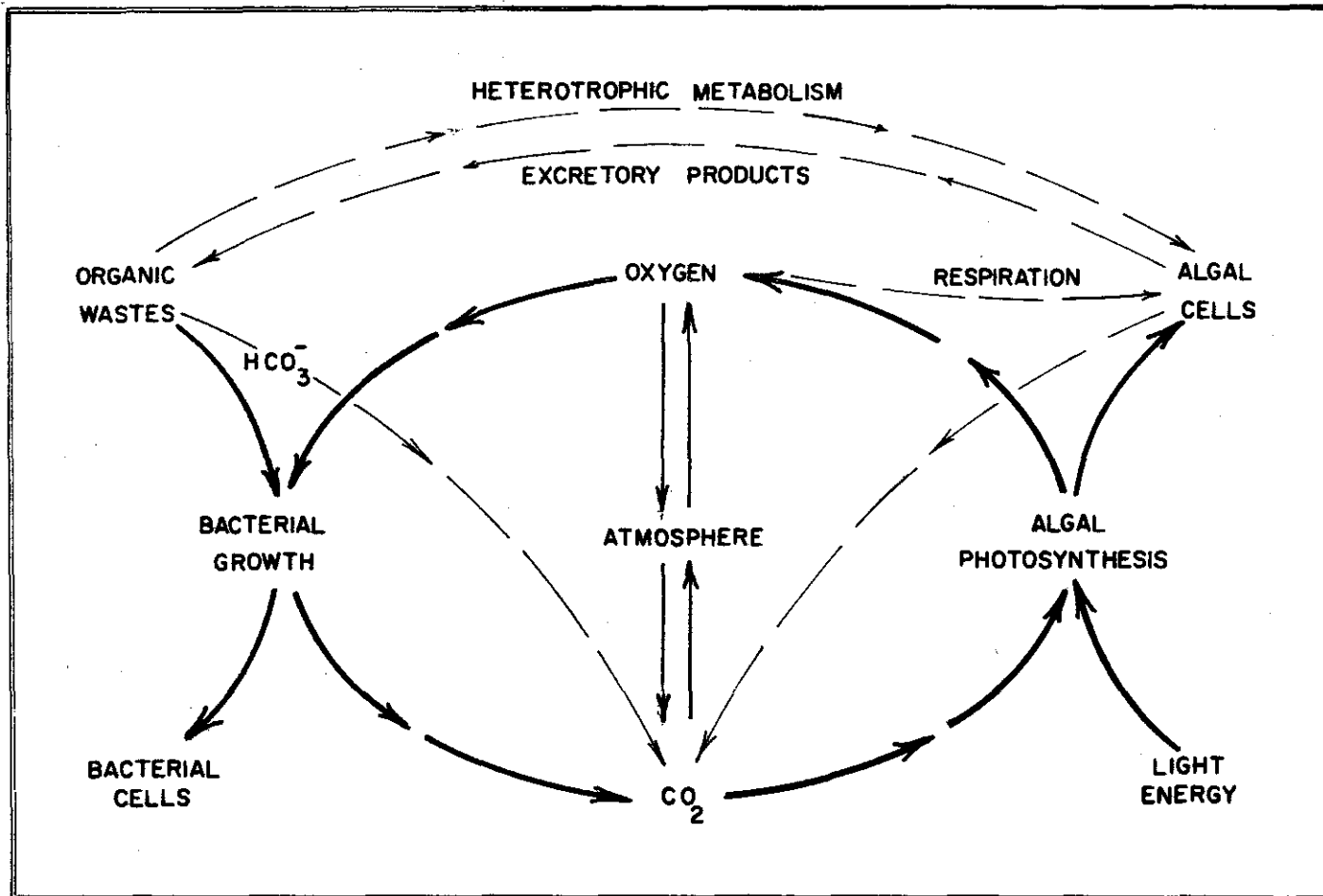


Figure 1 - The cycle of constituent processes in an aerobic oxidation pond.

(2) bacterial oxidation, (3) carbon dioxide, (4) light energy, (5) algal photosynthesis, and (6) dissolved oxygen are paramount factors in the functioning of aerobic oxidation ponds.

### Photosynthesis

The fact that the photosynthetic reactions consist of two distinct phases is well known, and detailed information can be found in many texts (15)(16). The "light" reactions are energetic in nature. The electromagnetic radiation energy of the light is absorbed by the photosensitive systems containing the chlorophylls, and this light energy is used to bring about the transfer of electrons from the water molecule. This results in the formation of TPNH and ATP with concomitant evolution of  $O_2$ . In the "dark" reactions, the available carbon dioxide is reduced to the level of sugar phosphates, at the expense of the co-factors formed in the light reactions.

### Light

From the above it can be discerned that light intensity exerts a controlling effect on the rate of photosynthesis through the "light" reaction. However, once the rate of the light reaction is equal to the rate-limiting step of the dark reactions, an increase in the light energy will not increase the overall rate of photosynthesis; therefore, it is reasonable to expect that the photosynthetic rate depends upon the intensity of light only within certain limits. The upper limit is imposed by the rate of



the dark reaction which, in turn, depends upon the species present, available substrate, and other environmental factors. The lower limit, the so-called compensation point, depends upon the species present and their physiological condition. At this light intensity, all of the oxygen produced is required to meet the respiratory demands.

Rodhe attempted to elucidate the influence of some environmental factors on the development of fresh water plankton algae (17). He conducted experiments with Ankistrodesmus falactus under different temperatures (15, 20, and 25°C) and light intensities (1000, 1700, and 3600 lux). The cultures were illuminated ten hours per day. The results indicated higher growth at 3600 lux (about 360 foot candles [ft-c]) at all three temperatures. However, he cautioned that the individual organisms probably do not have fixed temperature and light optima, but that their levels may be also dependent on the periodicity of the lighting.

Varma and Wilcomb published a paper entitled "Effect of Light Intensity on Photosynthesis" (18). They reported that they have made metabolic studies of algae using a Warburg respirometer and BOD bottles. They did not state what type of algae they used, or the source of algae. However, in a previous paper (19) Varma, et al. indicated that Oscillatoria was the predominating alga in their system. BOD bottles were incubated at 30°C under continuous lighting. The light intensities varied from 260 to 1200 ft-c.

The experiments in the Warburg apparatus were conducted at 37°C and at light intensities of 816 and 1450 ft-c. Though many essential details are missing, the reported results show that oxygen was evolved at increasing rates with increased intensities of light.

Lubbers and Parikh (20) studied the effect of light intensity and other factors upon growth of mixed algal cultures from experimental waste stabilization lagoons. They used raw, homogenized sewage having a 5-day BOD value of 350 to 650 mg/l. The "algal culture" would be expected to contain other microbes as well, since the culture was taken from experimental lagoons. The light intensity was varied from 10 ft-c to over 9000 ft-c. They reported the compensation point (light intensity when net oxygen production is zero) as slightly less than 20 ft-c. They stated that 720 ft-c "may be considered as a saturation value." The reason for this indefinite statement may be the design of the experiments. The experiments were conducted at about 10, 20, 40, 70, 140, 300, 720, 1000, 3300, 4500 ft-c, etc. The net O<sub>2</sub> production increased until 720 ft-c was reached; above this value it remained almost constant. They could have made a definite statement that at 720 ft-c (as at 1000 and 3300 ft-c) O<sub>2</sub> production was maximum. The saturation point was somewhere between 300 and 720 ft-c. From their arithmetic plot of the data one would estimate the value to be around 550 ft-c. This report would have been more useful had the authors indicated the concentration of the

culture in these particular experiments.

Oswald, et al. (12) experimented with Euglena gracilis, employing natural and synthetic sewage in semicontinuous flow units. The BOD of the natural sewage was 90 mg/l, and that of the synthetic sewage was 117 mg/l. The retention periods employed were seven and five days, respectively, and the range of light intensity was 100 to 2400 ft-c. They reported the value of the saturation point to be at or near 400 ft-c for the low BOD (natural sewage) system run at a detention time of seven days. However, on the synthetic sewage, the growth increased until the 2400 ft-c level was attained. Even with this high light intensity, growth was lower than the growth at 400 ft-c on the natural sewage system. They concluded that the optimum light intensity depends on retention time and strength of sewage. It is possible that their synthetic sewage was lacking or low in some essential growth nutrient.

Myers (13) (21) in 1946 presented data relating growth rate to light intensity. Chlorella pyrenoidosa was grown in a continuous culture, the intensity of light being varied from 6 ft-c to 360 ft-c in one series, and from 9 ft-c to 325 ft-c in another. Both sets of experiments indicated a levelling off of the growth curve at approximately 100 ft-c. He concluded that "at low intensities (<60 ft-c) growth is proportional to light intensity. At high intensities (>100 ft-c) growth is nearly independent of light intensity." Seven years later, the same author determined the light

intensity curve for growth of Chlorella pyrenoidosa in connection with the determination of growth rate in flashing light (22). In this work he estimated the value of the compensation point to be less than 24 ft-c. He stated that the growth rate does not have a light saturation, but continues to increase slowly with increasing intensity. His estimate of the "saturation value," wherein the growth rate attained ninety per cent of its observed maximum, was 600 ft-c. He accounted for this discrepancy in the saturation values by the difference in the nature of the illumination. The value of 100 ft-c was arrived at when the light was multilateral (from many directions) and the value of 600 ft-c was obtained when the illumination was unilateral (from the top only). The difference, to a certain extent, may be due also to changes in the lighting system and modification of the medium in the two studies.

In a book edited by Burlew, Myers discussed (23) the growth characteristics of algae in mass culture. He stated that "the minimum intensity required for maximum rate of growth of Chlorella is in the neighborhood of 400 ft-c under unilateral illumination."

Considering the above discussion on the effect of the intensity of light, it can be surmised that under normal conditions encountered in oxidation ponds (at a temperature of approximately 25°C), 400 ft-c is close to "saturation value."

### Carbon Dioxide

One of the "driving forces" of the "dark reactions" is the carbon dioxide present in the medium. The incorporation of  $\text{CO}_2$  can take place during the light period also. The nature of the initial carboxylation reaction and the regeneration of the  $\text{CO}_2$ -acceptor, ribulose diphosphate, were unraveled primarily by the experiments of Calvin and co-workers (24). The metabolic pathway proposed by them for the photosynthetic carbon cycle is widely, although not universally, accepted (25).

Since  $\text{CO}_2$  is the substrate for the initiating step of the photosynthetic carbon cycle, the rate of the dark reactions depends on the  $\text{CO}_2$  concentration, within certain limits. Much conflicting information is available regarding these limits. Emerson and Green studied the rate of photosynthesis (measured by the rate of  $\text{O}_2$  evolution) as a function of  $\text{CO}_2$  concentration in the medium using Chlorella pyrenoidosa (26). Their results showed that the photosynthetic rate varied linearly with  $\text{CO}_2$  concentration up to approximately 0.05 per cent, and thereafter remained constant up to the maximum tested concentration of 5 per cent.

Spoehr and Milner (27) compared the effect of bubbling  $\text{CO}_2$  at concentrations of 3, 5, and 10 per cent (in the aerating gas) on the yield of Chlorella pyrenoidosa under two light intensities. At low intensity the yields in three and five per cent showed no significant difference,

while at the higher intensity the yields were roughly proportional to the  $\text{CO}_2$  concentration. In the culture grown with ten per cent  $\text{CO}_2$ , the yield was less, amounting to about eighty per cent of the five per cent  $\text{CO}_2$  culture. Despite the fact that measurement of  $\text{CO}_2$  concentration is different in the two cases referred to above, the saturation limits are far apart.

Davis, et al. (28), in an effort to ascertain the effect of  $\text{CO}_2$  on the growth rate of Chlorella, conducted experiments at an incident light intensity of 350 ft-c. The concentrations of carbon dioxide dissolved in the culture medium in these experiments were 4.43, 2.19, 1.02, and 0.56 per cent. Within experimental variations, the growth rates were the same. They stated that with low concentrations of carbon dioxide, the gas must be supplied fast enough so that the culture medium is in equilibrium with the  $\text{CO}_2$  concentration in the gas stream.

Gaucher, et al., working with thermophilic Chlorella pyrenoidosa, attempted to control the photosynthetic rate by varying carbon dioxide and some other factors as the principal parameters (29). They used 0.5, 3.0, and 5.5 per cent  $\text{CO}_2$  in the aerating gas, and concluded that concentrations of  $\text{CO}_2$  around 0.5 per cent do not appear to limit the growth of the organism.

Even though the atmospheric air contains an abundant quantity of  $\text{CO}_2$ , its concentration is only 0.03 per cent. This is far below the "saturation value." Therefore, the

growth of algae in a body of water which is in continuous contact with the atmosphere, e.g., an open oxidation pond, may be limited by CO<sub>2</sub> concentration. Field and laboratory data support this contention (30) (31) (32) (33) by pointing out that in many instances carbon is the growth-limiting factor.

Ludwig, et al. studied the growth characteristics of Euglena gracilis in sterilized sewage which was reseeded with sewage (30). They conducted three series of experiments using air with 2.30, 1.75, and 0.03 per cent CO<sub>2</sub>. The maximum algal yields were 0.23, 0.22, and 0.08 grams/liter/day, showing a linear relation in a semi-log plot. In a similar study with Chlorella pyrenoidosa (31), analyses were made on algal cells and the sewage medium. Calculations showed that the sewage provides all nutrients in excess except carbon. Allen investigated algal growth in sewage oxidation ponds in California (32). She isolated a strain of Chlorella, CC-2, from a pond in Contra Costa, and studied its growth in sterilized sewage with and without CO<sub>2</sub>. The concentration of CO<sub>2</sub> was five per cent. After seven days, the growth was approximately three times higher in the culture with CO<sub>2</sub>.

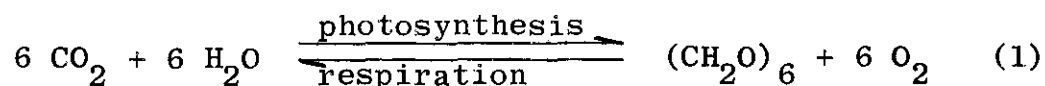
All of these studies have shown how algal growth rate can be increased by provision of additional CO<sub>2</sub>. However, the increased algal growth, per se, will not improve the efficiency of waste water purification. In the oxidation pond, bacteria are mainly responsible for the oxidation of

organic matter. In fact, during algal growth some organic materials are excreted into the medium (to be dealt with in some detail later) which will increase the pollutional load on the system. The prime function of algae in the ponds is to act as "biological aerators."

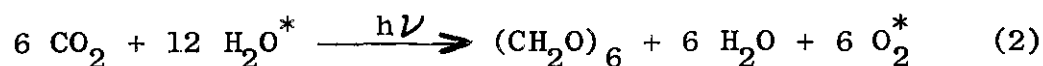
The effect of CO<sub>2</sub> availability on oxygen production can be seen from the following example. Hannan and Patouillet used a high temperature strain of Chlorella pyrenoidosa to relate CO<sub>2</sub> supplied with the oxygen produced (33). The input rate was adjusted by changing either the volume of flow or the concentration of gas. The CO<sub>2</sub> input was varied from 2310 to 3820 cc/hr. In four experiments out of five the oxygen produced could be correlated to the CO<sub>2</sub> supplied; furthermore, the density of the steady-state suspension could also be correlated to oxygen produced.

#### Photosynthetic Oxygenation

The classical definition of photosynthesis is usually given as "the reduction of carbon dioxide to carbohydrate." For convenience it is usually explained as the "reversal of respiration," and is denoted by the equation:



Since the source of the evolved oxygen has been found to be water, a more appropriate equation to describe photosynthesis is:





In accordance with this, 6 moles of  $\text{CO}_2$  are assimilated for the synthesis of one mole of carbohydrate with the simultaneous liberation of 6 moles of  $\text{O}_2$ .

Estimation of the oxygen evolved can be made directly by quantitative determination of the liberated  $\text{O}_2$ ; the Warburg apparatus has been used in such determinations (30) (31) (34). Extrapolation of the Warburg results to natural conditions may be of questionable validity. Apart from that, another unsolved and inherent difficulty is the effect of lighting on the normal respiration of cells. Hence, the determination by Warburg respirometer is the net oxygen production over and above the respiratory requirements, if any, under a different environment.

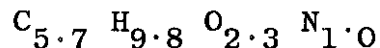
Another approach is the calculation of  $\text{O}_2$  evolved using the balanced photosynthetic equation, i.e., Equation (2). Assuming that the equation is correct, it can be seen that 6 moles of oxygen are evolved for 6 moles of  $\text{CO}_2$  consumed or for each mole of carbohydrate synthesized. Measurement of  $\text{CO}_2$  utilized for photosynthesis in purely photoautotrophic systems can be done with considerable accuracy. But it is not possible in heterotrophic systems or even when  $\text{CO}_2$  is fixed non-photosynthetically. The other alternative is the determination of the quantity of organic material synthesized and multiplication of this quantity by an appropriate factor.

There are two schools of thought concerning the multiplication factor. One is the "unit process" concept (35)

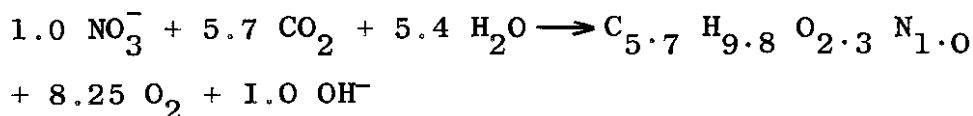
maintaining that the primary end product of photosynthesis is a hexose sugar even though the "first sugar" was not identified. This school of thought does take into account the fact that algal cells or, for that matter, any plant cells, are not composed of carbohydrate alone but contain other components as well. However, in accordance with this concept, nitrogen assimilation and other metabolic processes take place outside the photosynthetic cycle. If this is correct, the multiplication factor will be 6, and the assimilatory quotient ( $\text{CO}_2/\text{O}_2$  ratio) will be 1.0.

The other line of thought is the "meshed process" in which nitrogen assimilation and other metabolic processes are considered to be intimately related to photosynthesis. A quotation from Myers, who advocated this second possibility, is given below (34):

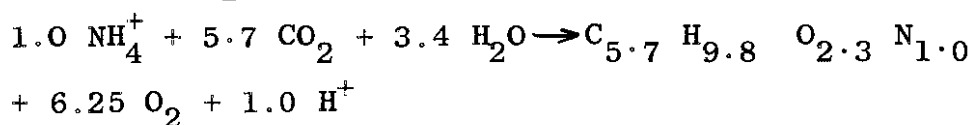
"Elementary analysis on Chlorella, as grown in our experiments, yields 53.0 per cent C, 7.5 per cent H, 28.5 per cent O, 10.8 per cent N, on an ash-free, dry weight basis. On dividing by the appropriate atomic weights, these percentages can be converted to the expression



If the nitrogen source is known, it becomes possible to write balanced equations for overall metabolism and thus predict the gas exchange. For instance:



$$\text{Quotient } \text{CO}_2/\text{O}_2 = - 5.7/8.25 = - 0.69$$



$$\text{Quotient } \text{CO}_2/\text{O}_2 = - 5.7/6.25 = -0.91."$$

It can be seen that the multiplication factor varies, depending not only upon the elemental composition of algal cells, but also on the nitrogen source.

Whether nitrogen assimilation takes place in the photosynthetic step may not be of much significance to the overall oxygen balance of oxidation ponds. The anticipated function of algae in the ponds is the provision of oxygen over and above their own respiratory needs. As such, the quotients estimated by considering actual composition and nitrogen source will be more appropriate. Oswald, et al. (12) changed over to this line of thought in the middle of their published series of reports (12) (30) (31). Taking ammonia as the nitrogen source for their Euglena cultures, they framed the equations and calculated assimilatory quotients and thereby the gross  $O_2$  production for light intensities from 100 to 2400 ft-c. Maximum  $O_2$  was produced at approximately 700 ft-c and greater than ninety-three per cent of the maximum production was evolved at light intensities between 400 and 1200 ft-c. Furthermore, when they developed design criteria (8), they related the weight of oxygen evolved to the weight of cell material synthesized using the equation at 400 ft-c. They found 1.6 grams of  $O_2$  were produced for each gram of new cell material, and that this value may vary from 1.5 to 2.0.

Another method which is widely used in field studies is the light and dark technique. Several airtight bottles containing the culture are suspended at desired depths.

One-half of the number of bottles (dark bottles) are wrapped with light-excluding material. The difference between the DO changes in the two groups is the net oxygen production. In this method it is assumed that the respiration is identical in the light and in the dark. Bartsch and Allum (36) presented field observations of two oxidation ponds. At one foot below the surface, where the light intensity was about 1000 ft-c, the net production was six times the DO required for respiration. At a depth of two and one-half feet the light intensity was 167 ft-c, and the net production was zero. At this point the DO consumed equalled the DO produced.

O'Connell and Thomas (37) studied the effect of benthic algae on dissolved oxygen in the Truckee River in Nevada. They evaluated the net oxygen contribution by two separate methods, and arrived at approximately the same values. In one method they used a modified sag equation containing a term for photosynthesis, evaluated all other terms, and thus determined the amount of photosynthesis. In the second method they measured the net oxygen change using airtight plexiglass-polyethylene sheet chambers of 10-liter capacity, which were immersed in the stream and from which samples could be withdrawn periodically. Oscillatoria, which was predominant in the reach of stream under study, was cultured in petri dishes. The petri dish, entirely covered with the alga, was placed in the chamber and flushed with river water. Test results were expressed

as mg of O<sub>2</sub> per hour per unit area of alga. Along with this measurement, the calculation of the bottom area covered by the alga and the water volume in each reach enabled them to express the results in units of mg/liter/hr. The results showed that the maximum photosynthetic oxygen production occurred at approximately one o'clock in the afternoon. At one and one-half hours after sunrise and one and one-half hours before sunset, the respiratory consumption equalled the photosynthetic production. On the average, 72.5 lb. of O<sub>2</sub>/acre/day was produced against the average respiratory demand of 65.4 lb. of O<sub>2</sub>/acre/day.

#### Heterotrophy

The ability of algae to grow in darkness utilizing an organic carbon source was known even in the last century. However, the species that have this heterotrophic capacity, the substrates they can oxidize, and the mechanisms of this metabolism are scantily categorized (38). A few selected works which have a bearing on this research are summarized in Table I.

Pearsall and Bengry (39) cultured a strain of Chlorella in the dark, using a medium containing 10 g/l of glucose. They found that the growth, at first, was exponential in character until the cell number reached approximately 6000 cells per cubic millimeter. The logarithmic growth rate in the dark was about sixty per cent of the logarithmic growth rate in the light. After this, the growth curve was nearly linear in nature, which may have been due

TABLE I  
HETEROTROPHIC METABOLISM OF ALGAE

#	Authors	Ref.	Algae	Substrate	Results
1	Pearsall and Bengry	39	<u>Chlorella</u>	Glucose	Retarded, yet positive growth; exponential followed by linear in nature
2	Samejima and Myers	40	<u>Chlorella pyrenoidosa</u> <u>Chlorella ellipsoidea</u> <u>Scenedesmus</u> sp. D3	Eleven sugars, three alcohols, sugar phosphates, and some fatty acids	The two chlorellae grew well only on glucose and galactose. The growth of <u>Scenedesmus</u> was good on glucose and slow on galactose, mannose, fructose, maltose, and lactose
3	Theriault	41	<u>Chlorella pyrenoidosa</u> 7-11-05	Single carbon source glucose, glycerol, galactose, sucrose, mannose, mannitol  Two carbon source glucose + (glycerol ) (galactose) (fructose ) (xylose ) (arabinose)	Glucose was used well; glycerol 52% of glucose. Galactose 25% of glucose. Others not appreciable  Fair Good Toxic
4	Lewin	42	<u>Chlamydomonas dysosmas</u> 1. Wild type 2. Mutant	Acetate, lactate, pyruvate Succinate, malate, citrate, glutamate, glycerol, glucose, G-1-P	Wild type grew well in acetate, slightly in lactose or pyruvate, and no growth in others, including glucose. Mutant, no growth
5	Parker, et al.	43	<u>Chlorococcum</u> <u>Bracteacoccus</u> <u>Spongiochloris</u> <u>Dictyochloris</u>	Glucose, acetate	<u>Bracteacoccus</u> , <u>Spongiochloris</u> , and <u>Dictyochloris</u> grew; <u>Chlorococcum</u> did not grow
6	Finkle, et al.	44	<u>Chlorella vulgaris</u> (Emerson strain)	Glucose	No growth
7	Karlander and Krauss	45	<u>Chlorella vulgaris</u>	Glucose	No growth
8	Myers	46	<u>Chlorella vulgaris</u>	Glucose and peptone	Production of pigments
9	Allen	32	<u>Chlorella Scenedesmus</u> , etc.	Sewage	No growth
10	Pipes and Gotaas	47	<u>Chlorella pyrenoidosa</u>	Sewage	Cultures having detention period less than three days were able to utilize some of the organic matter
11	Pipes	48	<u>Chlorella pyrenoidosa</u>	Glucose, fatty acids with less than six carbons, five amino acids, urea  Sewage fractions soluble in (1) water, (2) acid, (3) hydroxide, (4) carbonate. Insoluble, soap-like amorphous precipitate	Growth rate was increased compared to the growth rate in inorganic medium. Water soluble sewage fraction increased growth

to the limitation of oxygen supply.

Samejima and Myers (40) studied the heterotropic growth of Chlorella pyrenoidosa and two other organisms on various organic substrates. One per cent (weight/volume) substrate concentrations were employed. The specific logarithmic growth rate of Chlorella pyrenoidosa on glucose in the absence of light was almost one-half of its specific logarithmic growth rate in the light (0.46/day against 0.93/day). The growth rate on galactose was one-half the rate on glucose. When this strain was grown on a glucose and galactose medium, the growth rates were identical to the growth rates in medium containing only glucose, indicating a common rate-limiting reaction. If the cells have used glucose in preference to galactose (sequential substrate removal), that would explain the above observation. This could have been verified if the specific substrate removals had been followed.

Theriault (41) investigated the production of xanthophylls by Chlorella pyrenoidosa 7-11-05, grown heterotrophically. Glucose was the only sugar among the eleven sugars used as sole carbon source which gave appreciable growth. Glycerol and galactose utilization were approximately fifty-two and twentieth-three per cent of glucose utilization. Fructose, which was not consumed when used as a sole carbon source, was almost completely assimilated when it was present in combination with glucose. Galactose utilization was enhanced when it was used with glucose, but

did not match the growth in the fructose-plus-glucose system. This difference was more pronounced in cultures under dark than in light. The presence of either arabinose or xylose with glucose retarded the growth.

Comparing these results with those of Samejima and Myers (40), two anomalies are seen. Glycerol was assimilated in Theriault's studies, whereas it was not utilized at all in those of Samejima and Myers. Galactose utilization was about half of glucose utilization in the latter, compared to one-fourth in the former. Two explanations are possible for these anomalies. Samejima and Myers used the Emerson strain of Chlorella pyrenoidosa, whereas Theriault used Chlorella pyrenoidosa 7-11-05 (Sorokin). The strain 7-11-05 adapted to galactose after six transfers. The efficiency for utilization of galactose in this adapted strain improved to fifty-eight per cent of the glucose utilization, while that of the wild strain was about ten per cent. Theriault's further study with single carbon sources was accomplished under illumination. Since there was neither a CO<sub>2</sub> supply nor bacterial contamination, the growth was organotrophic. The presence of light might have had some effect.

Lewin (42) selected a facultatively heterotrophic species, Chlamydomonas dysosmos, and obtained a mutant (D.2075) induced by ultraviolet light, which behaved like an obligate photoautotroph. The growth of the wild type in the dark was good on acetate, slight on lactate or pyruvate,



and absent on other sugars tested, including glucose. The mutant, D.2075, did not grow on acetate, lactate, or pyruvate. Lewin concluded that the assimilatory system for acetate was present in the wild type, and absent in the mutant.

Inability of all of the sixteen known species of Chlorococcum to utilize either glucose or acetate has been reported (43). This quality of obligate photoautotrophy can be used to distinguish this genus from others. Nevertheless, in some other genera some species are heterotrophs and other species are autotrophs. Two strains of Chlorella vulgaris have been shown not to grow in the dark on glucose (one per cent solution) (44) (45). The growth did not stop immediately when cells were transferred from light to dark, but slowly decreased up to five days, after which it was completely stopped. To study the pigments produced by Chlorella vulgaris in darkness, Myers (46) grew it on 0.5 per cent dextrose and 0.2 per cent peptone. He found that the pigments produced by this strain of Chlorella vulgaris in darkness were the same as that produced in the light. Possibly peptone was used as substrate, or pigment production might have taken place without growth; no data were presented with respect to cell numbers, but it would appear from the results which were presented that production of the pigment was not dependent upon light energy.

In a study on algal growth in sewage oxidation ponds, Allen (32) made the following statement:

"It at first appeared possible that the dominance of Chlorella in the ponds could be due partly to its ability to grow during the hours of darkness. However, numerous experiments have shown that neither Chlorella nor other common pond algae (Scenedesmus, Chlamydomonas, Ankistrodesmus) can grow oxidatively on sewage in the dark, whether alone or in the presence of bacteria, so that it must be concluded that neither sewage nor its bacterial oxidation products contain materials utilizable by Chlorella in the dark in the pH range encountered in oxidation ponds. It was also found that growth of Chlorella on sterilized sewage in the light did not result in any decrease in oxidizable organic matter."

While convincing data were presented for the second finding, no data was shown for the first conclusion based on numerous experiments.

As if to verify the above statements, Pipes and Gotaas (47) made an attempt to determine the organic compound(s), if any, in sewage which can be oxidized directly by Chlorella. It is of interest to note that these workers used a strain of Chlorella pyrenoidosa isolated by Allen, though her work was not referenced. They conducted pure culture studies in continuous flow units under saturating light intensity (1000 ft-c) using sterile filtered sewage supplemented with 0.0 per cent, 0.03 per cent, and 0.1 per cent CO<sub>2</sub> air mixture. After the cultures had attained equilibrium, the dissolved volatile solids and BOD in the influent and effluent supernatant were determined. Any change in the concentration of these parameters was attributed to algal metabolism. They concluded that cultures of Chlorella pyrenoidosa having detention periods of

less than three days did utilize some of the organic matter present in sewage, and that older cultures (detention times of more than three days) excreted organic matter into the medium. The design of the experiment did not provide an ideal test, since provision of  $\text{CO}_2$  and light energy led to photosynthetic growth which introduced an unnecessary complicating factor. Hence, the experiments with 0.03 per cent and 0.1 per cent  $\text{CO}_2$  air mixture do not merit consideration with respect to assessing the utilization of organic substances by the algae. In the figures shown some points were not plotted at all, and some were incorrectly plotted. Considering the series with no addition of  $\text{CO}_2$ , the BOD and dissolved volatile solids values were less than the sewage value only for the cultures of one-day detention periods. For all other cultures, these values were equal to or more than the feed sewage. Furthermore, in the cultures having detention periods of more than two days, the BOD, dissolved volatile solids, and packed cell volumes were all increasing. The authors did not explain how this could happen. From a scrutiny of the basic data it seems that the reduction of alkalinity in these systems was the explanatory factor for the above phenomenon. The increase in pH gives additional support for this surmise. Because of the above criticism, the authors' conclusion about the utilization of organic matter would appear to be based upon inadequate data, and therefore their conclusions are of questionable value. However, their conclusion that the increase in the

supernatant BOD was due to secretion of organic substances by the algal culture of higher detention periods is supported by their data.

Pipes (48), the senior author of the paper discussed above, possibly was not certain of the findings. He later designed an investigation "to provide information which might indicate if stabilization pond algae do actually assimilate organic matter from waste waters," and other related conditions. He used the same organism, Chlorella pyrenoidosa, cultivated in an organic medium enriched with 0.03 per cent and 1.0 per cent CO<sub>2</sub> air mixtures under light. Growth rates in media with and without many organic substances were determined. The difference in the growth rates was attributed to the organic matter added. The increase in growth rates was more pronounced in systems with 0.03 per cent CO<sub>2</sub> than in the 1.0 per cent system. In the latter, the increase in growth rates was observed with organic nitrogen sources such as dilute urea. Pipes fractionated sewage into six parts, as shown in Table I, and tested them separately. The water-soluble fraction increased the growth rate. Here, also, the increment of growth was more apparent in the system aerated at the low CO<sub>2</sub> tension.

Solook (49) studied the responses of a mixed algal culture (predominantly Chlorella) to glucose. He developed the algal culture photo-autotrophically, added glucose, placed the culture in the dark, and tested for glucose

removal. He added no bacteria to the system, but did not use aseptic techniques. He stated that "the only bacteria present were those occurring as natural contaminants in the system." He noted an initial lag period in substrate removal. He attributed this lag 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 explanation, as given below, seems possible. The lag represented the time taken by the very few contaminating bacteria in the culture to multiply and attain a number sufficient to exert an observable utilization of the substrate. This surmise can be supported by Solook's statement that "in all systems there was a good correlation between the biological solids concentration and the viable bacterial counts." His observation indicated that bacterial growth occurred in all systems; therefore, the substrate removal which was observed could have been due entirely to bacterial growth from an initially very small population (which would account for the apparent lag in substrate removal).

In reviewing the literature on heterotrophic metabolism of algae, attention has thus far been focused on utilization of organic substrates without special attention to algal respiration. However, algal respiration, and in particular endogenous respiration, and the effects of various factors (e.g., intensity of light, organic

substrate, etc.) on them are important considerations which require further discussion in regard to depletion of the dissolved oxygen resource.

Gibbs (50) assembled and assessed the literature on algal respiration. Discussing the effects of added substrates on endogenous respiration, he pointed out that studies employing manometric techniques yielded evidence that the endogenous respiration is suppressed, whereas studies using isotopically labeled cells indicated that endogenous respiration is either unaffected or is somewhat stimulated. He concluded that "the effect of an external oxidizable substrate on the endogenous respiration is thus still unresolved. It is not unlikely that the situation may differ from one species to another, or within one species, according to the physiological state of the cells."

Solook (49) added 50, 100, and 125 mg/l of glucose to a photosynthetically grown Chlorella pyrenoidosa culture, and followed oxygen uptake in a Warburg respirometer. He calculated values of  $RO_2$ , rate of oxygen uptake in milligrams of oxygen per hour per gram of solids, and found that they were approximately equal for all three loadings. For the limited purpose of comparing the  $O_2$  uptake for the different substrate levels, the above unit of expression is quite sufficient. However, the unit implies that the oxygen uptake curve more or less followed a straight line, i.e., zero order kinetics, during the whole period for which the rate was calculated. The oxygen uptake curves were not

shown for these particular experiments; however, for other similar experiments the  $O_2$  uptake curves were shown. The uptake rate was higher at first, and then (probably after exhaustion of the substrate) reduced considerably. The values of cumulative oxygen uptake (mg/l) for a system with 150 mg/l glucose as taken from his Figure 10 were 37, 64, 83, 90, and 97 at 20, 40, 60, 80, and 100 hours. The increments of oxygen uptake were 37, 27, 19, 7, and 7 mg/l for each twenty hours. It can be seen that the uptake rate reduced considerably after sixty hours. This reduction in the  $O_2$  uptake rate  $RO_2$  (mg  $O_2$ /hr/mg solids) would be more pronounced when the increase of the solids in the system was taken into consideration. The results would have been more meaningful had the  $RO_2$  been calculated for the two portions of the curve, namely, for the periods during the active utilization of the substrate and after the apparent exhaustion of the substrate.

Allen, et al. (51) and Fitzgerald (52), using Chorella pyrenoidosa (Wis 2005) concluded that the respiration value depended on cell age and the nature of the culturing medium. Kutyrin, et al. (53) working with Chlorella and Elodea found that the respiration rate in the dark was directly proportional to the oxygen concentration. However, Myers (54), experimenting with Chlorella pyrenoidosa (Emerson's strain), found that the time for utilization of glucose was unaffected by the simultaneous occurrence of photosynthesis or by illumination in the

absence of carbon dioxide. The data of Ludwig, et al. (30) showed that the respiration (unit  $O_2$  uptake) of Euglena gracilis decreased as the retention period in a continuous flow unit was increased to fourteen days. At detention periods greater than fourteen days, algal respiration gradually increased, and finally, at a retention period of twenty-one days, it was equal to that of the three-day retention period.

#### Excretory Products

Excretion of organic substances into the medium by algae adds an additional load to the oxidation pond, and could adversely affect the efficiency of the system. The excretion of organic matter works in opposition to the heterotrophic properties of algae in oxidation ponds. Estimation of the quantity and the identification of the excretory products and their possible effects on the purification process is reviewed in some detail below.

Tolbert and Zill (55) made studies on Chlorella pyrenoidosa using  $C^{14}$  as a tracer. They found that glycolic acid, amounting to three to ten per cent of the total  $C^{14}O_2$  fixed, was excreted into the medium during short term photosynthesis. The concentration did not increase beyond 3-9 mg/l. This glycolic acid was re-absorbed by the cells, when the bicarbonate fixation stopped. They postulated that a bicarbonate-glycolate anion equilibrium existed across the cell membrane. Since this was a pure culture study, there was no possibility for





eighteen species examined. Fucose, xylose, and glucose were components of the polysaccharide in some systems.

Conducting investigations with a thermophilic strain of Chlorella pyrenoidosa and a mesophilic culture of Chlorella vulgaris, Maksimova, et al. (58) studied the phenomenon of secretion of organic substances. They also found that the higher the yield of algae, the greater is the quantity of material excreted into the medium. The two species tested behaved in the same manner.

Comparing the works of Maksimova, et al. (58), Lewin (57), and Allen (56) there were two significant differences in their findings. One difference pertained to variation in the percent of soluble organic material elaborated per unit of cell mass in a given time. Maksimova, et al. found that this value was approximately thirty per cent during the first two days, and decreased to five to ten per cent thereafter. The data of Allen and Lewin are not sufficient for an appraisal of the above change with respect to time. The indicated data did not show any significant variations. Whether this difference can be attributed to the difference in the genera Chlorella and Chlamydomonas or to any other reason is not clear.

Another difference was related to the light intensity. Maksimova, et al. concluded that light intensity had no appreciable effect on the quantity of excretory products in contrast to Allen's finding of increased quantity under higher light intensities. Both conclusions were based upon

observations at only two light intensities. Maksimova, et al. used 5000 lux (500 ft-c) and 10,000 lux (1000 ft-c), whereas Allen used 25 ft-c and 750 ft-c. There was no appreciable change in the first case, and the change in the second was six-fold. From this limited data it seems that the process of excretion, too, may have a limiting light intensity at or below 500 ft-c. If one takes as the limiting light intensity a value at or near 400 ft-c, the effects observed below 400 ft-c will be dependent on light intensity, whereas the effects observed above 400 ft-c will be independent of light intensity.

Maksimova and Pimenova (59) extended the above study, and determined qualitatively the composition of organic matter excreted by the algae. They did not find any nitrogen-containing compound in the filtrate. The constituents of the water-soluble polysaccharides were galactose, mannose, arabinose, xylose, ribose, fucose, and rhamnose. In addition, traces of glucose, fructose, and sucrose were detected.

Merz, et al. (60) assayed the extracellular products of twenty-two species of Chlamydomonas, Chlorella, and Scenedesmus. Of these, six were isolated from oxidation ponds. The CO<sub>2</sub> in the compressed air (0.03 per cent) was the carbon source. The COD of the supernatant medium was determined. Separation and qualitative assessment of products were made by paper chromatography. The COD of the soluble extracellular organics of ten systems was greater

than 100 mg/l, and in one system the COD was approximately 500 mg/l after twenty-four days. The average daily increases in COD concentration for Chlorella pyrenoidosa, Chlorella vulgaris, and Chlorella miniata were 5.43, 4.19, and 14.86 mg/l, respectively. These values for two species of Chlamydomonas were above 5 mg/l. The identified products included galactose, fucose, ribose, glucose, malic acid, oxalic acid, and glycolic acid.

Surprisingly, Merz, et al. 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." Many works, including their own, had shown that excretory products are of considerable quantity and of wide variety. It is quite reasonable to expect that these will have a significant effect on the efficiency of the pond; however, they did not conduct any experiments to arrive at their conclusion. Apparently they felt that 100 mg/l additional COD was an insignificant amount.

The growth of several microorganisms in cultures of Chlorella pyrenoidosa to which no organic substrate was added was studied by Vela and Guerra (61). The algal population and the "contaminating" (added) bacterial numbers were counted. The proliferation of bacteria was observed only when the algae were actively dividing. The organic compounds in the medium were fractionated, and they were tested in the Warburg respirometer for capability of supporting bacterial growth. The results showed that these

organics supported growth of bacteria, the rate being different for different species of bacteria. Ninhydrin-reactive materials and organic acids were detected by chromatographic analyses. The accompanying mass culture study indicated various types of contaminant bacteria, each type predominating at different ages of the algal culture.

### Inhibition

Retardation and inhibition of algal and bacterial metabolism may occur due to compounds present in oxidation ponds, resulting in decreased efficiency. Such compounds may be either brought into the ponds in the influent itself, or produced by microbial metabolism. Since, in the present research, the inflow quality was the same except for small changes in the components of the tap water, discussion is centered on the second source, namely, inhibition by excretory products of the microbial population.

Pratt and co-workers studied the growth of Chlorella vulgaris under continuous lighting in a nutrient medium, through which a five per cent CO<sub>2</sub> air mixture was bubbled. Their data indicated that: (1) the cells excreted a substance which retarded their own growth (growth was measured by increase in cell numbers) (62), (2) the growth-retarding substance, designated as Chlorellin, exhibited antibacterial properties against five tested bacteria, as seen by the zones of inhibition in the standard cup assay (63), and (3) the concentration of Chlorellin was at a minimum during rapid growth (64).

Shilo (65) reported that synthesis of toxic substances in Prymnesium parvum was greatest during the late log growth and stationary phases. Karlander and Krauss (66) attempted to determine the reasons for absence of growth of Chlorella vulgaris in the dark in the presence of glucose. Noting a decrease in pH of approximately two units, they suspected the excretion of organic acids. By gas chromatographic analysis of the supernatant, they identified acetic acid and formic acid as two of the components excreted in the dark. However, further experiments indicated that secretion of acid was one of the results of inhibition, and not the cause of it.

Pipes (48) measured the growth rates of Chlorella pyrenoidosa (cultured photosynthetically) in media enriched with various organic substances, including formic and acetic acids at three concentrations each. The specific logarithmic growth rates ( $k_g$ , day<sup>-1</sup>) are given below:

<u>Compound</u>	<u>Concentration</u>			
	0	10 <sup>-3</sup> M	10 <sup>-4</sup> M	10 <sup>-5</sup> M
glucose	1.400	1.490	1.460	1.465
formic acid	1.390	1.015	1.344	1.385
acetic acid	1.410	1.180	1.380	1.390

It can be seen that formic and acetic acids did have a retarding effect at the higher concentrations employed.

Leone (67) conducted an experiment for seventy-two days with Chlorella pyrenoidosa strain 7-11-05 in which the medium was recycled. He concluded that even though some

amount of autotoxic substances might have been produced, no buildup of them was noticed.

Oswald, et al. (31) found the BOD values of the supernatant from a Chlorella culture were higher than those of the supernatant from an Euglena culture. They attributed this result to the lower bacterial population in the former system. Their data are given below:

<u>System</u>	<u>Bacterial Colonies per ml</u>
1. Sewage + bacteria	$1 \times 10^8$
2. Sewage + bacteria + <u>Euglena</u>	$1 \times 10^7$
3. Sewage + bacteria + <u>Chlorella</u>	$1 \times 10^6$

As an explanation for the lower bacterial count in system 3, they reasoned that "some factor inhibits bacterial action upon the sewage substrate while the Chlorella cells are present." While the given explanation is quite possible, another reason also seems plausible. When there are two types of organisms capable of using the substrate in the system, there will be competition between them for the substrate utilization. The ultimate population reached by either type of organism in the combined system will be lower than that of the population attained by each organism when it is present alone. Substantiation for this line of reasoning is obtained by comparing systems 1 and 2, or 1 and 3. It is normal to expect that faster-growing organisms will attain higher numbers in the combined system; however, the numbers reached by the faster-growing organisms depend upon the relative growth rates of the two types

of organisms. Euglena has a lower growth rate than that of Chlorella. This fact was recorded by the authors themselves in another paper (30). As a hypothetical example, if Euglena had utilized twenty per cent of the substrate during the experimental period, Chlorella would have used forty per cent of the substrate, leaving eighty per cent and sixty per cent for the bacteria in systems 2 and 3, respectively. This would lead to a lower bacterial population in systems 3 than in system 2.

Discussing the reason for occasional low BOD removals in high rate ponds, Oswald, et al. (68) suspected the existence of higher pH values as the major reason. This explanation may be plausible and it could have been confirmed had the authors given data correlating pH values and BOD removal. Fitzgerald, et al. (69) studied the toxicity of approximately three hundred organic compounds on Microcystis aeruginosa, a bloom-producing species. Gloyna and Thirumurthi (70) tested the toxic effects of about sixty organic chemicals on Chlorella pyrenoidosa. The general pattern indicated by their results was that the straight chain compounds were more toxic than corresponding branched chain compounds. Among the straight chain fatty acids, those with odd numbers of carbon atoms possessed greater toxicity than those of even number. Furthermore, they found that the toxic effect of some chemicals can be nullified by certain inactivating chemicals.

Kott, et al. (71) studied the inhibitory effect of



chlorine and bromine on Chlorella pyrenoidosa. At a concentration of 0.4 ppm, both exhibited algicidal properties; the effect of bromine was greater than that of chlorine. This indicates that the presence of these residual halogens in the influent to an oxidation pond will affect the photosynthetic oxygenation process.

#### Efficiency of Oxidation Ponds

All of the factors and processes hitherto discussed in detail, and others, such as the type of waste, temperature, and bacterial metabolism, can be expected to affect the overall efficiency of oxidation ponds. The performance of the pond can be evaluated by employing various parameters, namely, oxygen demand satisfied, solids reduced, nutrients removed, etc. The selection of a particular parameter depends upon the purpose for which the pond is intended. Most oxidation pond installations are intended as secondary treatment devices. In those, reduction of oxygen demand is the usual, though not the exclusive, yardstick of measurement for efficiency.

Organic loading is reckoned by two parameters: BOD and COD. While it is not the intention of the author to discuss the advantages and limitations of these two parameters, some mention of the relation between them, with particular reference to oxidation ponds, is warranted. BOD is monitored extensively in the field, since these data are usually required by the regulatory agencies, while COD is used in an increasing number of cases in laboratory studies.

To compare studies using these two different parameters and to extrapolate the findings of numerous laboratory studies to the field conditions, correlation between BOD and COD would be quite useful.

Ballinger and Lishka (72) evaluated the BOD and COD of a sample containing glucose and glutamic acid (1.6 grams each in one liter) with a theoretical oxygen demand of 308 mg/l. The 5-day BOD value was 186 mg/l, and the COD was 281 mg/l; i.e., the BOD<sub>5</sub> was sixty-six per cent of the COD: however, this ratio is not a constant one. It is not only different for different wastes, but varies with the relative proportions of the constituents at various times within a particular waste. Loehr (73) determined the oxygen demand (BOD and COD) of the effluent from a lagoon treating animal feedlot wastes:

BOD <sub>5</sub> (mg/l)	1340	1420	1930
COD (mg/l)	4700	5500	7400
BOD <sub>5</sub> /COD (%)	28.5	25.8	26.1

McKinney (74) reported on the performance of an aerated lagoon treating predominantly domestic wastes. The median values of the parameters were:

	<u>Influent mg/l</u>	<u>Effluent mg/l</u>	<u>Reduction %</u>
BOD <sub>5</sub>	125	26	80
COD	360	165	54

The difference in the expression of performance was due to the difference in the BOD/COD ratio of the influent and effluent, which were 34.7 per cent and 15.7 per cent,

respectively. In an oxidation pond used in Kansas for the treatment of oil refinery wastes (75) the oxygen demand removed during summer and winter were eighty-three per cent and fifty-two per cent on the basis of BOD, and sixty per cent and thirty-eight per cent on the basis of COD. The percentage removal of oxygen demand in an oxidation pond receiving influent from trickling filters is given below (76); the determinations were made on seven days in a period of ten months:

As BOD	50	20	58	50	59	44	68
As COD	-2	2	0	22	7	7	39

The absolute COD values for the first reading giving negative reduction were 3232 and 3295 for influent and effluent, respectively, and showed an increase of 63 mg/l in the pond.

Results of a pilot plant study employing pasteurized partially skimmed milk as substrate have been reported (9). The detention time was ten days for these (semi-continuous) fill-and-draw type units, which were fed intermittently on alternate days. The 5-day BOD of the pond water immediately after a feeding ( $BOD_i$ ), and just before the next feeding after two days ( $BOD_e$ ), and the corresponding percent removals between feedings ( $R_B$ ) were as follows:

$BOD_i$	49	84	56	56	76	82	59	60	74	95	70
$BOD_e$	56	24	37	58	60	48	42	58	76	49	66
RB %	-14.3	71.4	34.0	-3.6	21.0	41.5	28.8	3.3	-2.7	48.5	5.7

The average BOD reduction in the test period of twenty-two days was only 21.2 per cent. The reduction, had it been

expressed on a COD basis, would have been still less (possibly negative).

Oswald, et al. (31) presented data on  $BOD_5$  of the influent sewage, the unfiltered effluent, and of the culture supernatant, at various retention periods. The whole effluent  $BOD_5$  increased with time of retention; for 1-day cultures it was about ninety per cent of the influent sewage, equal to the influent  $BOD_5$  for 2-day cultures and almost double in 7-day cultures. However, the BOD of the clear supernatant had a value of about forty-five per cent of the influent.

Pipes (77) studied BOD removal in laboratory stabilization ponds operated at various levels of pH, using synthetic media. The results showed two opposing trends of BOD reduction as a function of detention periods. When the pH was above or equal to 8.0, the BOD reduction increased with increased detention time, but when the pH was below or equal to 7.5, the effluent BOD increased with additional retention periods. It should be noted that in a system of given total alkalinity, an appreciable quantity of free  $CO_2$  is present when the pH is approximately 7.5 or lower.

Hermann and Gloyna (78) investigated two identical laboratory model oxidation ponds exposed to outside weather conditions in Austin, Texas. The daily batch feeding in one of them was double that of the other, resulting in a detention time of ten days in the former and twenty days in the latter. The BOD removals were ninety-eight per cent

and ninety-three per cent in low and high detention ponds, respectively. The authors noted this, and stated that "this phenomenon may be attributed to a considerable increase in algal population." Though not complete, their reasoning seems to be correct. The increased algal population in ponds with higher detention periods would have assimilated more  $\text{CO}_2$  from the atmosphere, and presumably would have secreted more organic substances. Further argument against the use of high detention times may be provided by analysis of the pond system treating oil refinery wastes referred to earlier (75). This pond system consisted of several ponds in a series, and it was possible to calculate BOD removal at various detention times. The maximum reduction in BOD occurred at approximately 20-day detention time, after which the effluent BOD increased. The authors indicated that "peak algal populations occurred in this region, and it is probable that the apparent BOD reduction rate was diminished after this time by the addition of organic compounds from algal decomposition." Some decomposition along with the excretion of oxidizable material could have caused the BOD increases which were observed to occur.

Wu (79) studied the effect of various organic loadings in laboratory oxidation ponds operated with detention periods of ten and twenty days. He concluded that the maximum allowable loading was near 62 lb/COD/acre/day for the maintenance of aerobic conditions and for reasonably good COD

removal efficiency. In his investigation he conducted two experiments with equivalent loading at the different detention periods, i.e., one with 300 mg/l at ten days detention, and the other with 600 mg/l at 20-day detention time. The percentage COD removal was greater in the 10-day detention pond (300 mg/l) than in the 20-day detention pond (600 mg/l). Furthermore, the "steady-state" dissolved oxygen levels were 4 mg/l in the pond with the shorter detention period (10 days) and 1 mg/l in the pond with the longer detention time (20 days).

## CHAPTER III

### THEORETICAL CONSIDERATIONS AND EXPERIMENTAL APPROACH

From the review of the literature it is apparent that for many cases the  $\text{CO}_2$  concentration was the growth-limiting factor for the algae (30) (31) (32) (33). In many instances the  $\text{CO}_2$  produced by bacterial metabolism might be expected to increase the  $\text{CO}_2$  concentration to values in excess of those needed for algal growth. However, this active bacterial oxidation occurs, in general, in the influent end of the pond. The excess  $\text{CO}_2$  produced can escape to the atmosphere, and hence all bacterial  $\text{CO}_2$  might not be fully available to the algae. Furthermore, it was seen that the minimum concentration of organic substances occurs in the middle of the pond, and the BOD often increases as the waste water progresses through the pond (75) (78). It was pointed out that the probable reason for this phenomenon is the continued growth of the algae and subsequent excretion of organic substances by them.

The oxygen balance could be improved by the increased algal utilization of  $\text{CO}_2$  produced by bacteria near the influent end of the pond and the increase of the BOD in the effluent end of the pond could be reduced by bringing these two phases together, i.e., possibly by more intimate

mixing. Without more intimate mixing, there would always be the tendency for bacteria to predominate where the organic substrate concentration is higher, namely, at the influent end, and for the algae to be in abundance in areas of less turbidity, (hence more light) at the effluent end. Any engineering expedient to counteract this natural separation and to keep these two phases together by supplying mixing energy results in additional expense. Also, mixing, if too turbulent, would strip  $\text{CO}_2$  and  $\text{O}_2$ , thus militating against its possible advantages. It is possible that the desired advantages could be achieved by an alternate means. For example, if a pond were closed to the atmosphere (but not shielded from light), the super-saturation of  $\text{CO}_2$  and  $\text{O}_2$  could not be relieved by escape to the atmosphere. This concept for oxidation pond operation might be practically engineered by employing a transparent covering material such as polyethylene. It seems possible that the advantage of the "closed oxidation pond" with respect to oxygen transfer might even outweigh the advantages with respect to  $\text{CO}_2$  tension. The rates of oxygenation and deoxygenation depend on the difference of  $\text{O}_2$  concentration in the pond and in the atmospheric air. In an aerobic oxidation pond the DO in the early morning hours would be near zero. The maximum oxygen gain to the pond is due to a driving force equal to the solubility of  $\text{O}_2$  in the medium, i.e., approximately 8 mg/l. It is quite common to encounter DO concentrations of 20 mg/l and above during the afternoon



hours. Some of this excess (around 12 mg/l) dissolved oxygen is lost, due to stripping, relieving the supersaturation. On the whole, the open pond may therefore lose oxygen to the atmosphere, a situation which could be prevented in a closed pond. Furthermore, the curtailment of evaporation loss in the closed pond would be an additional advantage in areas where water is scarce and the stream flow consists largely of the pond effluent.

Thus far in this development of some of the theoretical concepts pertinent to oxidation pond operation, it has been assumed that algal aeration is more critical than  $O_2$  transfers across the liquid-atmospheric interface. Also, it has been assumed that transfer of  $O_2$  to the atmosphere takes place at a rate approximately equal to transfer into the liquid. The same line of reasoning has been assumed for  $CO_2$ , although the problem here is not so critical because of the carbonate balance reactions which, depending upon the pH, may entrap some  $CO_2$ . In addition, the driving force for transfer of  $CO_2$  into the liquid is very small, and one need not consider  $CO_2$  from the atmosphere as a major contributor to the  $CO_2$  pool.

An experimental verification of the hypothesis that an open pond may lose excessive amounts of oxygen to the atmosphere leads to the need for determination of reaeration rates for the experimental ponds. Recently, Isaacs and Gaudy (80) have shown the necessity of determining the true saturation values for obtaining correct  $K_2$  rates. Hence,

in addition to the measurement of the apparent saturation values, a check by the method described by Isaacs and Gaudy (81) would provide a useful tool for the present research. For the determination of the saturation values in the reaeration experiments, the equations of Isaacs and Gaudy given below could be used directly:

$$\alpha = \frac{(D_1)(D_2) - (D_3)^2}{D_1 + D_2 - 2D_3} \quad (3)$$

$$C_s = C'_s - \alpha \quad (4)$$

where

$C_s$  = the true saturation value

$C'_s$  = assumed saturation value

$\alpha$  = error in the assumption, or the correction factor

$D_1, D_2, D_3$  = apparent deficits at times  $t_1, t_2$ , and  $t_3$

when  $t_3 = \frac{t_1 + t_2}{2}$

However, for the determination of the saturation values in deaeration experiments, only Equation (3) can be used directly, and Equation (4) must be modified as follows:

$$C_s = C'_s + \alpha \quad (5)$$

While at first sight this may seem contradictory, the following analysis will show the equivalence of both equations. Consider two hypothetical systems having the same  $K_2$  rates. One system is reaerating, and its true saturation value is 8 mg/l. The other one is deaerating, and its true saturation value is 10 mg/l. Figure 2 shows the reaeration and

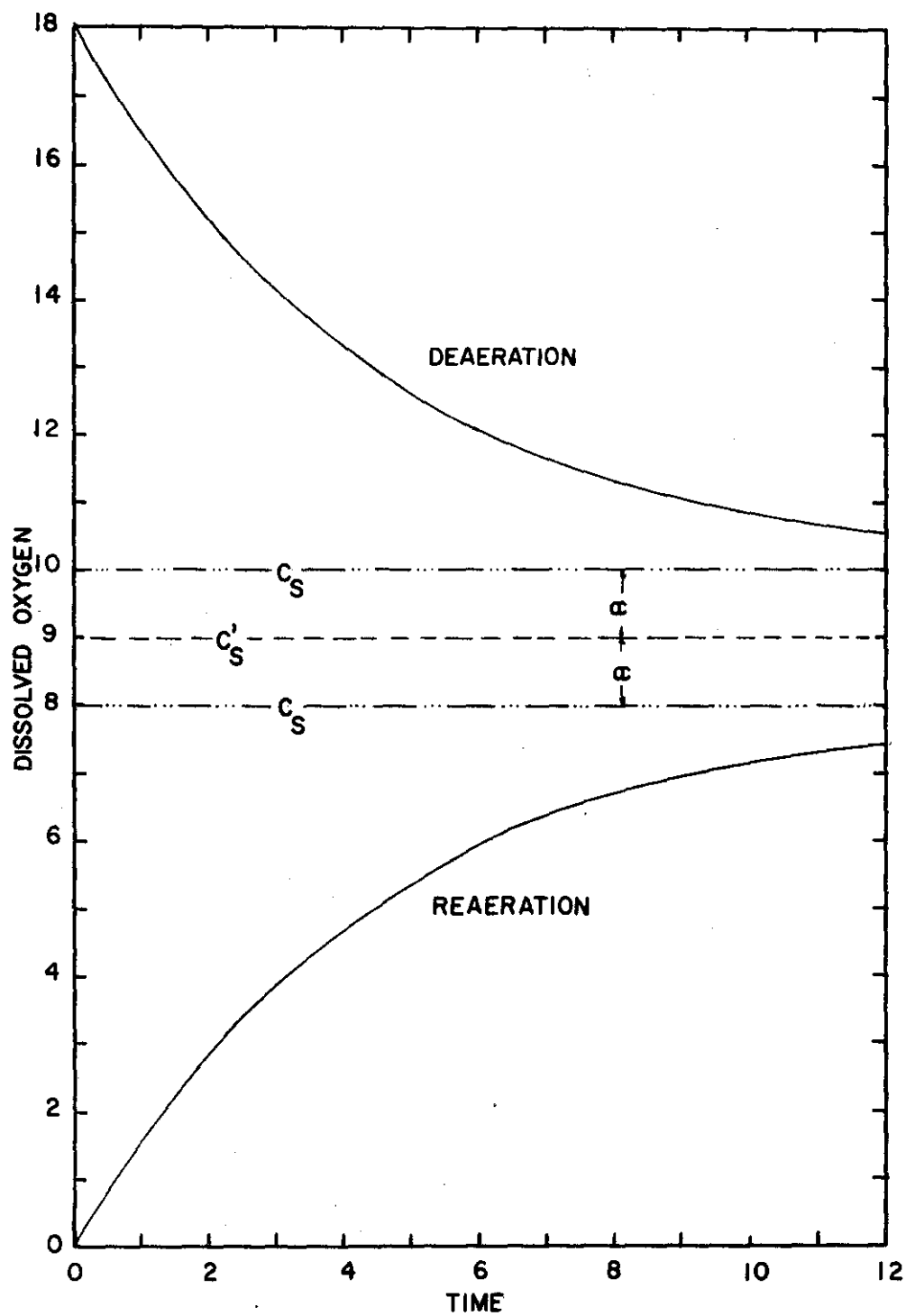


Figure 2 - Reaeration and deaeration curves of two hypothetical systems of the same  $K_2$  rates.

deaeration curves for the systems. Let the assumed saturation value for both the systems be 9 mg/l. The value of  $\alpha$  which could be calculated using Equation (3) is 1. To get the saturation values, this must be deducted from the assumed value of 9 mg/l in the reaerating system, whereas it must be added for the deaerating system.

The correction factor  $\alpha$  is the distance of the asymptotic line from the line of assumed saturation, and represents the difference in DO levels between the true and assumed values. The value of  $\alpha$  will be positive when the two lines do not cross, and negative when they cross. The reference point is the line of assumed saturation for both the deaerating and reaerating systems. However, the true saturation levels of the two cases lie on opposite sides of the reference line. Hence, the sign change in Equations (4) and (5). This change in sign can also be explained by taking into account the direction of measurement. The Y axis, on which DO concentrations are plotted, is positive upward. The deficits in the reaeration curve are measured downward from the saturation line, whereas the excesses in the deaeration curve are measured upward from the saturation line. In one case (deaeration), the differences in concentration are measured along the direction of the axis, and in the other (aeration) they are measured against the direction of the axis. Hence the sign of  $\alpha$  is different in the two cases.

In oxidation ponds a steady state condition with

respect to oxygen concentration (in the strict sense of the word) cannot be achieved, since the light intensity (an energy yielding "substrate" for phototrophic organisms) is subject to constant change. Nevertheless, since the light and dark periods alternate with some degree of regularity, a cyclic pattern, increasing during the day and decreasing during the night, can be expected. In laboratory experiments where the factors such as the influent, detention time, and lighting can be controlled, this regularity can be made more rigorous.

In such controlled studies it might be expected that the system would be approaching a "balanced condition" wherein the DO increase during the light period might be more or less equal to the DO decrease during the dark period. The fluctuation in DO might be represented as shown below:

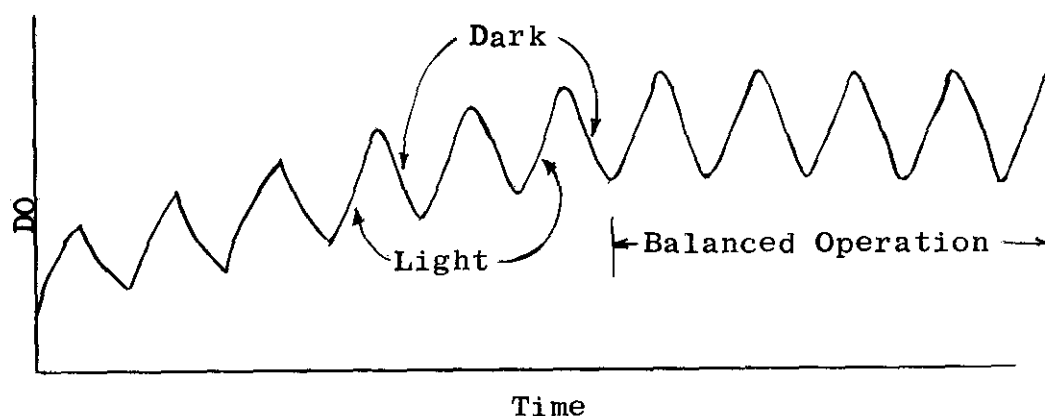


Figure 3 - Dissolved oxygen in a pond under balanced operation.

This figure is intended to show a general pattern, and does not necessarily represent the kinetic mode of change during the light and dark periods.

### Experimental Approach

The dissolved oxygen concentration present in oxidation ponds is a result of two groups of opposing forces, one adding to and the other deleting from the oxygen resource. The significant processes tending to increase the DO are the physical transfer of oxygen from the atmosphere into the pond where the DO in the pond is below saturation and the photosynthetic oxygenation by algae during the light period. In the other group, which reduces the DO concentration, the most important processes are utilization of oxygen by the organotrophic bacteria, the possible organotrophic metabolism of the algae, and the loss of oxygen by stripping during the period of supersaturation.

The experimental approach taken in the present research was one which attempted, insofar as possible, to assess the course of each process independently. In order to gain deeper insight into a few of the constituent processes, additional investigations were made. To relate the findings to previous studies on oxidation ponds in the bio-environmental engineering laboratories of Oklahoma State University by Wu (79), substrate loadings employed in these studies were in the general range of those employed therein. During the course of the present research effort, some experiments were conducted in laboratory oxidation ponds of

geometric configuration similar to that used by Wu in his study of organic loadings.

## CHAPTER IV

### MATERIALS AND METHODS

#### A. Development and Description of the Equipment

The experimental equipment used in this investigation consisted of the Warburg apparatus, BOD bottles, and laboratory oxidation ponds. The description of each and the phase of study for which they were used are given below.

Pond A (Figure 4A) was an aquarium tank of the following dimensions: 48.6 x 28.5 x 27.2 centimeters with a surface area of 1385 square centimeters. The sheet glass sidings were mounted in aluminum frames, and the top was open. The tank was used for preliminary studies; rubber tube syphons were used for sampling.

Pond B (Figure 4B) was devised as an improvement on the above pond. Entry or exist ports were fitted at three levels on the narrow sides; the tank was fitted with an airtight cover. A valve and a small opening in the cover, which could be closed to the air, provided for airtightness. This tank was used in deoxygenation studies employing bacteria alone (i.e., without the presence of algae).

Pond C (Figure 4C) was a rectangular plexiglass tank of the following dimensions: 50 x 30 x 30 centimeters. The corners of the tank were joined with brass countersunk



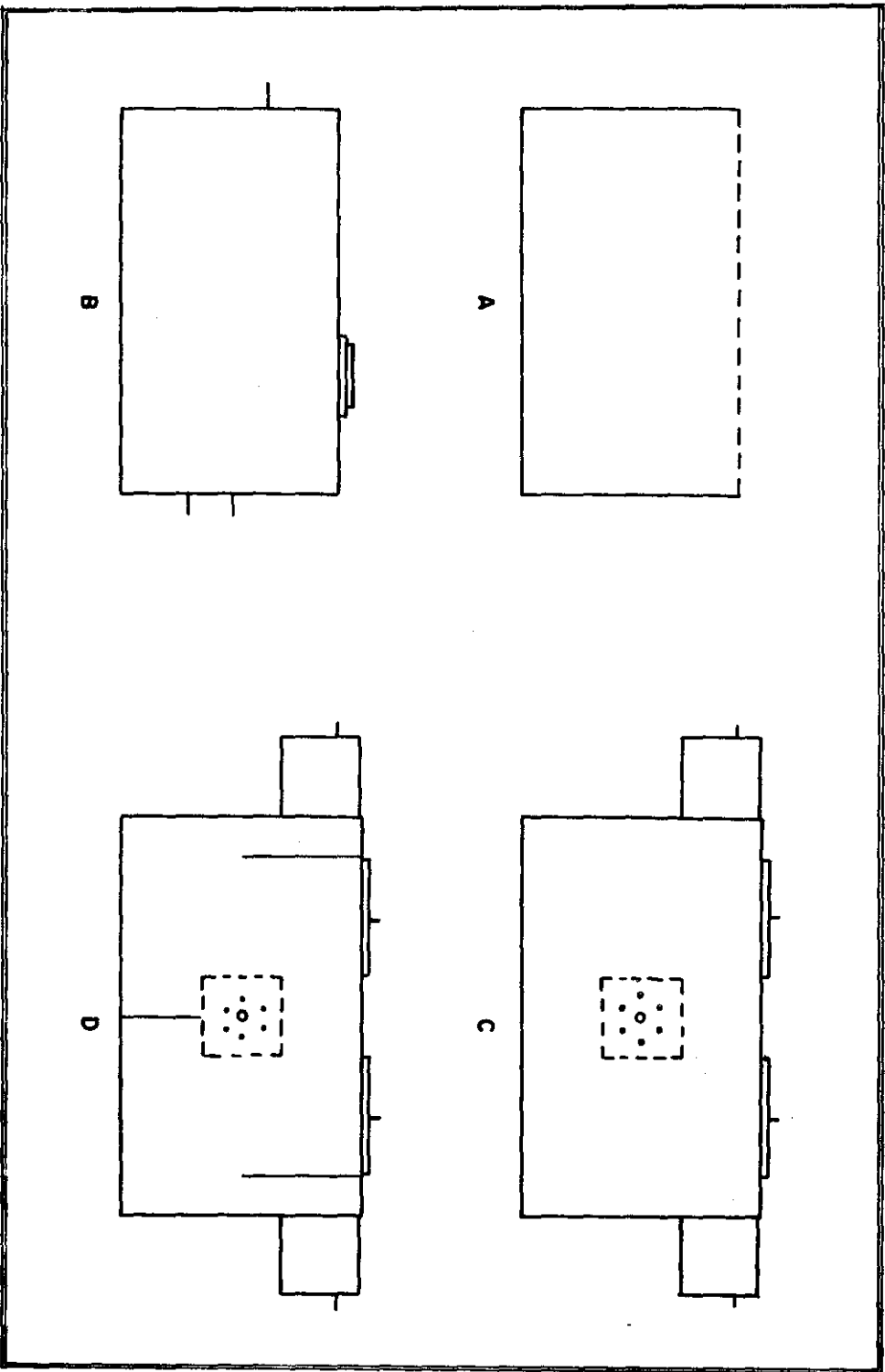
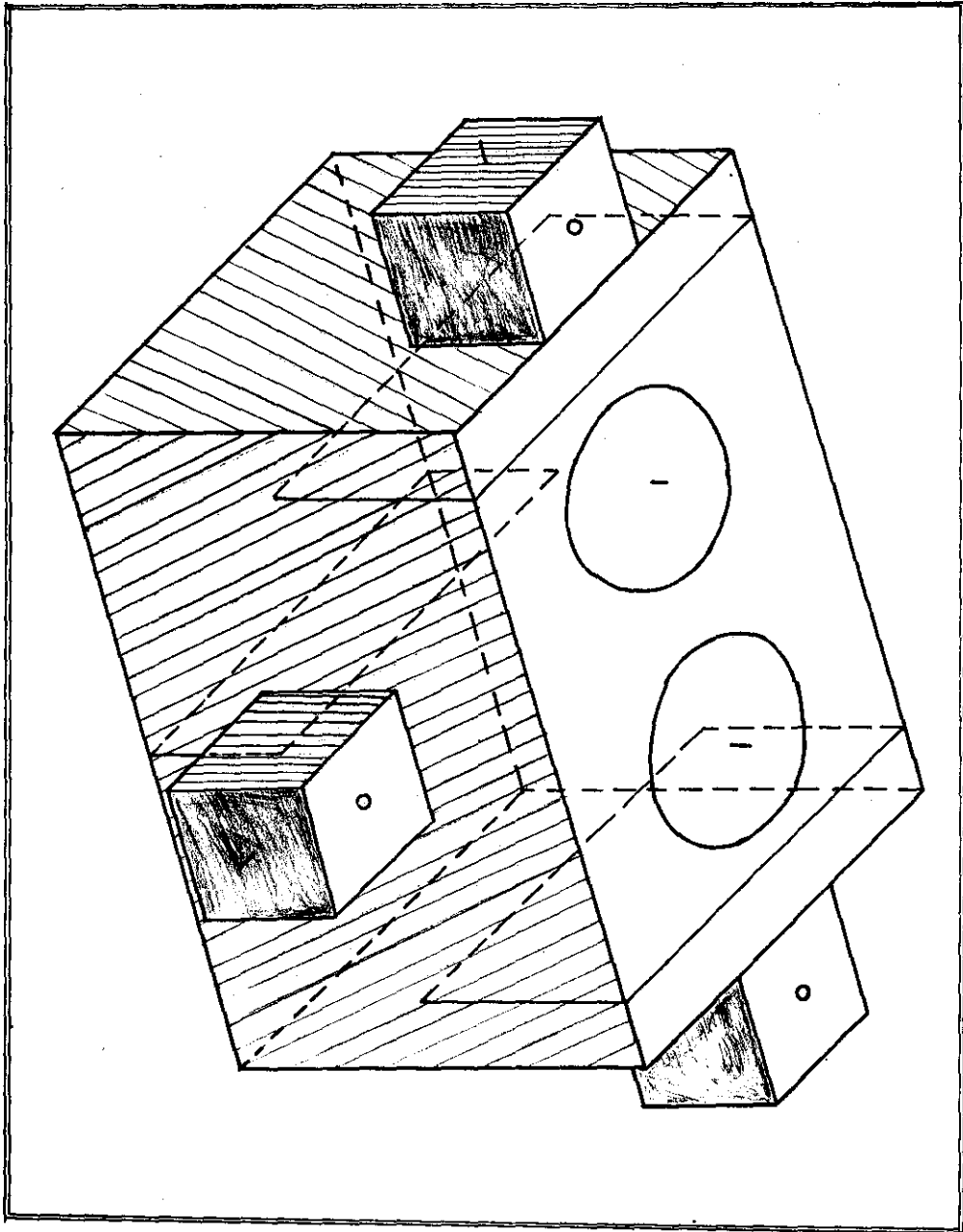


Figure 4 - Sketches of experimental oxidation ponds.

screws in addition to chemical bonding. There were three side wells 10 x 10 x 10 centimeters attached to three sides. Six holes, one centimeter diameter each, arranged in a hexagonal manner, connected these side wells to the main tank. The two side wells on the two narrow sides were fitted at the middle of the top edge, while the one on the broad side was attached at the center of the wall. All three side wells had an opening in the top, through which probes to register dissolved oxygen (electrometrically) could be inserted. In addition, the side wells were fitted with ports controlled by needle valves. Stirring magnets 2" x 3/8" were placed in all three wells. In the top of the chamber there were two 12.5" diameter openings. Lids to close these openings had valves in the center. A circular rubber ring positioned in a groove on the top surface of the tank and in the lid, and six tightening screws provided an airtight container. This tank was used in physical reaeration and continuous flow studies.

Pond D (Figure 4D and 5) was similar to C, except that it had three baffles in the main chamber. Two plexiglass baffles were attached to the top, parallel to the narrow sides, at a distance of five centimeters from each end. They extended to a depth of fifteen centimeters. Another one, ten centimeters high, was fixed to the base at the center, parallel to the other two. The provision of baffles eliminated any possibility of the formation of pockets. This tank was used in continuous flow and reaeration studies.

Figure 5 - Isometric view of experimental pond D.



## B. Seed

Oxidation ponds contain bacteria and algae of various species, and it would be ideal to use a heterogeneous culture of bacteria and algae. A bacterial seed was obtained from the effluent of the primary clarifier of the waste water treatment plant at Stillwater, Oklahoma, in addition, at times effluents from all of the experimental units in the bioenvironmental engineering laboratories at Oklahoma State University were mixed together and used for seeding. A heterogeneous algal population obtained from the botany department was used in the preliminary experiments. This seed caused practical (experimental) problems; the algal solids were not in uniform suspension. As a result, the reliability of the solids concentration determined by taking samples at particular locations in the pond was reduced. Furthermore, there was excellent opportunity for change in algal predominance during the long period of investigation. It was felt that heterogeneity in the bacterial population would provide ample variations. It was decided to use a pure culture algal seed which is commonly found in oxidation ponds, and which grew in even suspension.

Allen (32) reported on the specific kind and abundance of algae in sewage oxidation ponds at various localities in the State of California. Chlorella predominated under various operating conditions. Scenedesmus, Chlamydomonas, and Euglena were also found in considerable numbers.

Laboratory studies also confirmed the same trend. In Egypt a pilot plant oxidation pond study (9) was conducted for a period of five months. Chlorella, Scenedesmus, and Chroococcus were the abundant genera. Myers (82) made laboratory and field studies on fifteen sewage lagoons in Texas. He reported finding unicellular green algae and green flagellates in four ponds; colonial green algae, large spiral blue-green algae, and colonial blue-green algae in three. He did not identify the genera, but from their morphological description based upon microscopic examination, the predominant genera observed were probably Chlorella, Chlamydomonas, Scenedesmus, Spirogyra, and Gloeocarpa. Further evidence for the predominance of Chlorella in oxidation ponds is available in the literature (83) (74).

Shilo (65) reported that blue-green algae could be decomposed by a number of bacteria, while Chlorella was not affected by them. Holm-Hansen (84), giving reasons for selecting algal species for experimental material, stated: "Green algae such as Chlorella and Scenedesmus are small and unicellular, and can be readily grown in clonal culture, permitting work with larger populations which minimizes the effect of individual variation; a suspension of such algae can be pipetted accurately, and can be easily controlled in regard to temperature, light intensity, etc. Equally important is the fact that for these two genera there is an abundance of available

information on their growth and physiology."

Taking into consideration all of the above-mentioned information, it was decided to select Chlorella pyrenoidosa as the experimental alga. At the time of the present study, the agronomy department of Oklahoma State University was using Chlorella pyrenoidosa in a study to ascertain the effectiveness of some weed-controlling chemicals. They were not interested in keeping the culture pure. As such, it was possible that other species of algae might have been in their suspensions also. Seed was obtained from the agronomy department and maintained in the bioenvironmental engineering laboratory by periodic transfer into fresh medium. The seed developed in this way was used for all experiments except for those designated as pure culture studies, for which Chlorella pyrenoidosa obtained from the American Type Culture Collection was used.

### C. Medium

In most of the research with algae, the CO<sub>2</sub> was provided by bubbling a 5 per cent CO<sub>2</sub>-95 per cent air mixture through the culture. This CO<sub>2</sub> concentration is approximately one hundred sixty times the normal concentration in air. Bicarbonate was added to the medium as an additional source of carbon, since carbonates are normally found in oxidation ponds.

In order to gain insight into the concentration of bicarbonate to be added, two preliminary experiments were conducted in BOD bottles at two different initial DO levels.

Algal cultures were grown in medium containing different concentrations of  $\text{NaHCO}_3$ , varying from 0 to 500 mg/l, under light intensity of 80 ft-c. The DO and solid production indicated that a concentration of 100 mg/l would serve the purpose well.

Various workers have reported different media for growing Chlorella pyrenoidosa. Some of them, together with the medium used in the bioenvironmental laboratories of Oklahoma State University for activated sludge studies, were compared when fortified with 100 mg/l of bicarbonate. The composition of the modified media are given in Table II.

Six 4-liter volumetric flasks were used to grow the algal culture for this comparative study. One liter of each medium was used to which algal seed of 20 ml was added. The light intensity was 80 ft-c, provided by fluorescent lamps which were on at all times during the experiment. Samples were taken approximately once each twelve hours, and optical density was measured in Coleman (Model 6D) spectrophotometer at 540  $m\mu$ .

After 174 hours, the growth in media I and V was less than in the others, and they were discarded. In the second transplantation only media II, III, IV, and VI were tested. After 192 hours, the growth in media II and IV was lagging behind the other two (III, VI). Optical density measurement was continued for an additional 312 hours. The growth was far greater in medium VI. Another transplantation was made in media III and VI, and the growth was followed for

TABLE II  
COMPOSITION OF VARIOUS MEDIA TESTED

Constituents	Concentration - mg/l					
	I	II	III	IV	V	VI
NaHCO <sub>3</sub>	100	100	100	100	100	100
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	500	500	-	-	-	-
(NH <sub>4</sub> )NO <sub>3</sub>	-	-	1500	-	-	-
(NH <sub>4</sub> )Cl	-	-	-	50	-	-
NaNO <sub>3</sub>	-	-	-	1000	182	1000
K <sub>2</sub> HPO <sub>4</sub>	4280	4280	-	250	21.75	1000
KH <sub>2</sub> PO <sub>4</sub>	2108	2108	-	-	8.5	-
Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O	-	-	-	-	33.4	-
MgSO <sub>4</sub> ·7H <sub>2</sub> O	100	100	-	513	22.5	200
FeCl <sub>3</sub>	0.5	1.5	-	3	0.15	-
FeSO <sub>4</sub> ·7H <sub>2</sub> O	-	-	-	-	-	50
K <sub>2</sub> SO <sub>4</sub>	-	-	1500	-	-	-
MnSO <sub>4</sub>	10	10	-	-	-	-
MnCl <sub>2</sub> ·4H <sub>2</sub> O	-	-	-	-	-	2
CaCl <sub>2</sub>	7.5	7.5	-	50	27.5	20
NaMoO <sub>4</sub>	-	-	-	-	-	1
PH salt mixture-	-	-	1500	-	-	-
Tap water	100ml	100ml	100ml	100ml	100ml	100ml
Distilled water to make up to the required volume						

- I. Medium used in bioenvironmental engineering laboratories for activated sludge units
- II. Modification of I; FeCl<sub>3</sub> was increased to 1.5 mg/l from 0.5 mg/l
- III. Medium of Eyster, et al. used by Wu (81)
- IV. Allen's medium (51)
- V. Dilution water medium (51)
- VI. Medium recommended by Stanier, et al. (85).



287 hours. In this experiment, also, the growth was greater in medium VI, and medium VI was selected for use in further experiments.

#### D. Experimental Procedures

##### 1<sub>a</sub>. Continuous Flow Study with Algae and Bacteria (12 Hours Light, 12 Hours Dark)

Tanks C and D were used for this study; Figure 6 shows the experimental setup. A modified one-liter beaker was used as a constant head tank. A one-liter beaker was fitted with an outlet at the bottom on the side, and a glass tube of one inch diameter at the center on the bottom. The line from the feed bottle delivered medium to the annular space between the glass tube and the sides of the beaker. The outlet from the beaker served as the inlet for the reactor. When the feed level rose above the top of the central tube, the feed overflowed through the central opening to the waste collector. Thus, the head was always kept constant, causing an even flow irrespective of the level of the feed in the feed bottle. Lighting (overhead) was provided by thirteen GRO-LUX fluorescent lamps (Sylvania), 40 watts each, hung side by side. In addition to the reflectors in the fixtures, a sheet of cardboard wrapped with aluminum foil was placed on top of the fixtures. The light intensity at the top level of the reactor was between 425 and 475 ft-c. A 12-hours-on and 12-hours-off lighting cycle was employed.

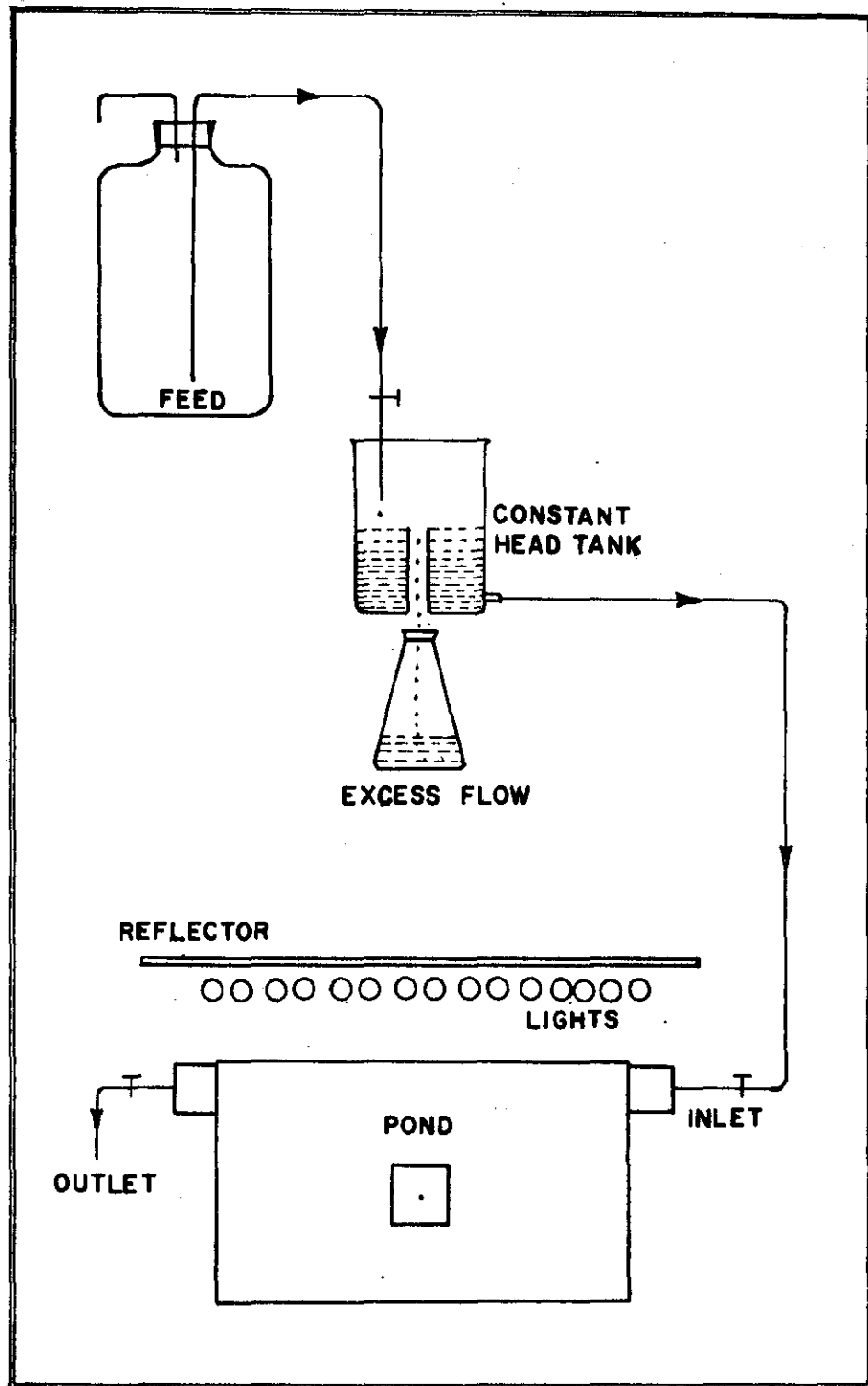


Figure 6 - Schematic diagram of experimental setup for continuous flow studies with algae.

The feed bottle, constant head tank, and the lines were autoclaved each time they were put into use. Generally, these were changed once in thirty-six hours. The effluent was collected and measured in order to have a check on the flow rate. Sewage and Chlorella pyrenoidosa were used as seed. After one week of operation, regular samples were taken at the beginning and end of the lighting period. Dissolved oxygen, solids, oxidation reduction potential, pH, and filtrate COD of the effluent were measured. The influent DO and COD were checked occasionally. The flow rate for a 10-day detention time in the closed system was 3.27 ml/min. At this rate it took about two hours to collect approximately 500 ml for DO determination. To avoid this problem, the rate of flow was increased during sampling. The valve in the outlet line was opened more to draw off more effluent. The decreased feed level in the constant head tank was brought to the rim of the central tube by withdrawing feed from the feed bottle.

Experiments were conducted with varying glucose concentrations with a detention time of ten days in two systems; i.e., one open to the atmosphere, and the other closed. A closed system with a detention time of twenty days and a glucose concentration of 500 mg/l was also studied.

#### 1<sub>b</sub>. Continuous Flow Study with Bacteria (No Algae Present)

Pond C was used for this study, which was conducted in the dark; settled sewage was used as seed. A Sigmamotor

pump was used to pump the feed to the influent side well. The detention period was ten days. Dissolved oxygen, pH, and COD of the effluent were determined twice daily. The effluent sample was taken from the exit side well of the pond. At times DO was measured for samples taken at the influent end and at the center of the main chamber. The reactor liquor was syphoned into standard 300 ml BOD bottles for determination of DO. Experiments were conducted with various glucose concentrations in the feed.

## 2. Physical Deaeration and Reaeration

Studies on physical reaeration were conducted in the dark, using the experimental ponds C and D. Both the rates of deaeration and reaeration were evaluated by conducting experiments with initial DO levels above and below the saturation values, using tap water at temperatures of  $22 \pm 2^{\circ}\text{C}$ .

### 2<sub>a</sub>. Deaeration

The rate of deaeration (stripping) was determined using water supersaturated with DO. The experimental pond was filled; pure oxygen was bubbled into the water through the port in the central well for about two hours. All openings were kept closed overnight. On the day of the experiment, four liters of the oxygenated water were drained through the central port into a bottle; then the exit port of the pond was opened and the extra water was drained off. The water level in the tank was the same as that of the medium in the tank for open system experiments.

An adjustable flow pump was used to circulate the water from one end well to the other end well to simulate the condition in the continuous flow experiments described earlier. The intake tube of the recirculation pump had two branches. One end was placed in one side well of the pond three inches below the water surface. The other end was placed in the bottle with oxygenated water three inches below the surface of the water in it. Other than at sampling times, the line from the bottle was closed. Whenever DO was determined by the chemical method, 500 ml of water was taken out of the pond. The same quantity of water from the bottle was pumped into the tank through the end of the line which was in the bottle.

The outlet tube of the pump ended in an airtight lucite cylinder. On the top of this lucite cylinder a DO probe was attached for electronic recording of DO, and a magnetic stirring bar was placed at the bottom of the cylinder. The cylinder assembly was placed on top of a magnetic stirrer. The effluent water from this cylinder was carried to the other side well of the pond, and it was let out three inches below the surface of the water.

Twice daily, DO concentrations were determined chemically. Standard 300 ml BOD bottles were used, and the water which was removed was replaced from the bottle, as mentioned previously. DO was recorded continuously, using a Galvanic cell oxygen analyzer and a Sargent recorder. The DO probe was calibrated before the start of each experiment, and the calibration was checked twice daily whenever DO was determined chemically. At the end of the experiment,

water from the pond was withdrawn into a 2-liter flask. The flask was thoroughly shaken, and the DO of the water was determined. The DO determinations were repeated daily until two consecutive readings were the same.

### 2<sub>b</sub>. Reaeration

The rate of reaeration (diffusion of oxygen from the atmosphere) was determined, using water devoid of oxygen. The experimental pond was filled; sodium sulfite in slight excess was added for chemical removal of the DO. Cobalt chloride (0.02 mg/l) (80) was used as a catalyst. The experimental setup and procedure were the same as described for the deaeration experiments.

## Biological Deoxygenation Studies

### 3<sub>a</sub>. Oxygen Utilization by Organotrophs

These studies were conducted in the dark and in the absence of algae. Experimental pond B was used for these experiments; BOD bottles were used for experiments which accompanied the work in the ponds. Samples were taken from one end of the tank through the valve arrangement previously described; an elevated distilled water reservoir was connected to the other end of the tank. Normally, both the inlet and outlet valves in the tank were kept closed. During sampling, both ports were opened and the required amount of sample was withdrawn. By this process an equal volume of distilled water replaced the sample volume. Then the ports were closed, keeping the system out of contact

with the atmosphere. Preliminary experiments indicated that the increase or decrease in the DO concentration due to the replacement was very small. In one experiment it ranged from 0.228 mg/l to 0.028 mg/l.

All normal precautions in running the BOD determinations were taken: scrupulous cleansing of bottles and pond, careful siphoning of samples, incubation in the dark, etc. The only omission was that the temperature was not precisely controlled; the bottles and pond were set up in a humid room in which the temperature range was  $25 \pm 2^{\circ}\text{C}$ .

#### Experiments in BOD Bottles

All constituents of the medium previously mentioned, excepting  $\text{NaHCO}_3$  and glucose, were mixed and made up to approximately 35 liters in a large carboy. Pure oxygen was passed through the medium for about three hours; then  $\text{NaHCO}_3$  and seed were added and mixed gently. Finally, the required amount of glucose was added, mixed gently, and the medium was carefully siphoned into BOD bottles (approximately ninety bottles were used). Almost one hour was required from the time of addition of substrate to complete filling of the BOD bottles; therefore, some change in initial DO was expected in different bottles. To minimize this effect, sampling was accomplished as follows: The bottles were arranged in the order in which they were filled. One from each end and another from the center of the row were taken for DO determination. Two more bottles were taken on different occasions from the center of the

above positions. Thus, it was expected that the variations in DO, if any, would be somewhat random.

### Experiments in Ponds

Making up the medium and seeding were done in the same way as described in the previous section. The sample volumes were measured each time in order to determine the volume displaced and consequently, the change in DO concentration which sampling might impose on the system. To keep the replacement at a minimum level, bottles of 150 ml capacity were used in place of standard BOD bottles (300 ml capacity).

#### 3<sub>b</sub>. Oxygen Utilization by Photo-autotrophs

These experiments were conducted in a Warburg respirometer at a temperature of 25°C. The test organism, Chlorella Pyrenoidosa, was obtained from the American Type Culture Collection. It was grown on sporulation agar (86) and was transferred to a shaker flask (250 ml) and aerated for seven to ten days. Then 50 ml of this culture was used to seed the medium in a larger culture vessel. Air containing five per cent CO<sub>2</sub> was used as an additional carbon source. The CO<sub>2</sub> cylinder was connected to the culture vessel through a cotton trap. The intensity of the light was 300 ft-c.

#### 3<sub>b1</sub>. Heterotrophic Metabolism

Two-liter flasks were used as culture vessels. One liter of medium was used. The flask with the medium (devoid of carbonate, phosphate, and ferrous sulfate, which



were autoclaved separately and added), stopper, and the connections from the CO<sub>2</sub> cylinder were autoclaved. After cooling, 50 ml of seed from the shaker flask was added to the medium. The culture was used for experimentation after two weeks of growth.

Two Warburg flasks were set up with the seeded medium for measuring endogenous O<sub>2</sub> uptake. Glucose was added to the seeded medium to give a substrate concentration of 500 mg/l, and portions of it were placed in other Warburg flasks. Oxygen uptake was followed for forty-eight hours. Concentrations of the biological solids and COD and carbohydrate content of filtrates were determined initially and whenever the flasks were removed from the Warburg apparatus. Microscopic examinations were made to ensure that there was no bacterial contamination.

### 3.<sub>b2</sub>. Endogenous Metabolism

Eight liters of medium were used in a 9.5-liter narrow-mouthed bottle for growing the algal culture. The autoclaving and seeding procedures were the same as described in the previous section. The culture was grown for fifteen to twenty days and used for experimentation.

On the day of the experiment, solids from 7.5 liters of the culture volume were harvested using a Sharples centrifuge. The centrifuge bowl, feed bottle, and all parts coming into contact with the algae were previously autoclaved. The harvested algae were re-suspended in autoclaved medium. Required volumes were placed in Warburg flasks,

and oxygen uptake was recorded for five to seven days. Duplicate flasks were taken off the Warburg apparatus at various intervals. Concentration of solids and COD of the filtrate were determined initially and whenever flasks were removed. Samples of the filtrates were subjected to gas chromatography in an attempt to detect metabolic products which might be excreted by the cells. Microscopic observations were made to check for contaminants.

#### E. Analytical Procedures

The experimental parameters examined and their methods of determination are given below.

##### 1. Dissolved Oxygen Concentration

Dissolved oxygen was determined chemically, as described in Standard Methods (87). The Alsterberg modification of the Winkler method was used. At times a galvanic cell oxygen analyzer (Precision Scientific Co.) was used to register DO electronically. A Sargent recorder was used for continuous reading.

##### 2. Oxygen Uptake

A Warburg respirometer, manufactured by the Gilson Medical Electronic Company was used for the measurement of oxygen uptake. Forty ml of culture fluid were used in the Warburg flasks of approximately 140 ml capacity. Experiments were run at a temperature of 25°C, and a shaker rate of 110 osc/min.

### 3. Biological Solids

The weight of biological solids was determined by the membrane filter technique.

### 4. Oxidation Reduction Potential (ORP) and pH

The pH was measured using a Beckman expanded scale pH meter. Oxidation reduction potential was measured by using a Beckman Zeromatic pH meter.

### 5. Chemical Oxygen Demand

The organic load was determined by using the COD test as described in Standard Methods (87). Both catalysts, silver sulfate and mercuric sulfate, were used. For lower concentrations, 0.025N dichromate, and for higher concentrations, 0.25N dichromate were used.

### 6. Carbohydrates

The anthrone test as described by Gaudy (88) was used to determine the carbohydrate concentration of the filtrate.

### 7. Glucose Concentration

The Glucostat test was used to determine the glucose concentration in the membrane filtrate. This was accomplished in accordance with the literature accompanying the enzyme sent by Worthington Biochemical Corporation (89).

## CHAPTER V

### RESULTS

#### 1. Continuous Flow Studies in Experimental Oxidation Ponds with Algae

Detention times employed in all continuous flow experiments are ten days, unless otherwise stated.

##### Mode of Presentation of Results

The general arrangement of the results of the studies with algae, with ponds open and closed to the atmosphere, is as follows: All parameters, observed and computed, are plotted against time. In the lower half of the figures the observed values of ORP and DO are plotted. The difference between the DO concentrations at the beginning and at the end of the light period was calculated and is shown on the figures as DO increase. Similarly, the difference between the DO concentrations at the beginning and at the end of the dark period was computed, and this is shown as DO decrease. The DO line is shown by a solid line during lighting, and by a dashed line during darkness. The light and dark periods are also shown. In the upper half of each figure the observed values of filtrate COD, biological solids, and pH are shown.

### Open Systems

Figure 7 shows the behavior of the open pond at an inflowing substrate concentration of 100 mg/l glucose, with a detention period of ten days. For the first fourteen days the DO concentrations averaged approximately 9.7 mg/l at the end of the light period, and the range was between 8.5 and 10.8. At the end of the dark period, the average value was 1.9 mg/l, with a range between 0.9 and 2.9 mg/l. The average change in DO concentration was 7.9 mg/l. The ORP gradually increased from 120 to 180 millivolts. The pH remained at the same level, i.e., around 7.1. The biological solids concentration exhibited a diurnal variation for the first seven days, increasing during the light period, and decreasing during the dark period. Following this, the solids concentration varied irregularly from approximately 110 to 60 mg/l. The filtrate COD fluctuated, but in general there was an increasing trend from about 110 mg/l to 140 mg/l.

Figure 8 shows the behavior of the open pond for a continuous loading of 150 mg/l of glucose. The average DO concentrations at the beginning and at the end of the light period were 0.4 and 4.7 mg/l. The ORP increased from 80 to 170 millivolts before dropping to 125 millivolts. The pH dropped slightly as the run progressed. After three days the biological solids concentration averaged approximately 92 mg/l. The effluent COD was approximately 90 mg/l.

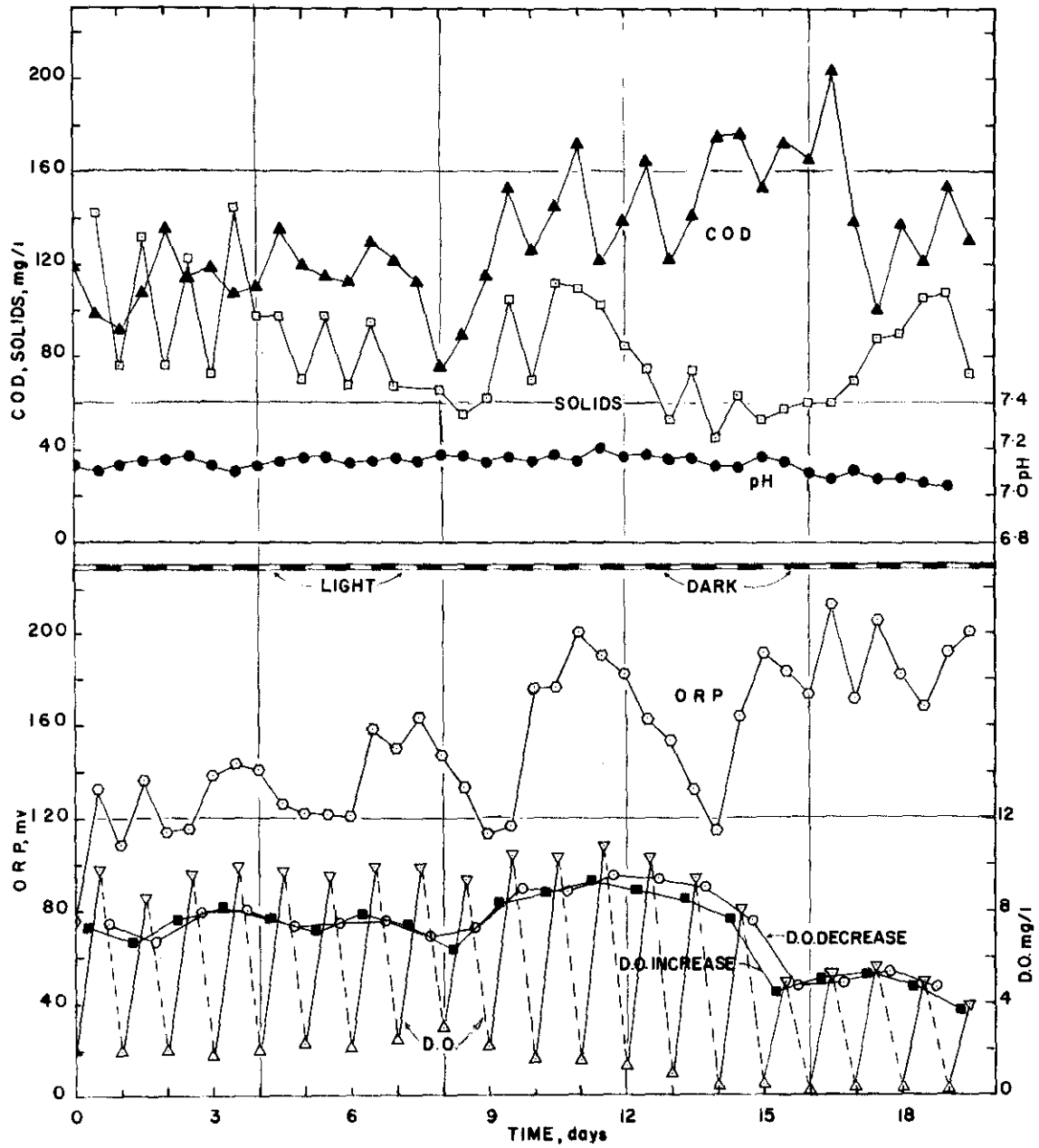


Figure 7 - Response of an open system to a continuous loading of 100 mg/l glucose.

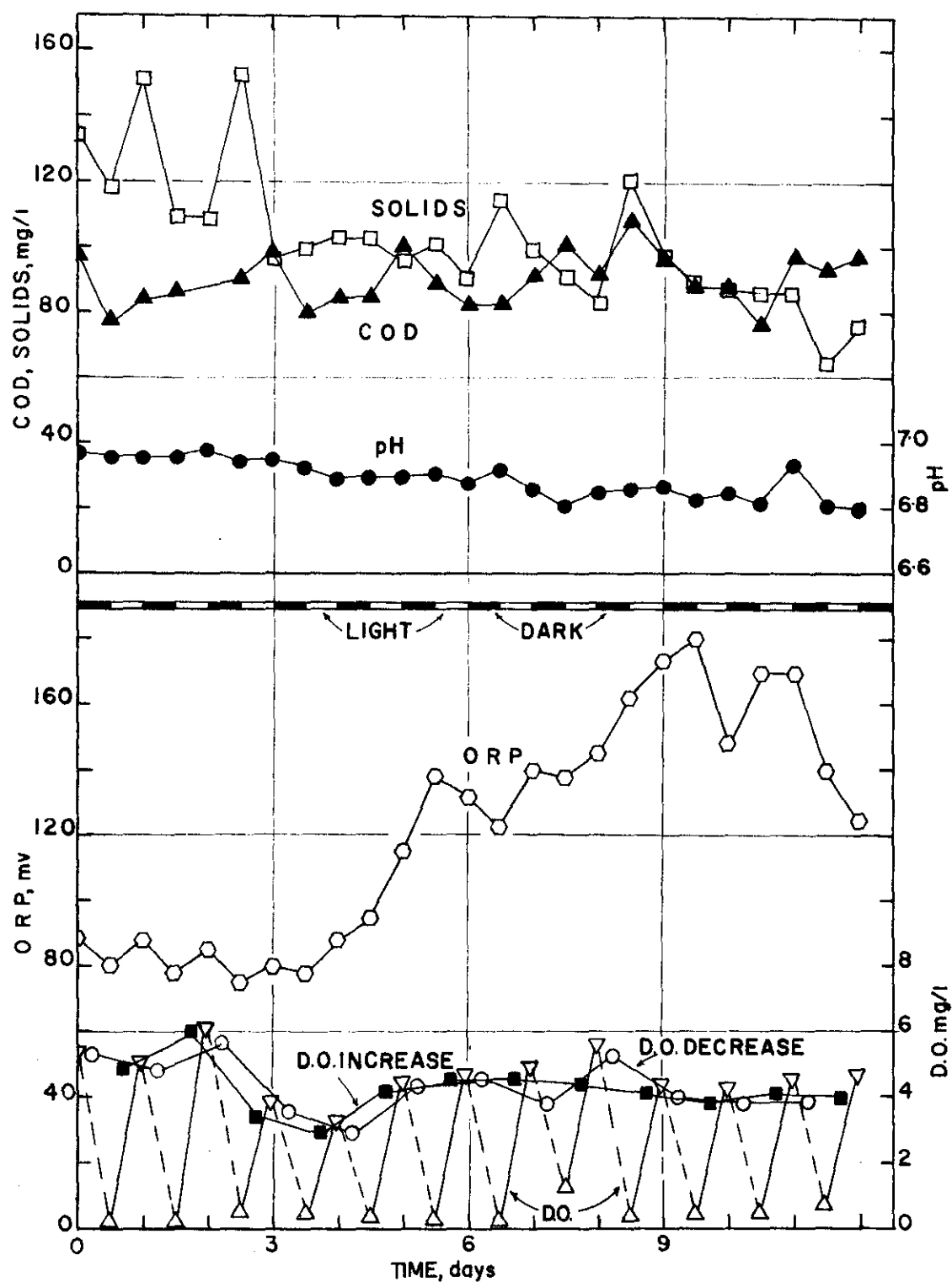


Figure 8 - Response of an open system to a continuous loading of 150 mg/l glucose.

Figure 9 shows the response of an open pond to a continuous loading of 250 mg/l of glucose. The DO at the end of the dark period was always zero, and all of the DO accumulated during the light period was removed during darkness. Therefore, both the DO increase and DO decrease are equal to the DO at the end of the light period. For this reason the DO curve was not plotted. Even though there was no DO at the end of the dark period, the DO at the end of the light period attained supersaturation levels for the first seven days (a maximum of 11.3 mg/l for one day). The changes in ORP were considerable (from +200 to -280 mv). After the third day, the ORP showed a diurnal change similar to the change in DO, increasing in the light and decreasing in the dark. The average increase during the light period was 210 millivolts, and the decrease during the dark period was 215 millivolts.

The biological solids concentration also fluctuated; most of the time it ranged between 40 mg/l and 90 mg/l. Only a slight pH change was noted. The COD was comparatively steady, gradually increasing from 60 to 75 mg/l. A comparative analysis and discussion of these systems follows in the next chapter.

#### Closed Systems

Figures 10, 11, and 12 show the response of the closed pond for a continuous loading of 100 mg/l of glucose. The variations in the diurnal fluctuations of DO were small for the first nine days. After fourteen days and until the



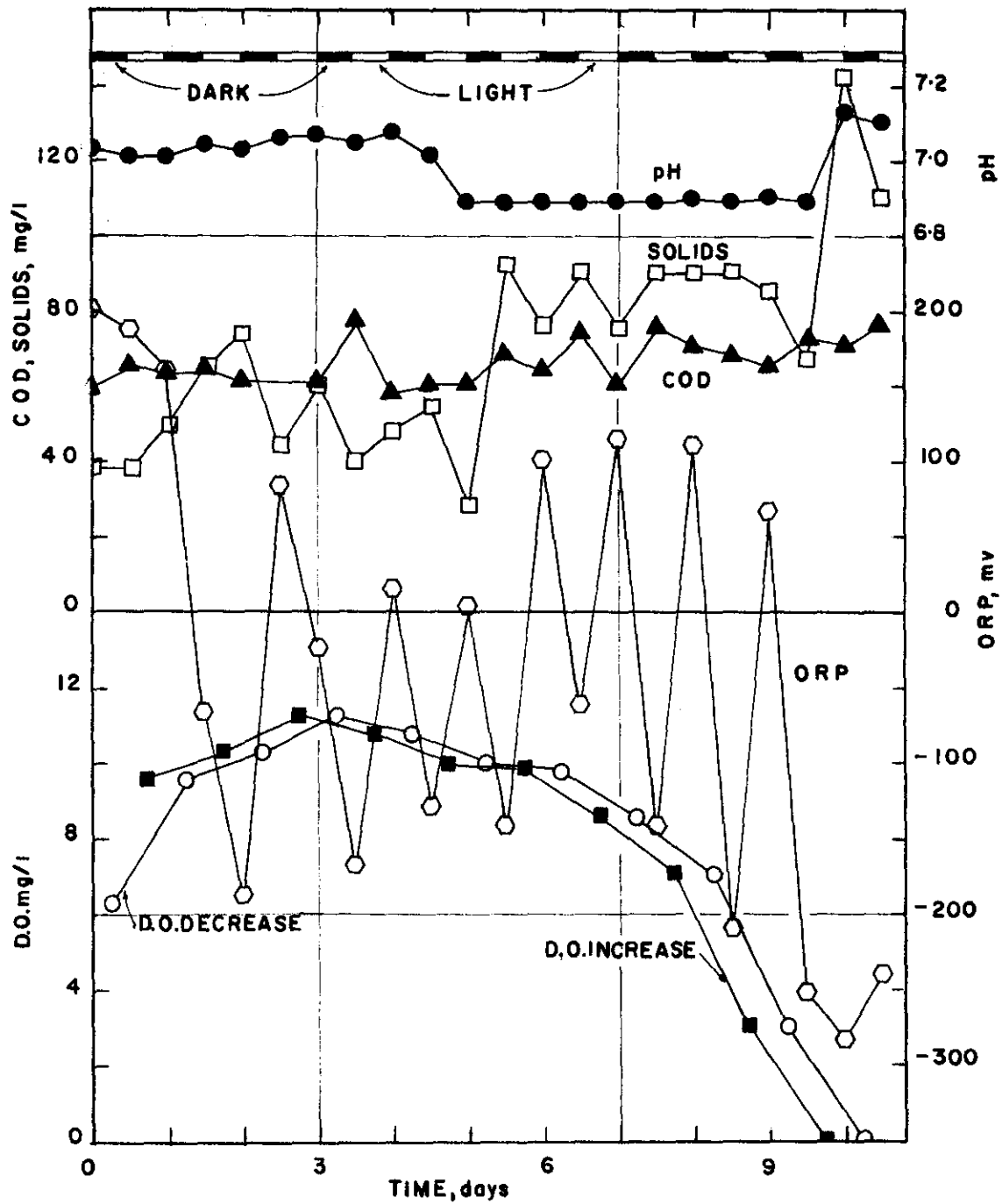


Figure 9 - Response of an open system to a continuous loading of 250 mg/l glucose.

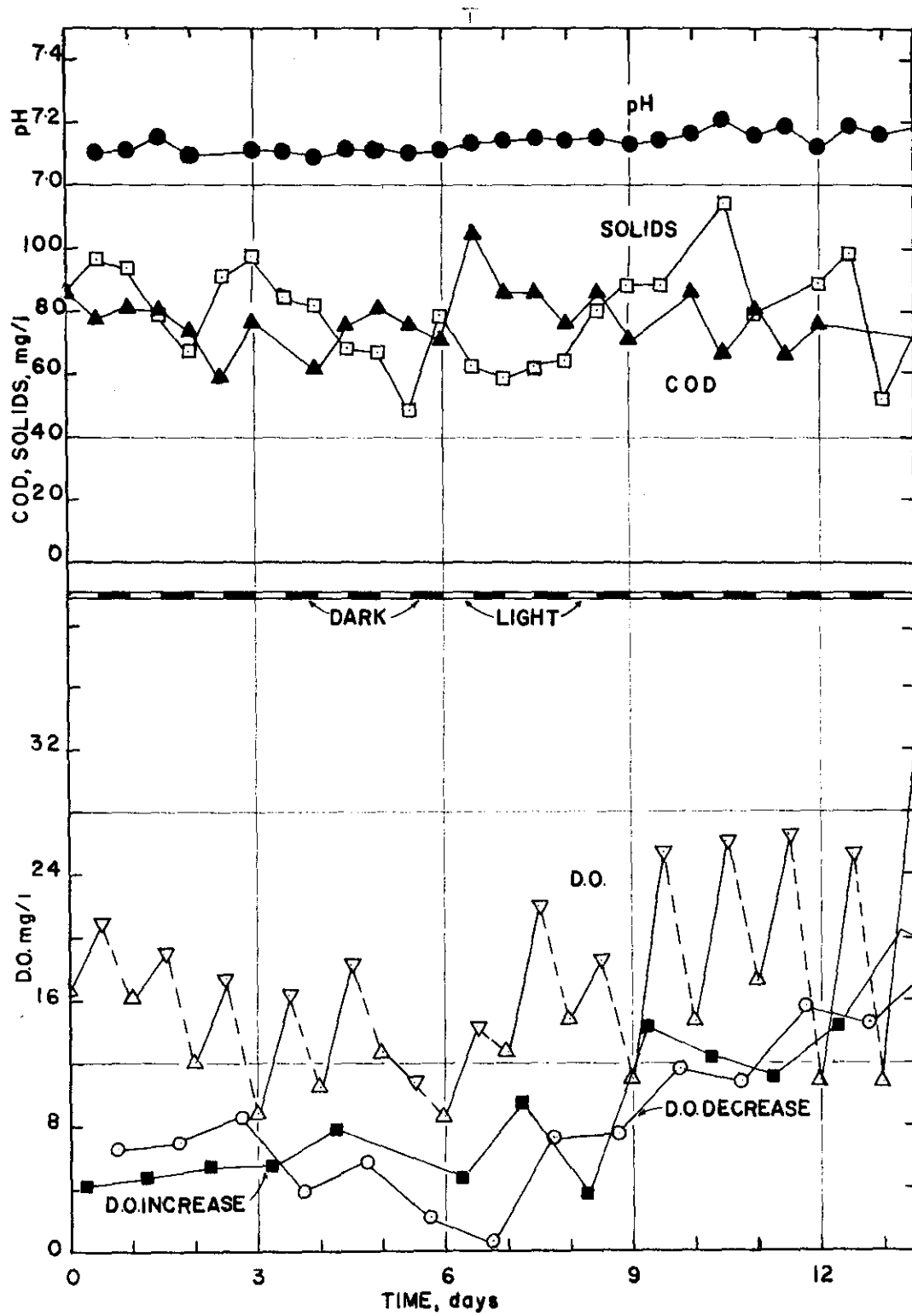


Figure 10 - Response of a closed system to a continuous loading of 100 mg/l glucose. (0-12 days)

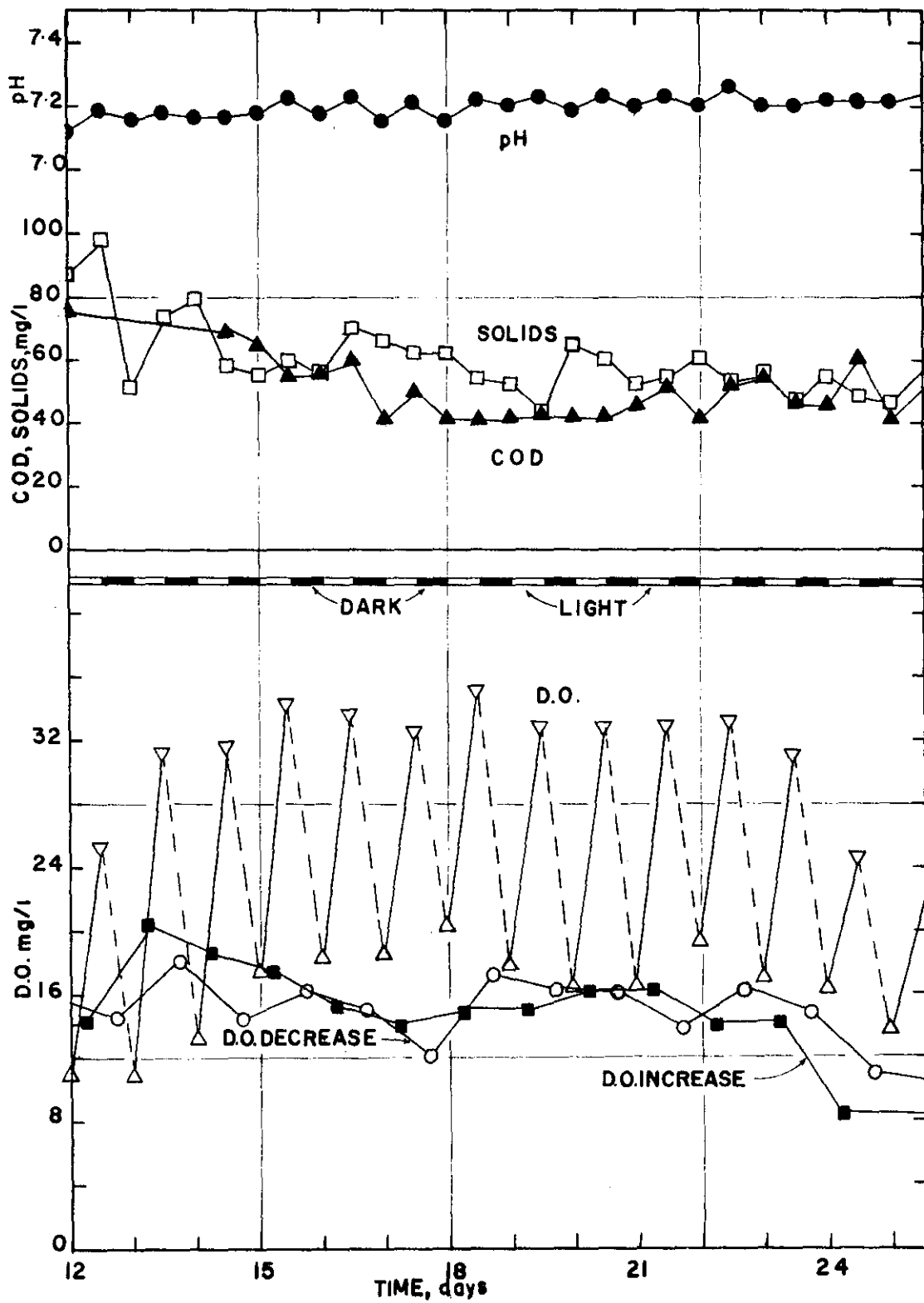


Figure 11 - Response of a closed system to a continuous loading of 100 mg/l glucose. (12-24 days)

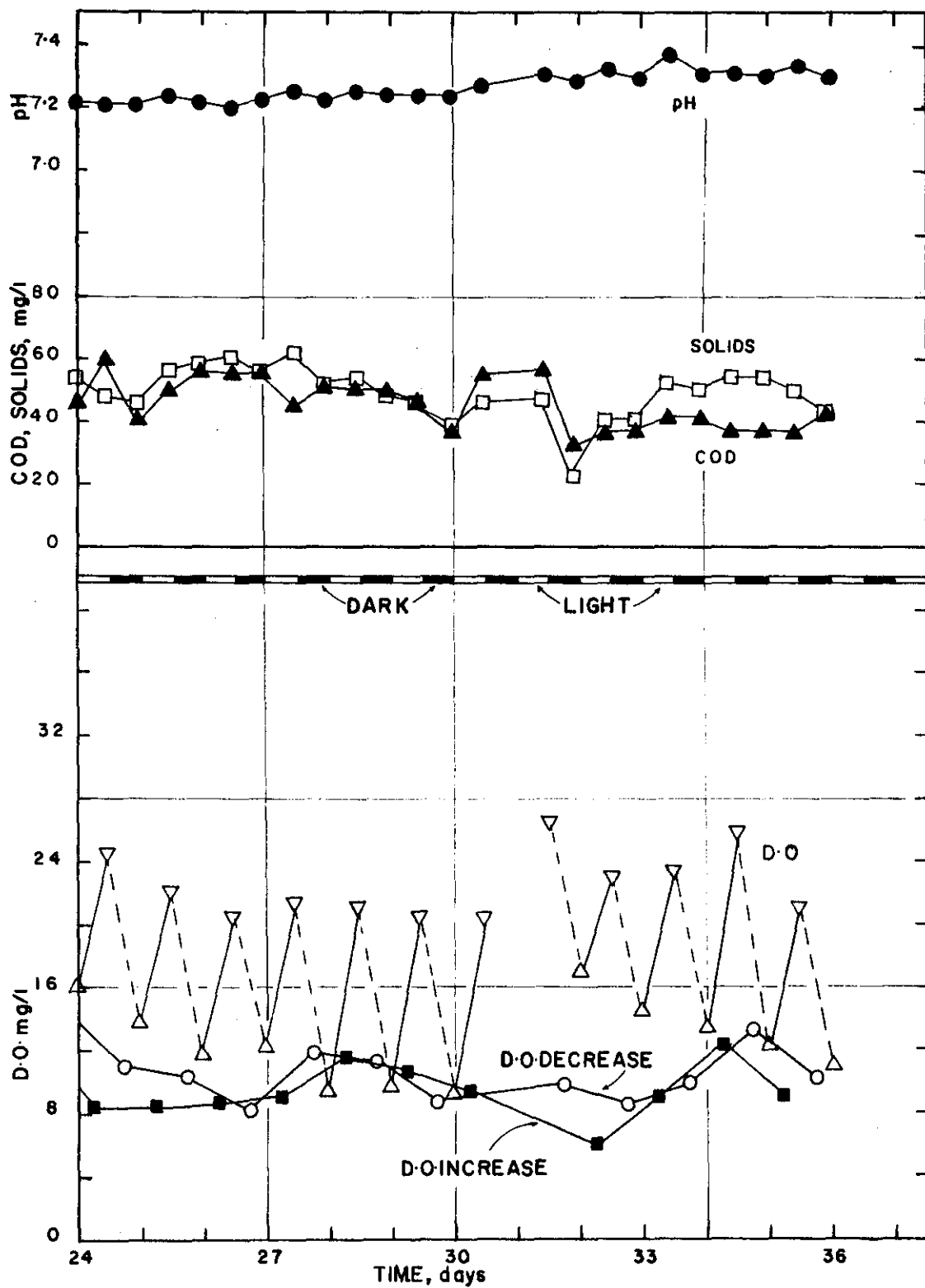


Figure 12 - Response of a closed system to a continuous loading of 100 mg/l glucose. (24-36 days)

twenty-third day, the system approached a condition of "balanced operation." During this nine-day period the average maximum DO was 33.1 mg/l, with a range of  $\pm 2.0$ . The minimum DO concentrations ranged between 16.6 and 20.4 mg/l, and the average was 18.0 mg/l. The average change in DO during this period amounted to 15.2 mg/l. The pond attained another "balanced condition" from the twenty-fourth to the thirty-sixth day of operation. The DO during the last twelve days was slightly lower, compared to the previous days. The maximum DO was in the range of 20.5 to 26.7 mg/l, with an average of 22.6. The minimum DO was in the range of 9.5 to 16.9 mg/l, with an average of 12.8 mg/l. The average amplitude during this period was 9.8 mg/l. Regarding pH, there was a slight rise (approximately 0.25 units) as the run progressed. During some days the pH varied diurnally, increasing about 0.05 units in the light, and decreasing by about the same amount in the dark. The solids showed marked oscillations for fourteen days, after which they were almost steady. The average solids concentration between the fourteenth and the twenty-third day was 57 mg/l. During the last ten days the average solids concentration was reduced to about 50 mg/l. The filtrate COD stabilized after twelve days. The average COD during the first "balanced operation" was 48 mg/l, which was reduced to about 45 mg/l during the second "balanced period."

Figures 13 and 14 show the response of a closed pond to a continuous loading of 150 mg/l of glucose. As in the

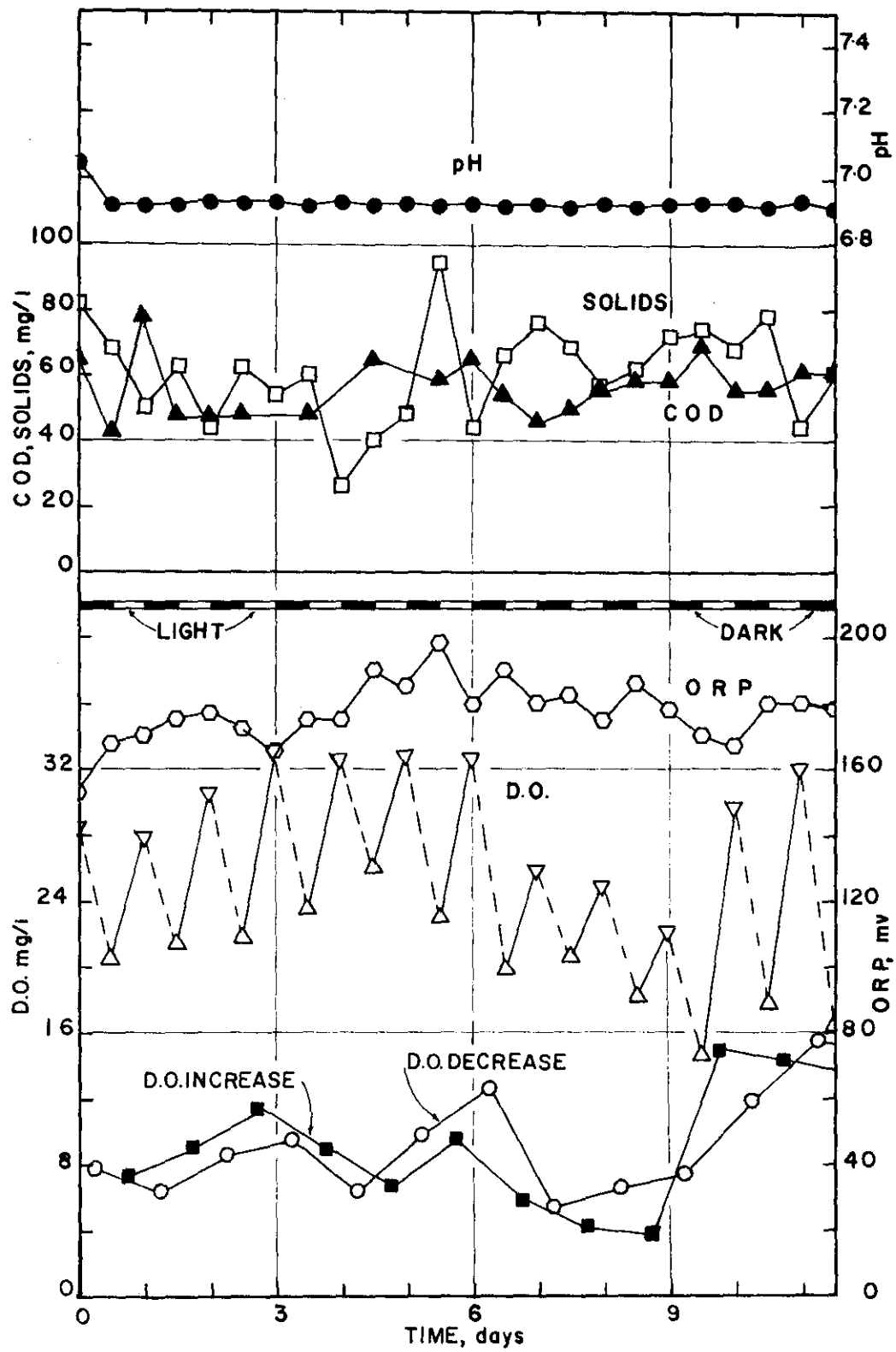


Figure 13 - Response of a closed system to a continuous loading of 150 mg/l glucose. (0-10 days)

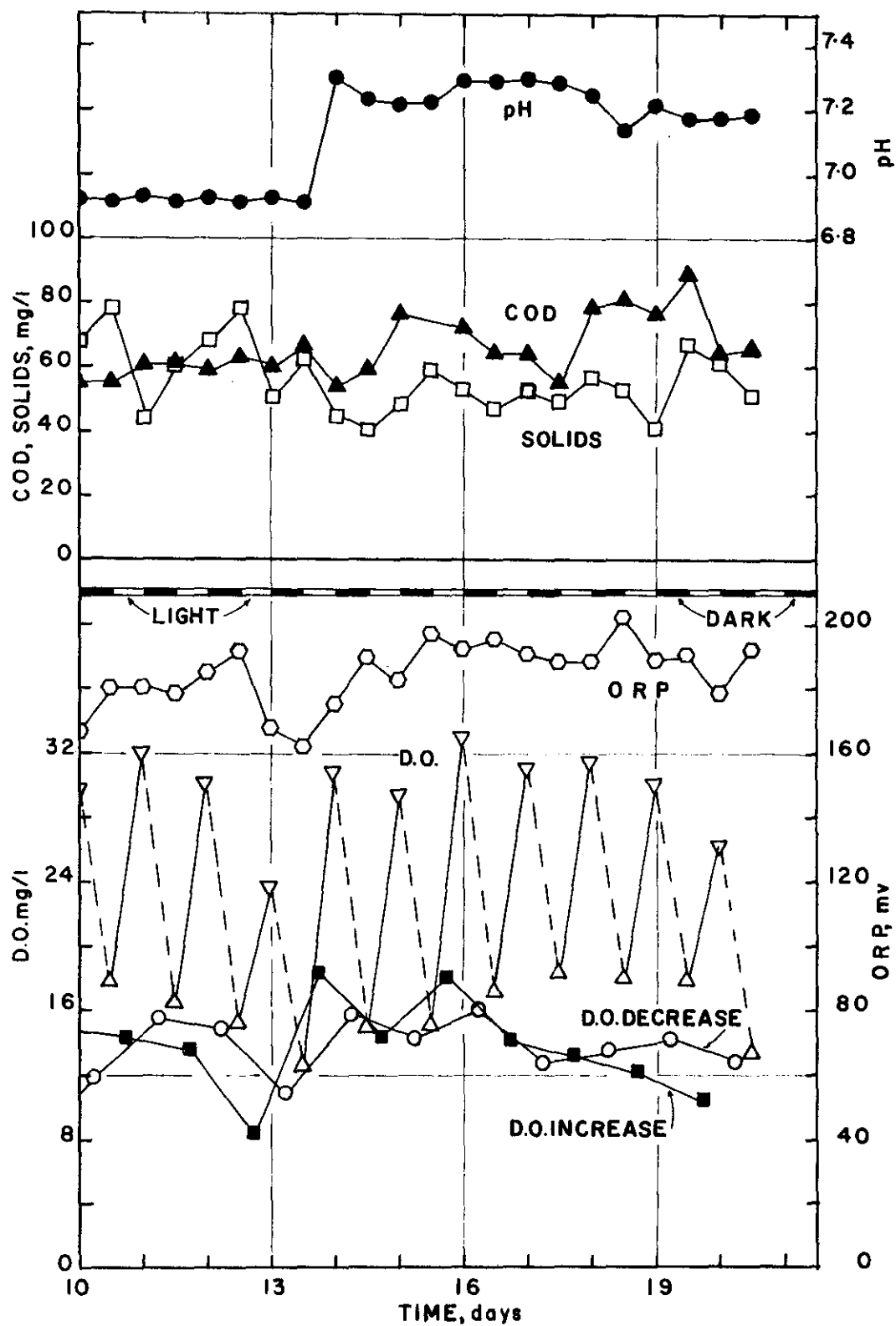


Figure 14 - Response of a closed system to a continuous loading of 150 mg/l glucose. (10-20 days)

previous case, the diurnal fluctuations were small for the first ten days. During the second ten days the DO attained a somewhat steady condition, i.e., it oscillated between approximately the same limits. The average maximum DO was 30.1 mg/l, and the average minimum DO was 16.0 mg/l. The average change was 14.1 mg/l; the ORP increased gradually from +153 to +185 millivolts in five days and, for the most part, remained at this level.

The pH remained constant for the first thirteen days at 6.9, and on the fourteenth day rose sharply to 7.3. The biological solids concentration was approximately 60 mg/l throughout the experimental period. The effluent COD varied between 50 and 80 mg/l, with an average of 65 mg/l.

Figures 15 and 16 show the response of a closed pond to a continuous loading of 250 mg/l of glucose. The DO at the end of the light period was approximately 35 mg/l for the first nine days of operation, and dropped to approximately 29 mg/l for the next three days. During this period the DO increase and the DO decrease in the same day were more or less equal, although the magnitude of the change dropped from an average of 29 mg/l during the first nine days to 14 mg/l during the last three days. Thereafter, the DO decrease remained at approximately 13.5 mg/l, whereas the DO increase was only 8.5 mg/l. As a result, the maximum and minimum DO followed a decreasing trend, and the DO dropped to zero during the dark period after eighteen days. The overall decrease in pH was very small,



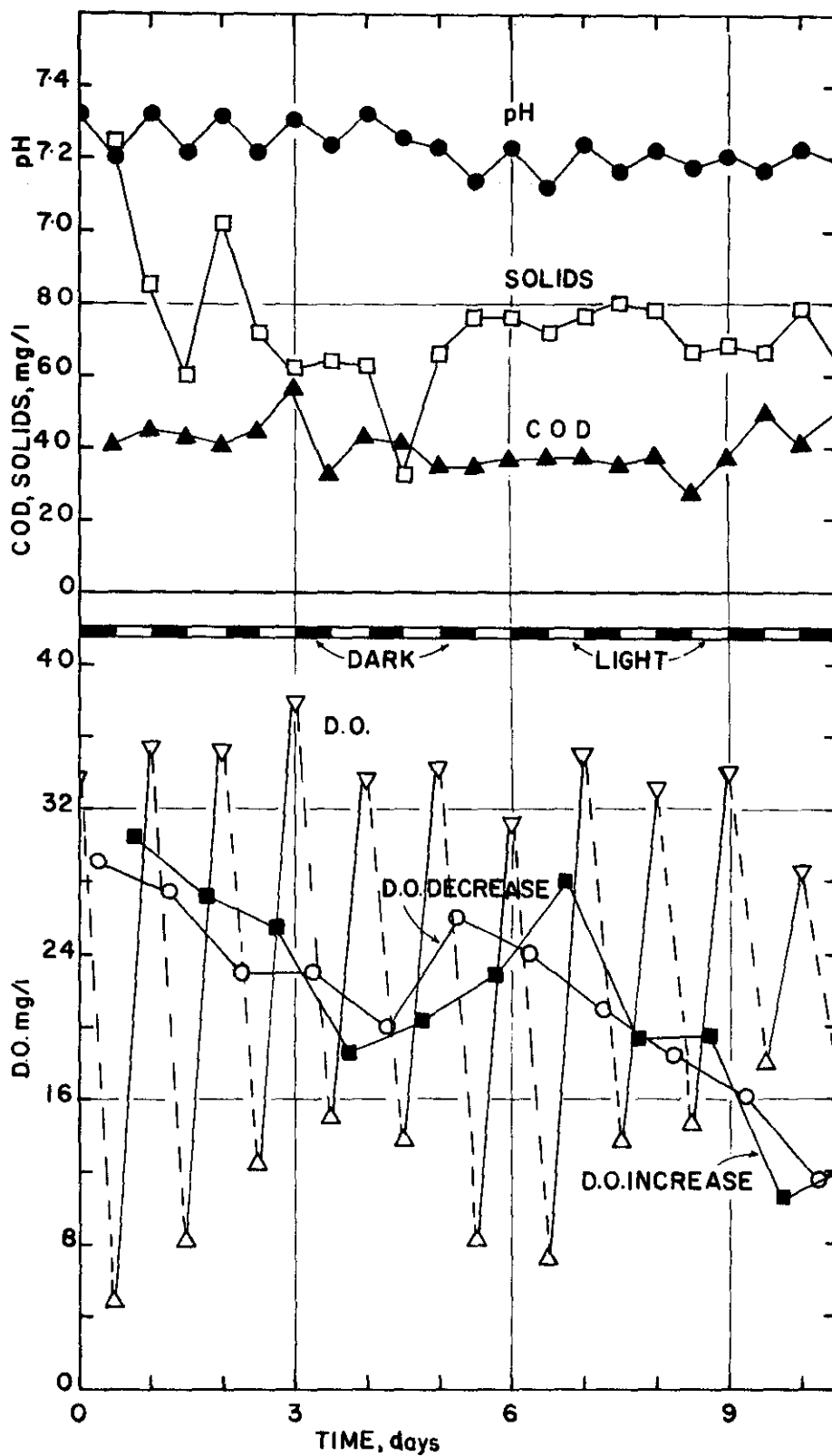


Figure 15 - Response of a closed system to a continuous loading of 250 mg/l glucose. (0-10 days)

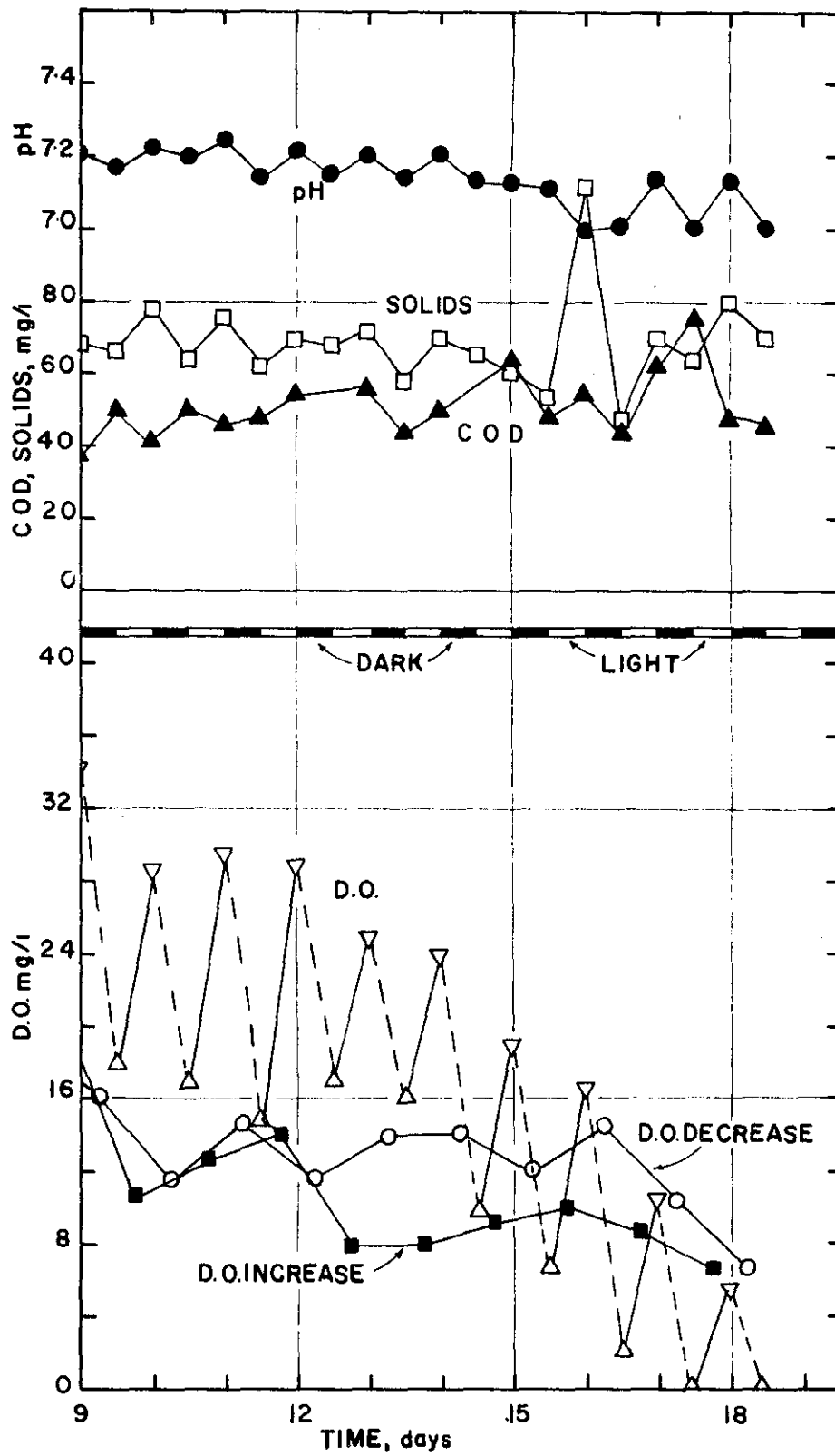


Figure 16 - Response of a closed system to a continuous loading of 250 mg/l glucose. (9-18 days)

amounting to about 0.18 units. However, pH followed a pattern of diurnal variation, increasing during the light period and decreasing during the dark period. Out of eighteen light periods, pH increased during fifteen, the increment ranging from 0.12 to 0.03. In nineteen dark periods pH decreased in eighteen, the decrease ranging from 0.12 to 0.02. The solids concentration ranged between 60 and 80 mg/l. The effluent COD (filtrate) averaged approximately 40 mg/l.

When the DO in the pond reached zero, an attempt was made to regenerate the system. For three days the lights were left on. For the following 3-day period the lights were on for fifteen hours in each twenty-four hours, i.e., the duration of dark periods was only nine hours. On the seventh day the system was brought back to the original light cycle of 12-hours-on, 12-hours-off. Figure 17 shows the performance of the regenerated system after the regular light schedule of 12-hours-on and 12-hours-off was adopted. The DO at the end of the light period ranged from 10.8 to 18.6 mg/l, averaging 13.9 mg/l. At the end of the dark period, some DO was present; the average DO was slightly in excess of 0.3 mg/l. Although the ORP dropped during the fifth day of operation, it averaged approximately 195 millivolts. The pH remained steady around 7.0. In this system, also, the pH showed, in general, a regular diurnal pattern of increase during light periods and decrease during dark periods; however, the maximum increase was very

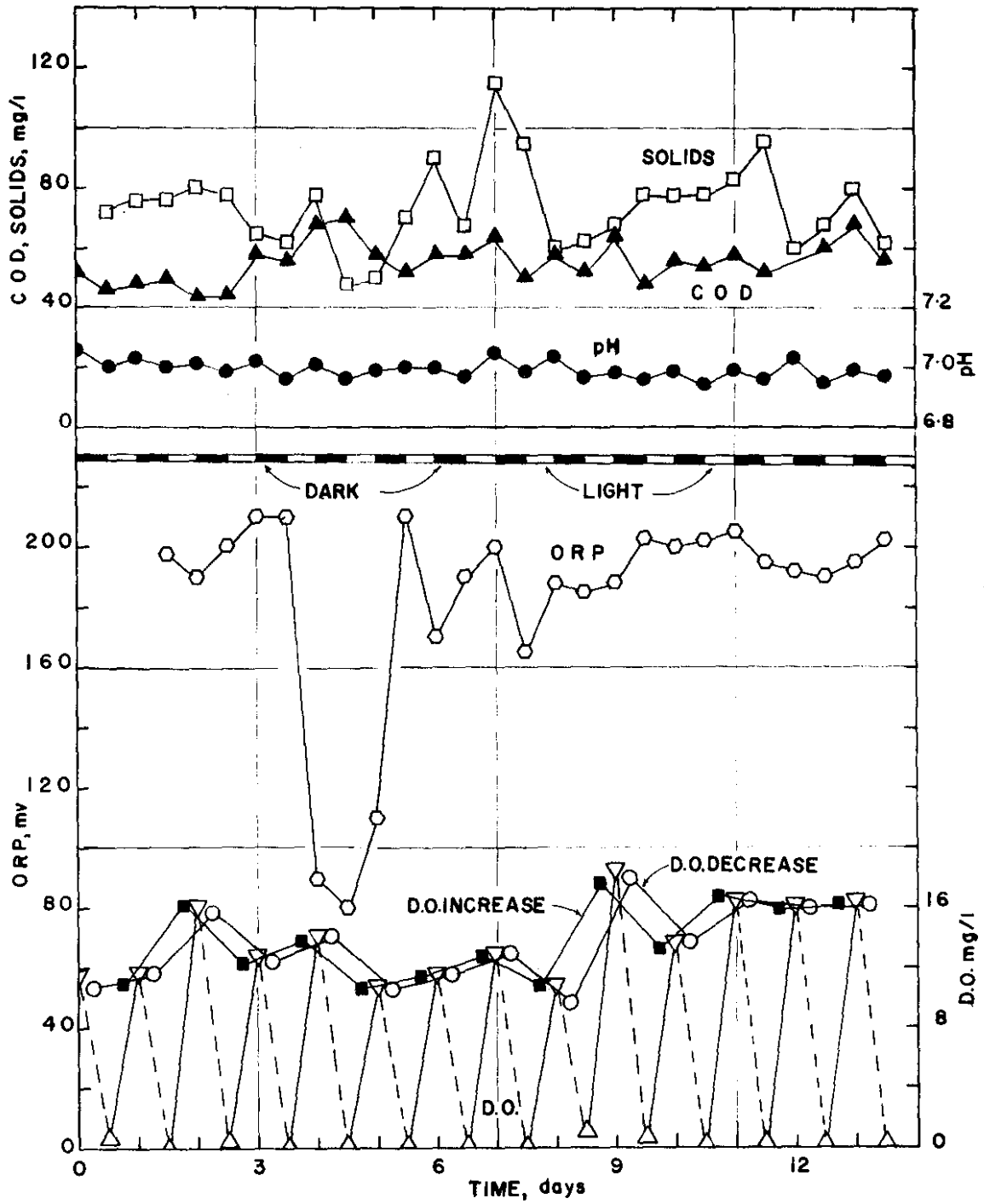


Figure 17 - Response of a "regenerated" closed system to a continuous loading of 250 mg/l glucose.

small. The solids concentration ranged between 60 and 80 mg/l, and the filtrate COD was  $55 \pm 10$  mg/l during most of the operational period.

Figure 18 shows the performance of a closed pond when subjected to a continuous loading of 500 mg/l glucose with a detention period of twenty days. No dissolved oxygen was detected during the experiment; the pH remained steady at about 6.9. The ORP was always negative (average value -310 millivolts). The solids showed a downward trend, while the COD decreased for the first two and one-half days and then increased. Since the system was anaerobic throughout the monitored period, observation was not continued.

## 2. Continuous Flow Studies in Open System Without Algae

Figure 19 shows the response of the open pond without algae to an incoming glucose concentration of 50 mg/l. At the beginning the pond was filled with medium containing substrate (50 mg/l) to the operating volume. Acclimated seed, started from settled sewage, was added in an amount of 5 ml/l, and the influent feed was pumped into the pond. The detention time was ten days. The dissolved oxygen in the effluent decreased to the zero level after two days, and the effluent was devoid of DO until the fourth day of operation. The increase in the effluent DO was very small for five and one-half days; thereafter the increase was gradual up to the tenth day, when it reached 3.6 mg/l. Beyond the twelfth day of operation the effluent DO remained almost at the same level (approximately 3.7 mg/l). The DO

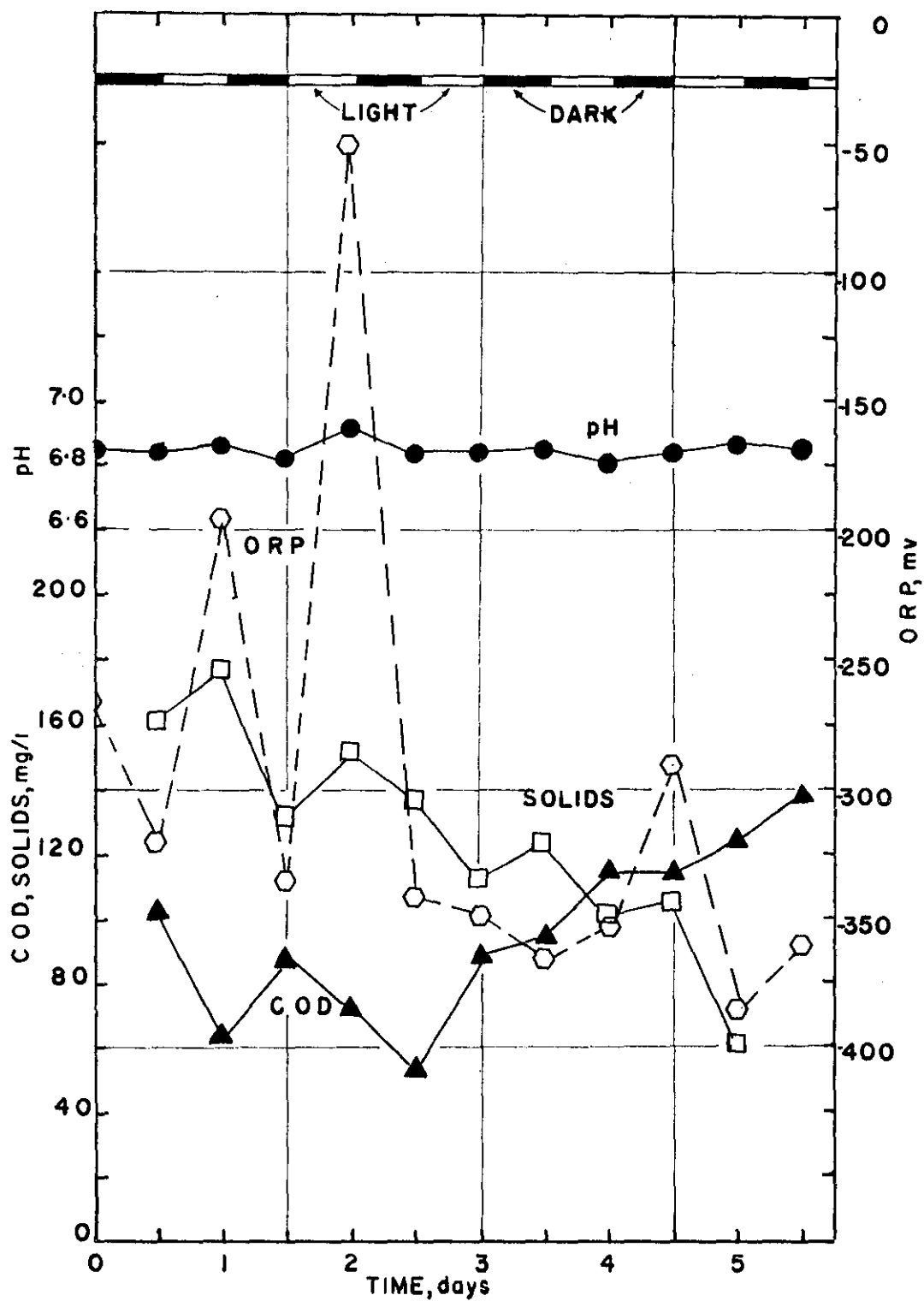


Figure 18 - Response of a closed system to a continuous loading of 500 mg/l glucose; detention period 20 days.

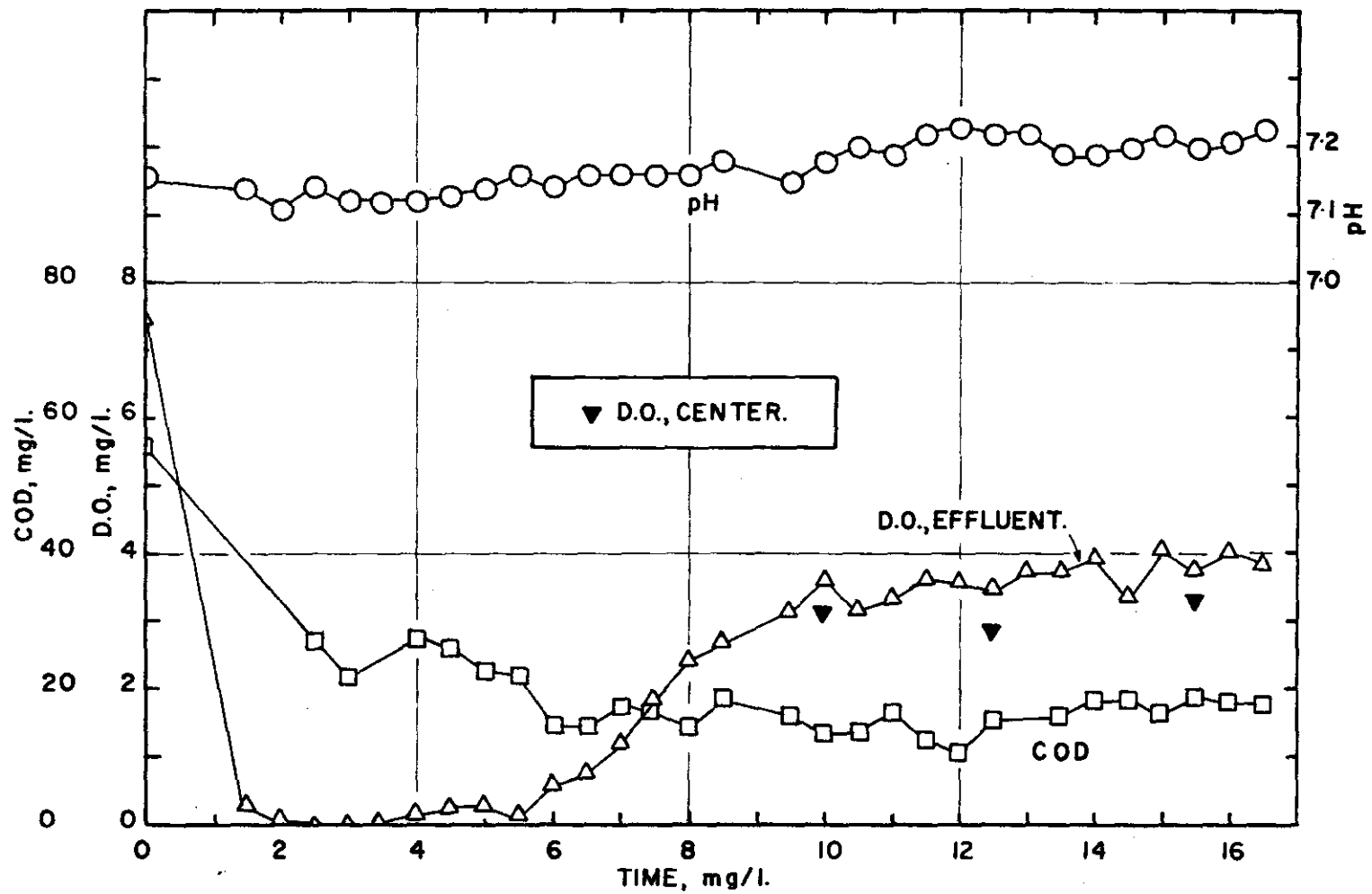


Figure 19 - Response of an open system without algae to a continuous loading of 50 mg/l glucose.

concentrations from samples at the center of the pond (three inches below the surface) were determined three times after the system recovered. They were approximately 0.5 mg/l lower than the corresponding concentrations in the effluent; the average influent COD was 56 mg/l. The effluent COD decreased sharply for three days and then decreased gradually up to the sixth day. After the sixth day the effluent COD remained steady at an average value of 17 mg/l; the removal was about 70 per cent. Only a very small increase in pH was noted.

Figure 20 shows the behavior of the pond when it received a glucose concentration of 80 mg/l. These results were obtained after changing the feed concentration from 50 to 80 mg/l glucose in the system shown in the previous figure. The effluent DO (3.8 mg/l) remained the same for thirty-six hours after the increase in the substrate concentration, and decreased to 3 mg/l during the next twenty-four hours. Thereafter it remained at approximately 3 mg/l for the remainder of the operational period, whereas the DO at the center of the pond was approximately 2 mg/l. The average DO at the influent end of the main chamber was approximately 0.3 mg/l. The effluent COD remained at the initial level of 17 mg/l for about five days, then increased gradually to approximately 20 mg/l the next day. Beyond this time, the effluent COD was more or less constant at approximately 20 mg/l. The average influent COD was 81 mg/l; the removal was approximately 75 per cent. There



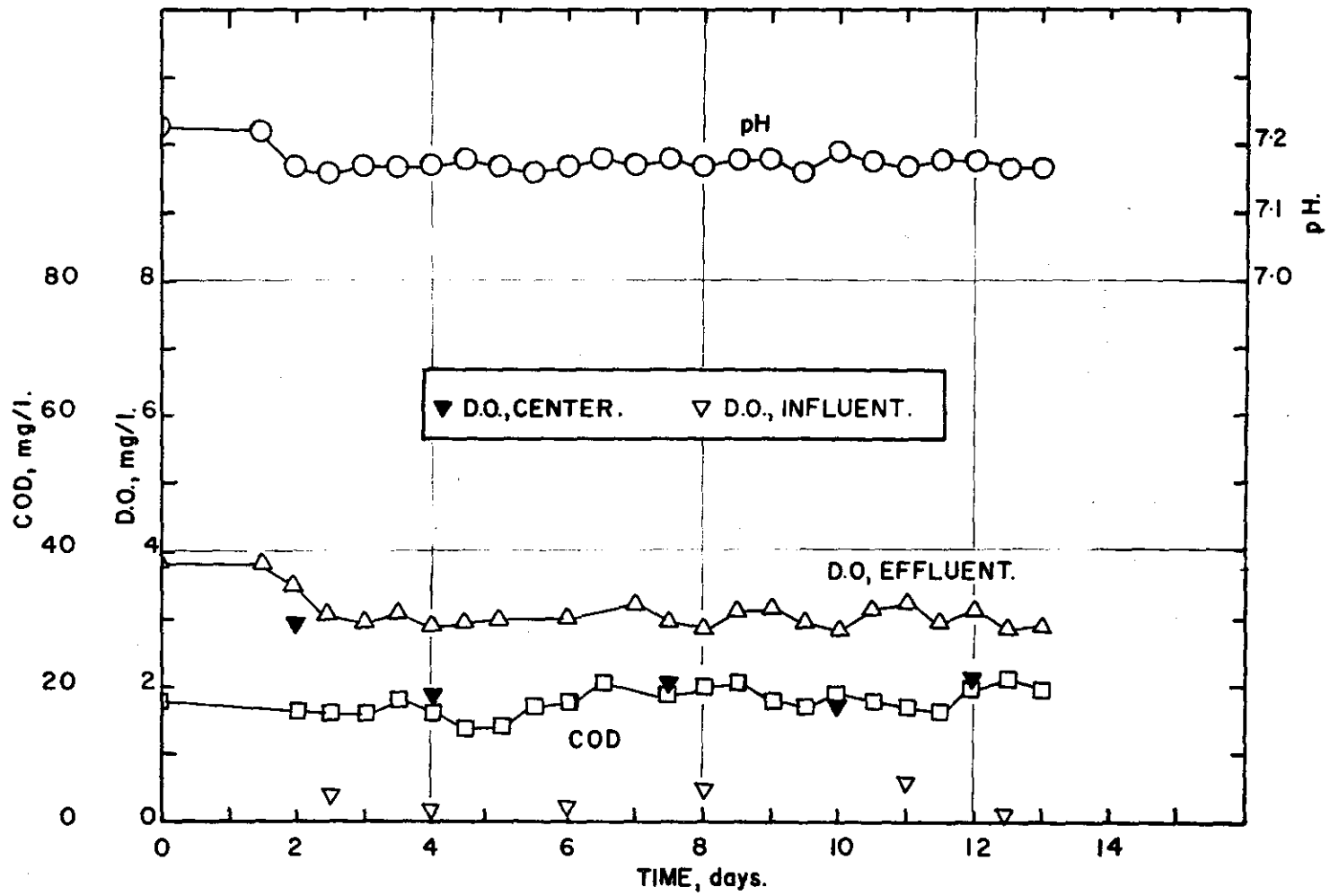


Figure 20 - Response of an open system without algae to a continuous loading of 80 mg/l glucose.

was a very small decrease in pH (0.05 units) during the first two days; thereafter the pH was steady throughout the experiment.

The response of the system to a continuous loading of 120 mg/l glucose is shown in Figure 21. This was also a continuation of the previous system. The substrate concentration was increased from 80 mg/l glucose to 120 mg/l; the figure shows the performance of the system after the increase. The effluent DO was reduced from approximately 3 mg/l to 0.5 mg/l in four days. The DO concentration increased to about 1.5 mg/l during the next three days, and once again decreased. From the tenth day onward the effluent DO was near 1 mg/l. The DO concentrations at the center of the pond were zero for all of the days tested, except that a trace amount was recorded for one day. Typical anaerobic conditions existed in the side well receiving the influent, as evidenced by the production of hydrogen sulfide and the black color of the reaction liquor. The settled sludge in the side well was black in color, and there was an accumulation of settled solids (approximately 3/4 cm) in the side well of an area 10 cm x 10 cm. The effluent COD increased to 29 mg/l on the fifth day, an increase of approximately 10 mg/l from the initial effluent COD. The COD curve was not "steady," in comparison with the previous systems, and averaged approximately 26 mg/l. The average influent COD was 133 mg/l; the pH remained more or less at the same level.

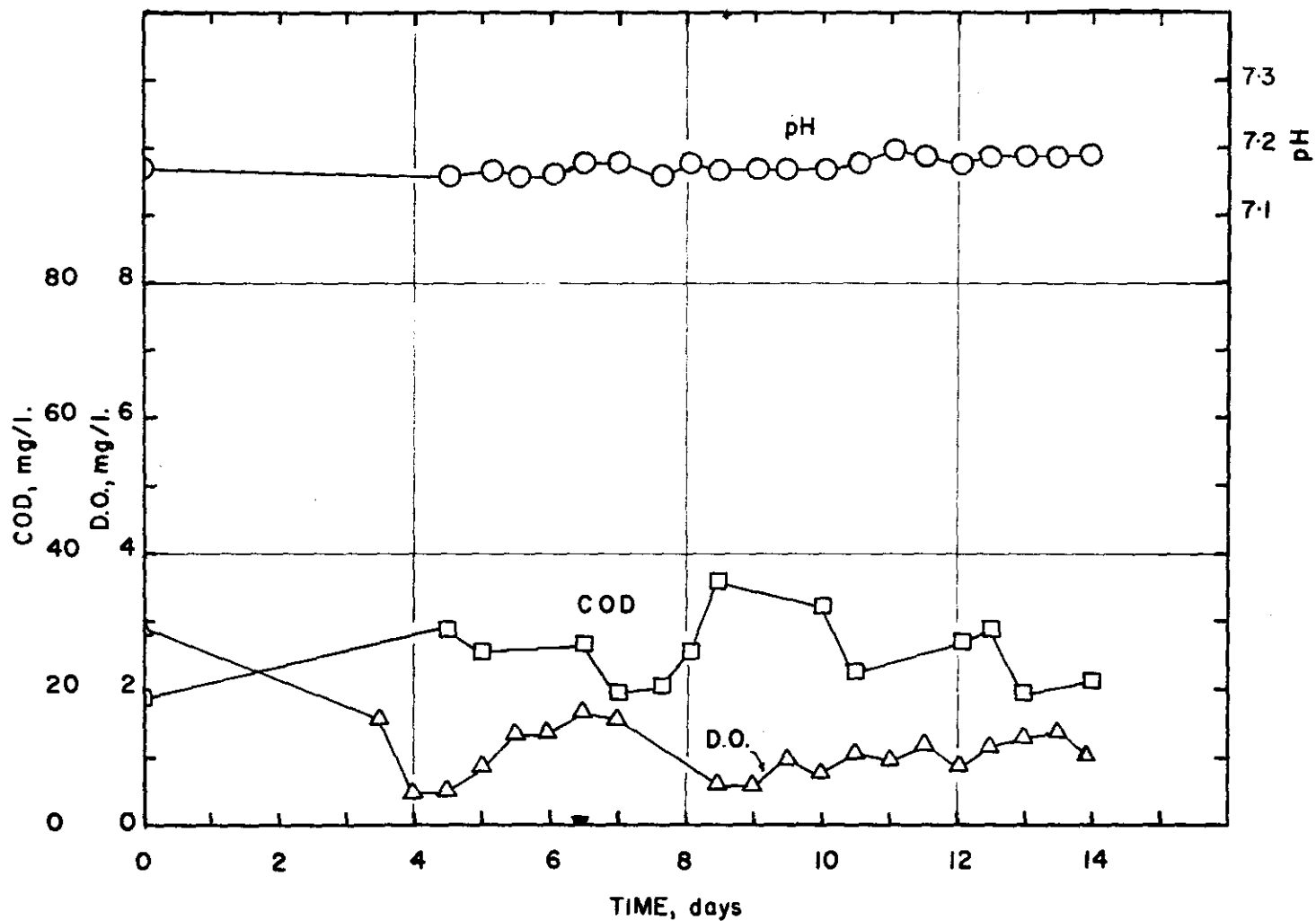


Figure 21 - Response of an open system without algae to a continuous loading of 120 mg/l glucose.

### 3. Physical Oxygenation and Deoxygenation Characteristics of the Experimental Ponds

#### Mode of Presentation of Results

The observed values of the DO concentrations for each experiment are plotted against time in the lower half of each figure on arithmetic paper. At the end of each experiment the saturation values of the DO concentration were determined (observed values). The differences between these values and the observed values of the DO concentration at various time, i.e., deficits or excesses, were computed. These deficits or excesses were plotted against time in the upper half of each figure on semilogarithmic paper with time on the arithmetic scale. The slopes of the lines in the upper half of the figures were calculated; these values represent the reaeration or deaeration rate constants. The saturation values for DO concentration were also obtained by calculation from the DO-time curve, using the  $\alpha$  method (81) as described previously. Deficits or excesses were computed based on these values; using these differences in DO concentrations, the rates of reaeration and deaeration were also determined.

#### A. Deaeration

Figure 22 shows the course of deaeration in Pond C. The observed saturation value was 8.32 mg/l, and the deaeration rate was  $0.305 \text{ day}^{-1}$  ( $0.0127 \text{ hr}^{-1}$ ). The computed values were 7.81, 0.292, and 0.0122, respectively. Figure 23 shows the deaeration in pond D. The observed

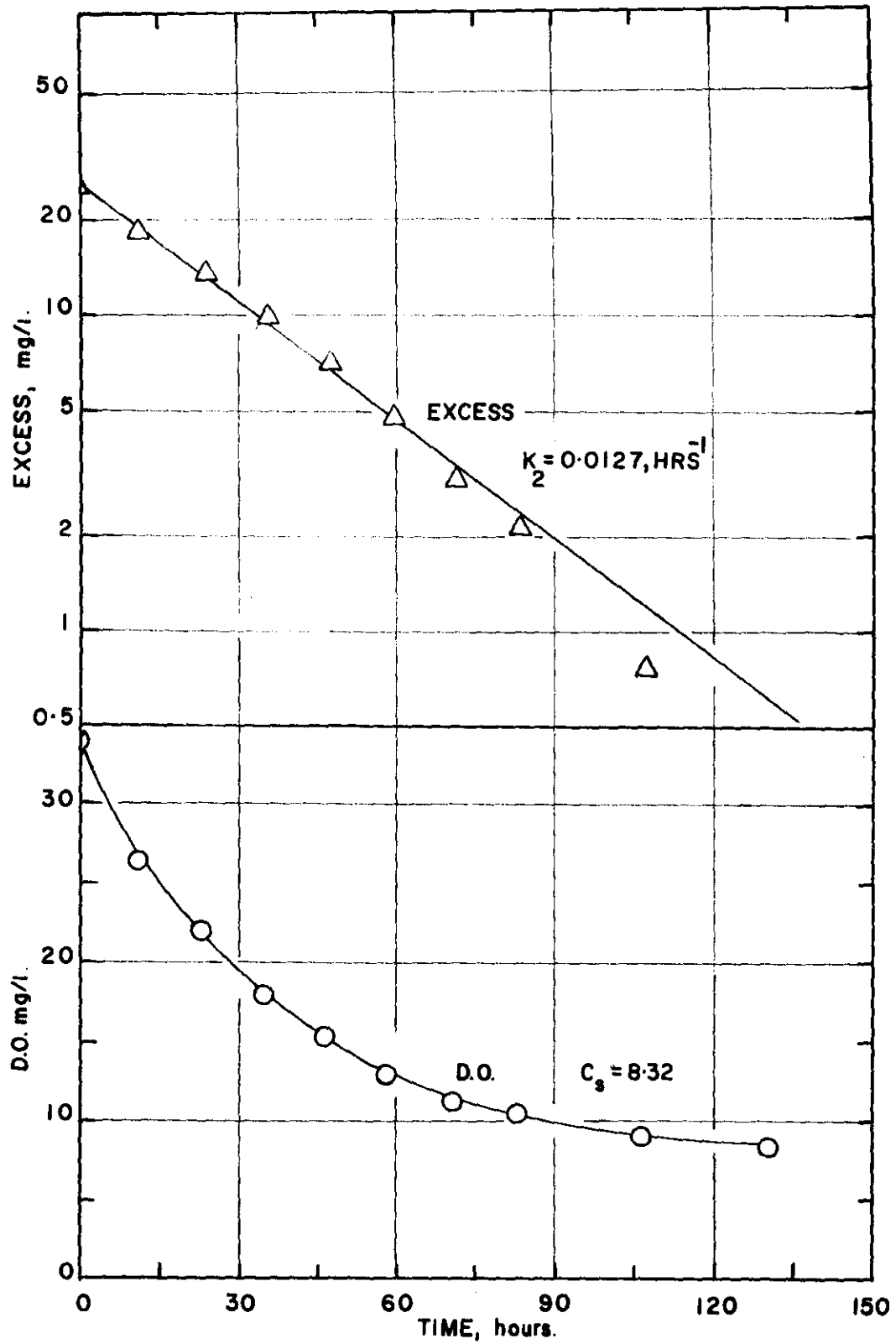


Figure 22 - Deaeration characteristics of Pond C.

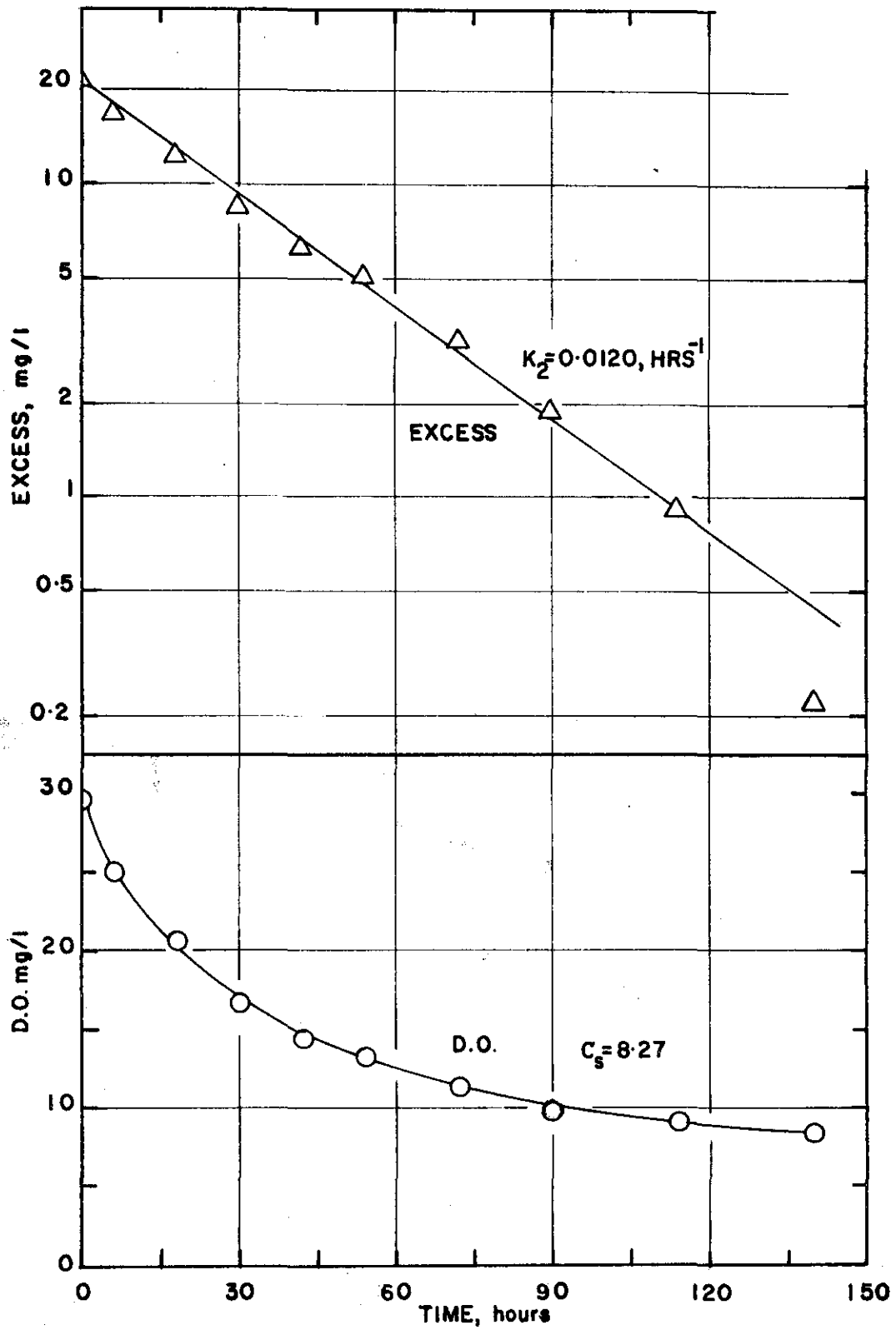


Figure 23 - Deaeration characteristics of Pond D.

saturation value was 8.27 mg/l, and the  $K_2$  rate based thereon was  $0.288 \text{ day}^{-1}$  ( $0.0120 \text{ hr}^{-1}$ ). The computed saturation value was 8.21 mg/l, and the  $K_2$  rate based on this value was  $0.282 \text{ day}^{-1}$  ( $0.0118 \text{ hr}^{-1}$ ).

#### B. Reaeration

Figure 24 shows the reaeration in pond C, the pond without baffles. The observed saturation value of the DO concentration was 8.15, and the value computed by the  $\alpha$  method was 8.26 mg/l. The corresponding reaeration rates were  $0.298 \text{ day}^{-1}$  ( $0.124 \text{ hr}^{-1}$ ), and  $0.290 \text{ day}^{-1}$  ( $0.0121 \text{ hr}^{-1}$ ).

Figure 25 shows the reaeration in pond D, the pond with baffles. The observed and computed saturation values of DO were 8.18 and 8.14 mg/l, respectively. The corresponding reaeration rates were  $0.308 \text{ day}^{-1}$  ( $0.0128 \text{ hr}^{-1}$ ) and  $0.316 \text{ day}^{-1}$  ( $0.0132 \text{ hr}^{-1}$ ).

### 4. Biological Deoxygenation Due to Organotrophic and Photo-autotrophic Organisms

#### A. Oxygen Utilization by Organotrophs

##### Mode of Presentation

For each substrate level the results for two typical experiments are shown. The DO concentrations at various times, both for BOD bottle systems and for the pond are shown. The oxygen uptake values at various times were calculated by deducting the DO concentrations at those times from the initial DO concentration, and these calculated values are plotted with respect to time for both

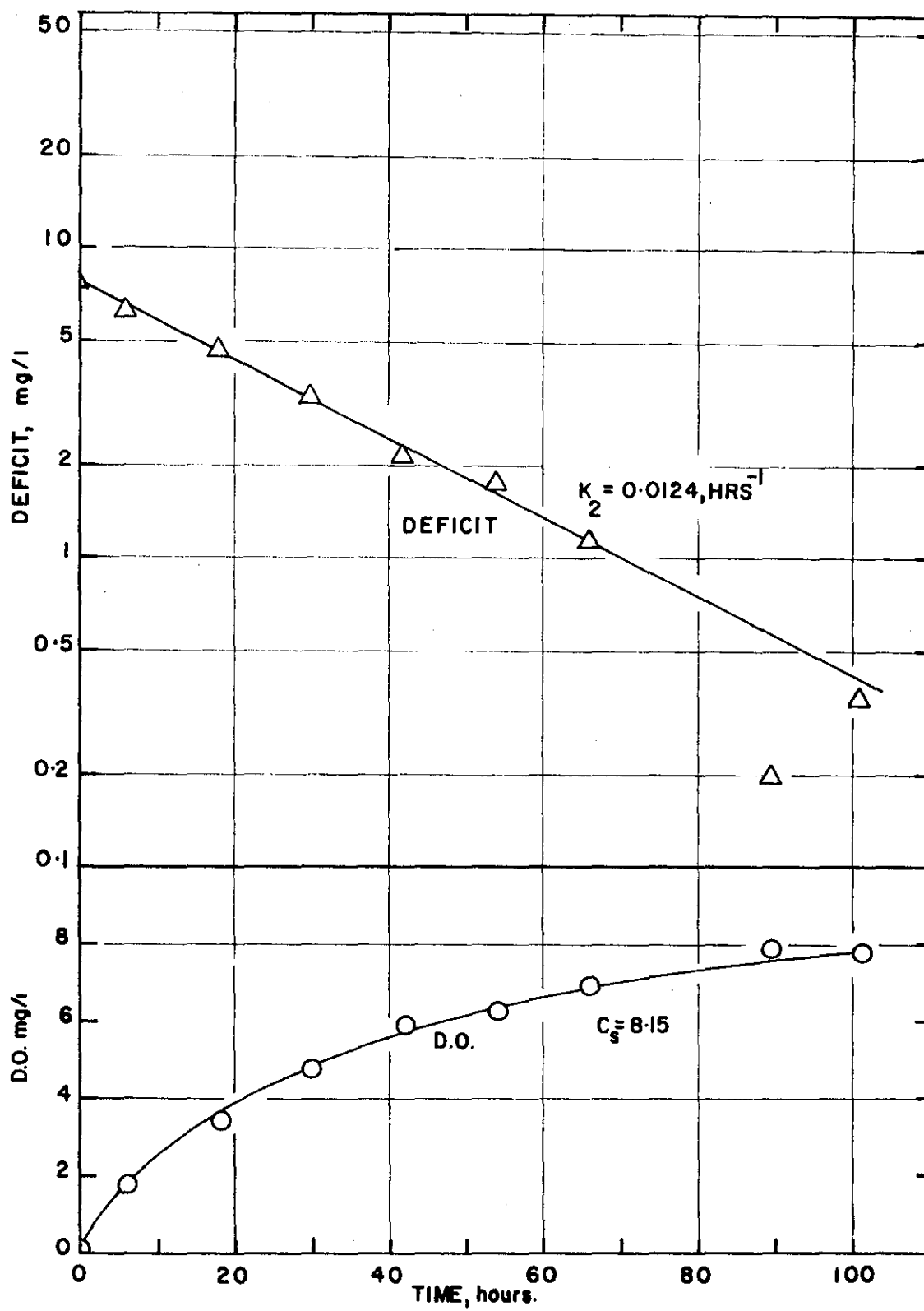


Figure 24 - Reaeration characteristics of Pond C.



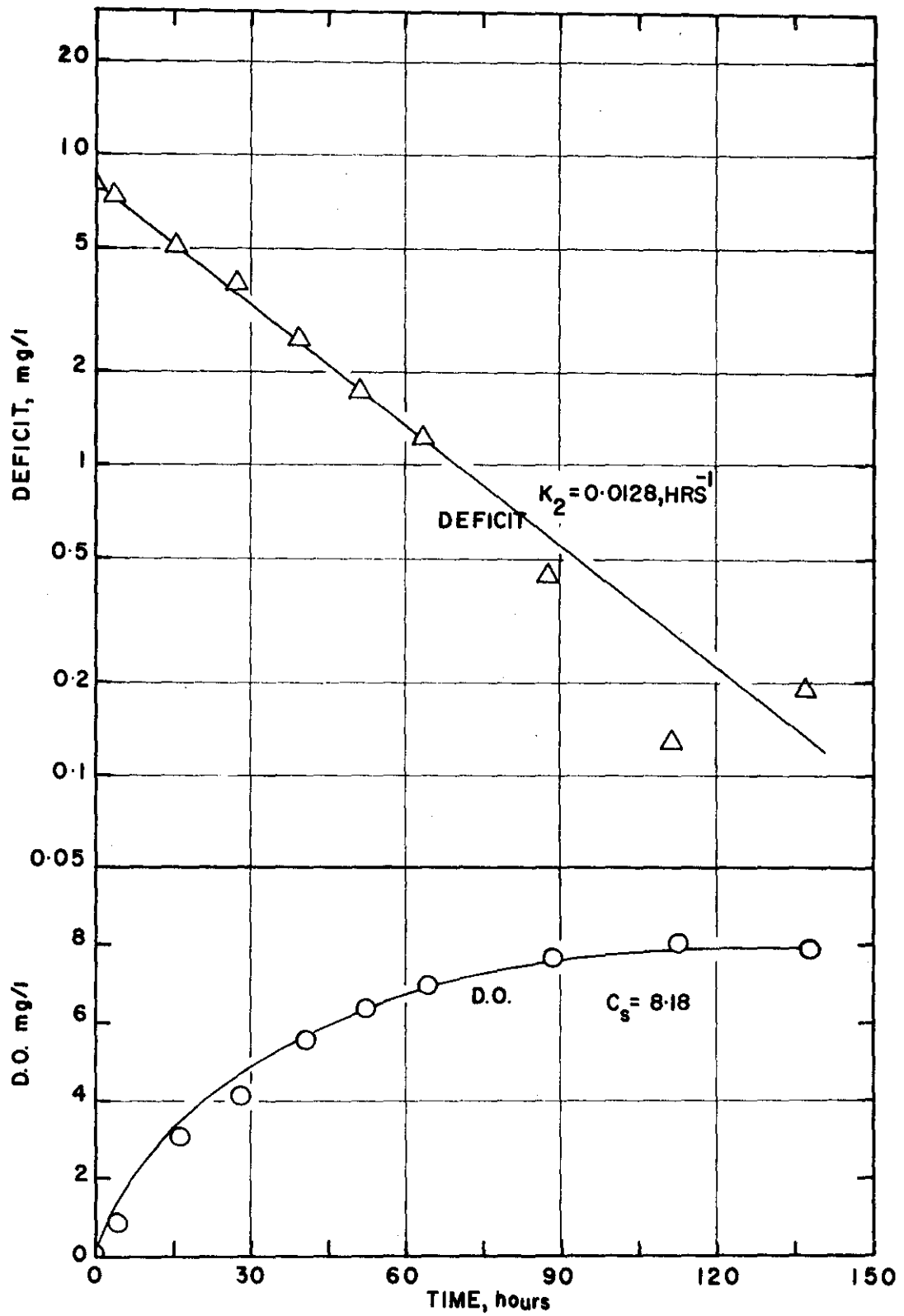


Figure 25 - Reaeration characteristics of Pond D.

systems to show the oxygen uptake curve. For the calculation of the uptake rate, the oxygen uptakes were plotted on semilogarithmic paper. The points for this plot were taken from the arithmetic plot of oxygen uptake. The slope of the line on semilogarithmic paper during the logarithmic growth phase (increasing first order rate) was calculated and designated as  $K_1$ .

Figure 26 shows the results of an experiment with 40 mg/l glucose. The  $K_1$  values for the pond were  $0.104 \text{ hr}^{-1}$ , and for the BOD bottles,  $0.082 \text{ hr}^{-1}$ .

Figure 27 shows the deoxygenation pattern in the pond for a duplicate run with the same substrate concentration (40 mg/l). The value of  $K_1$  for the pond for this experiment was  $0.113 \text{ hr}^{-1}$ .

Figures 28 and 29 show the results of experiments with a substrate loading of 60 mg/l. The  $K_1$  values (in  $\text{hr}^{-1}$ ) for the pond were 0.128 and 0.117, and for the BOD bottles they were 0.134 and 0.076, respectively. Figures 30 and 31 show the response of the systems to a loading of 80 mg/l. The deoxygenation constants were  $0.162$  and  $0.115 \text{ hr}^{-1}$  for the pond, and  $0.116$  and  $0.102 \text{ hr}^{-1}$  for the BOD bottles.

Figures 32 and 33 show the course of oxygen removal for a loading of 100 mg/l in both systems. The  $K_1$  values (in  $\text{hr}^{-1}$ ) were 0.161 and 0.135 for the pond, and 0.123 and 0.084 for the BOD bottles.

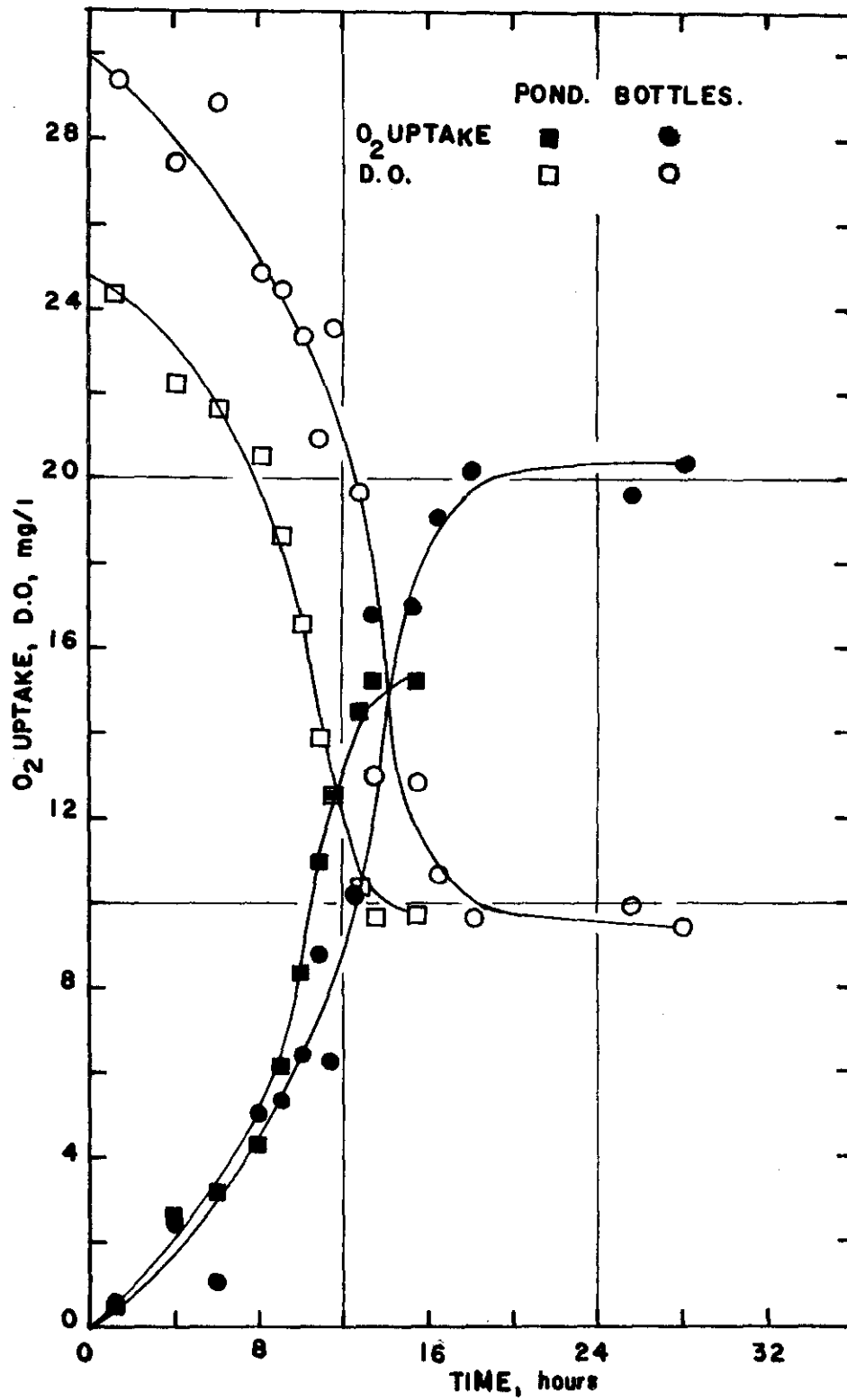


Figure 26 - Deoxygenation by organotrophs in systems with 40 mg/l glucose.

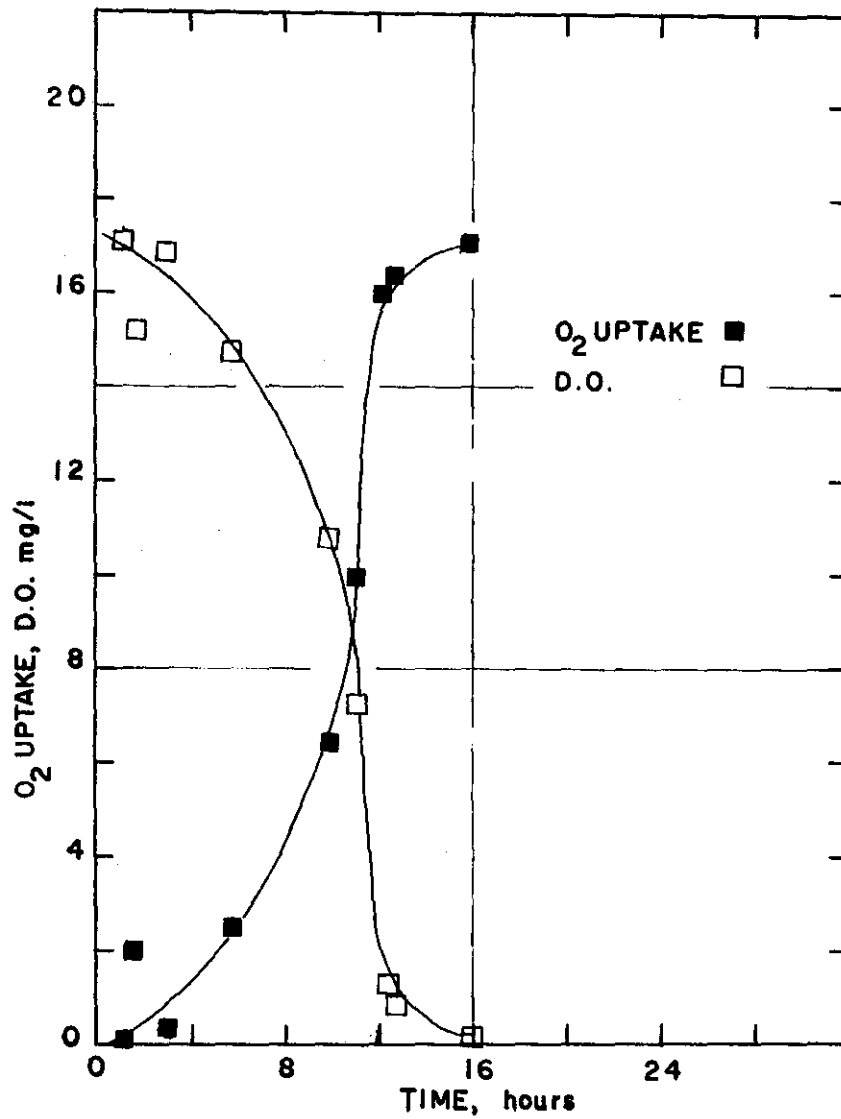


Figure 27 - Deoxygenation by organotrophs in pond with 40 mg/l glucose (duplicate run).

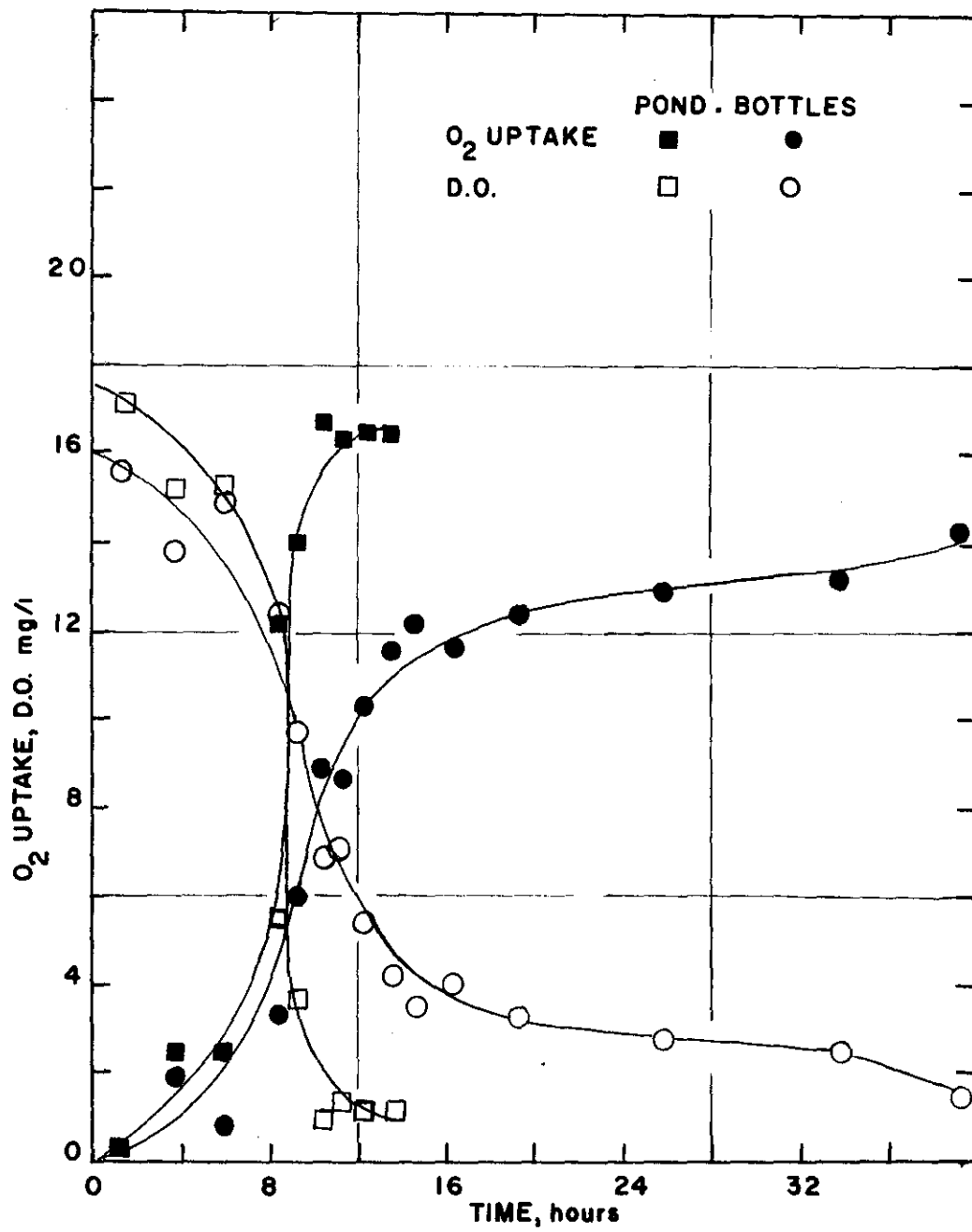


Figure 28 - Deoxygenation by organotrophs in systems with 60 mg/l glucose.

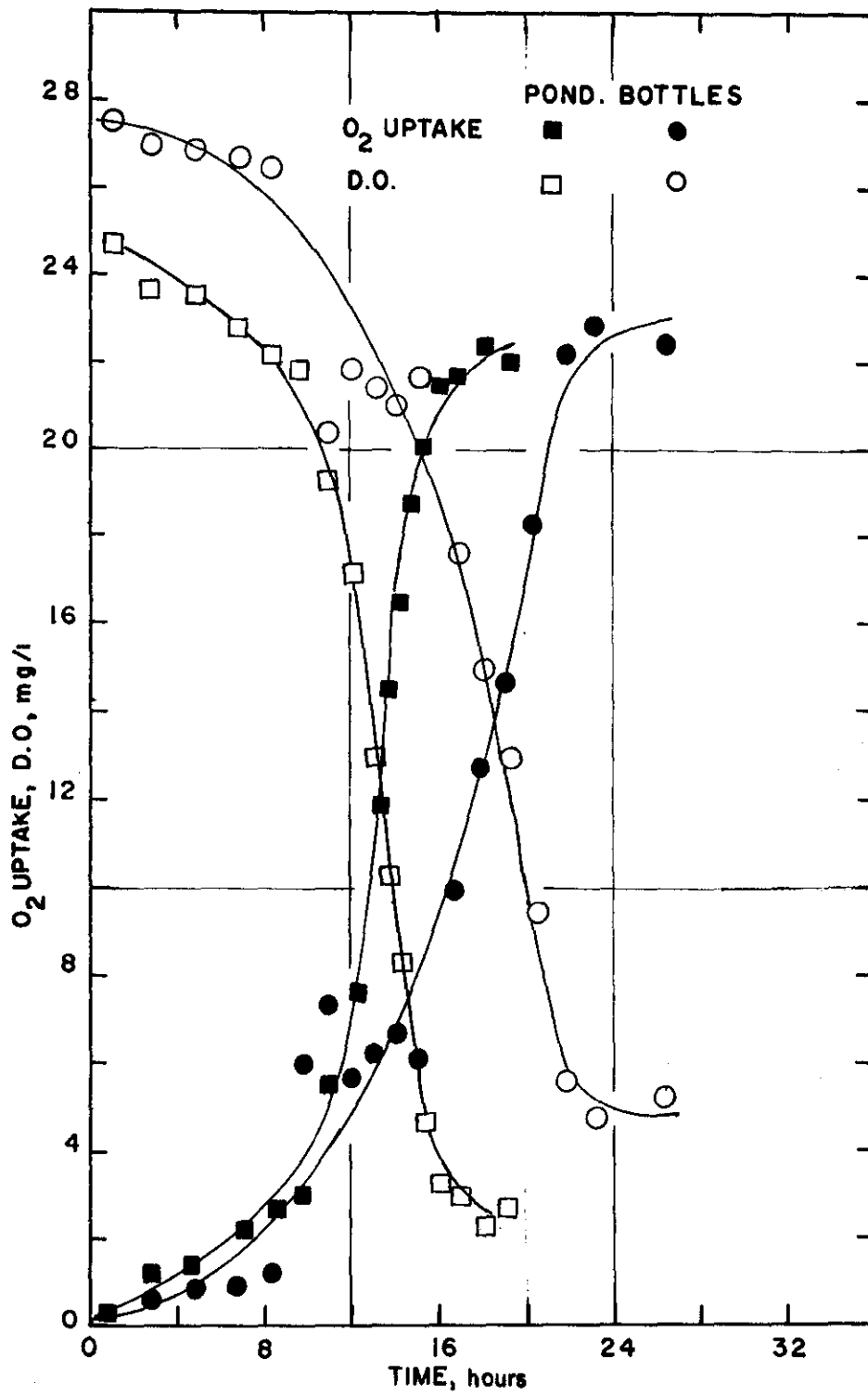


Figure 29 - Deoxygenation by organotrophs in systems with 60 mg/l glucose (duplicate run).

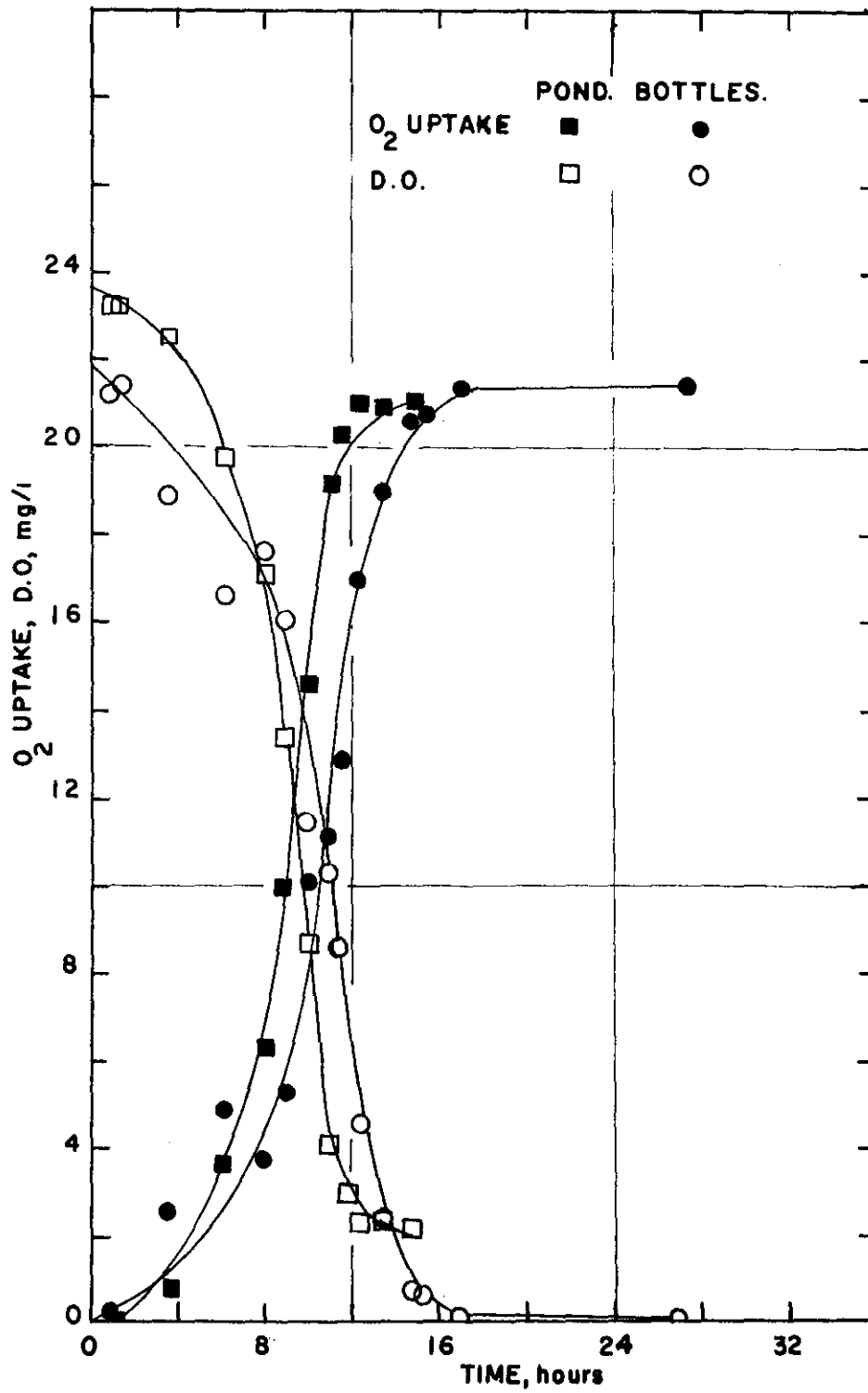


Figure 30 - Deoxygenation by organotrophs in systems with 80 mg/l glucose.

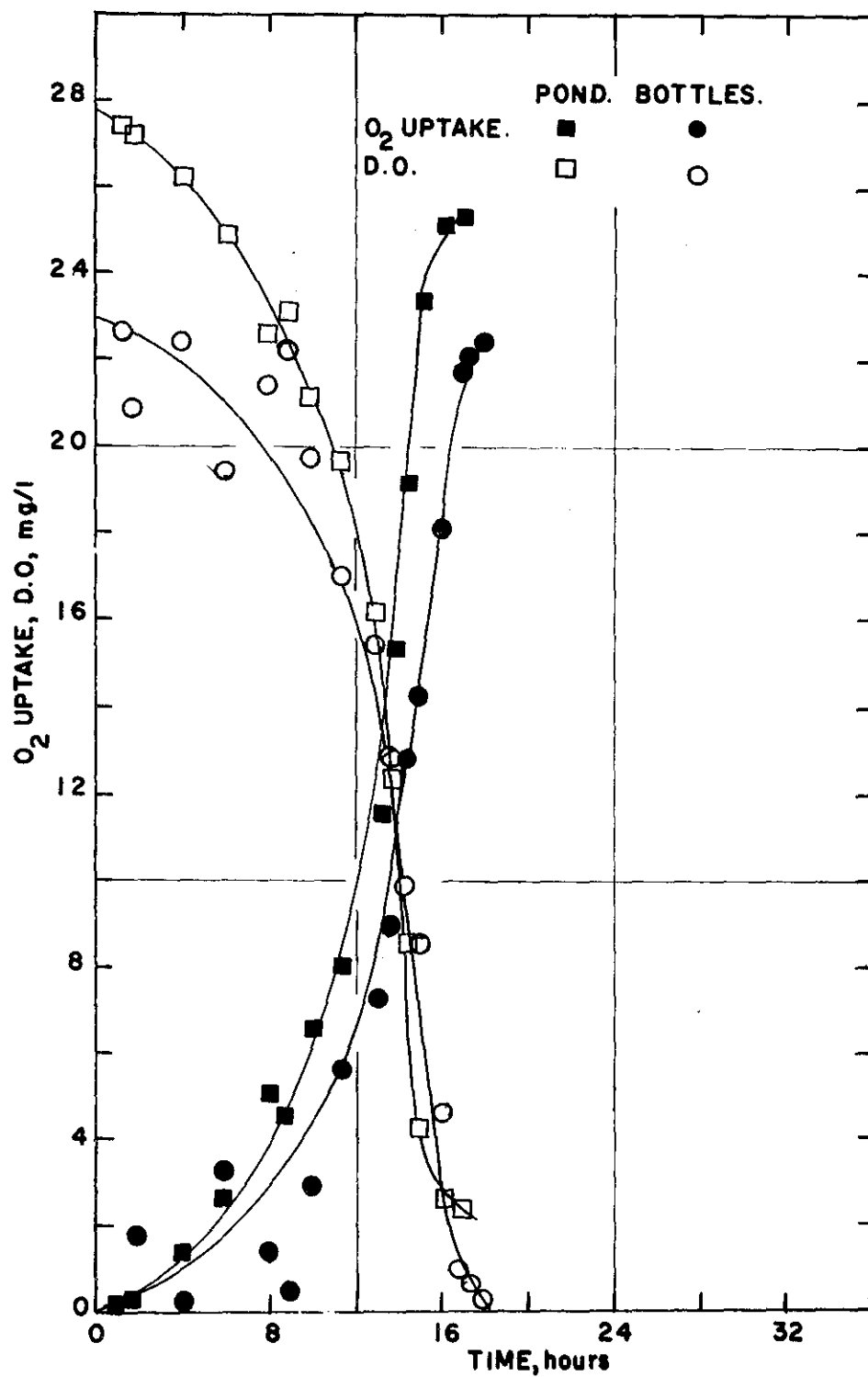


Figure 31 - Deoxygenation by organotrophs in systems with 80 mg/l glucose (duplicate run).



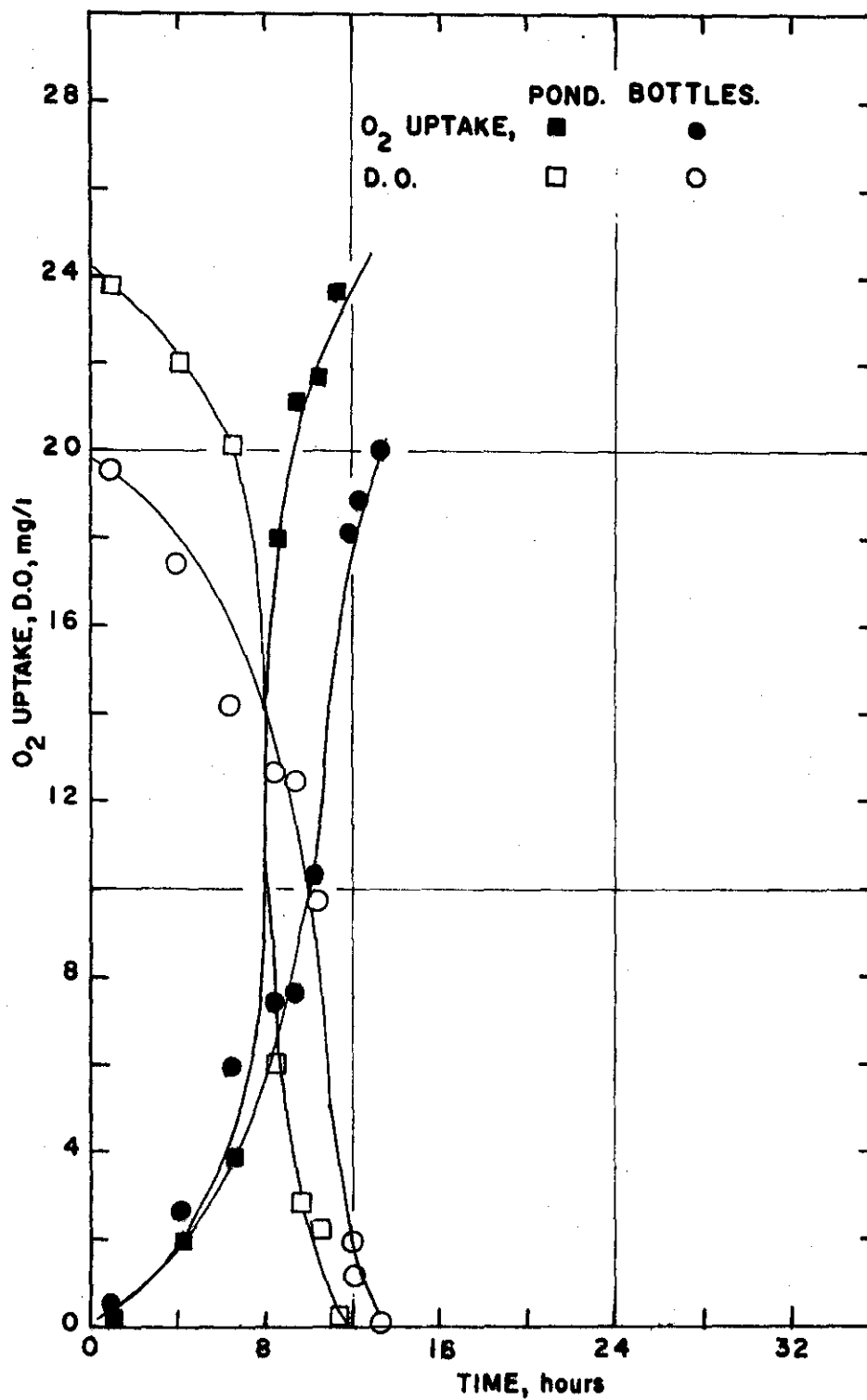


Figure 32 - Deoxygenation by organotrophs in systems with 100 mg/l glucose.

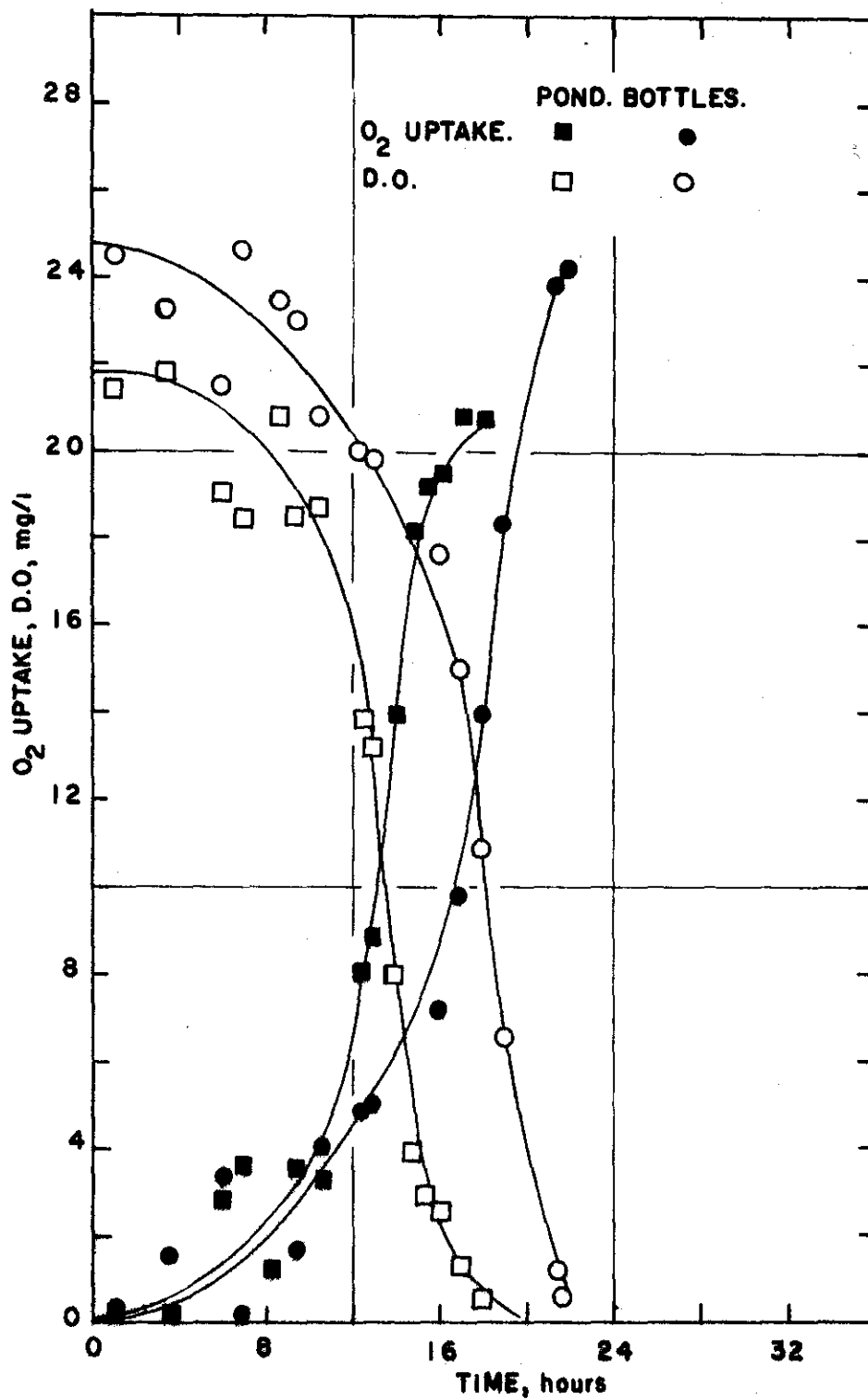


Figure 33 - Deoxygenation by organotrophs in systems with 100 mg/l glucose (duplicate run).

## B. Oxygen Utilization by Photo-autotrophs (*Chlorella pyrenoidosa*)

### Heterotrophic Metabolism

Figures 34 to 38 show the oxygen utilization and other related parameters for *Chlorella pyrenoidosa* in batch systems (Warburg flasks) containing an initial concentration of 500 mg/l glucose with various initial solids concentrations. Figure 34 shows the response of a system with an initial algal concentration of 896 mg/l. COD removal was accomplished within seven hours. The COD increased slightly for the next thirty-five hours, and then decreased slightly during the last six hours of the experiment. The solids increased considerably during the first seven hours. After reaching a maximum at nine hours, the biological solids concentration decreased a very small amount during the period in which the COD showed a slight increase.

The oxygen uptake curve followed zero order kinetics during active substrate removal, and then dropped off at a decreasing rate; however, during the last six hours the curve turned upward. In view of the decrease in both COD and solids during this period, it seems that the second stage of oxygen utilization might have proceeded due, partially, to secondary growth using the lysed products of the cells. The carbohydrate curve shows that metabolic intermediates and/or end products produced during active substrate removal were in very small amount, and that the residual COD was not due to carbohydrate.

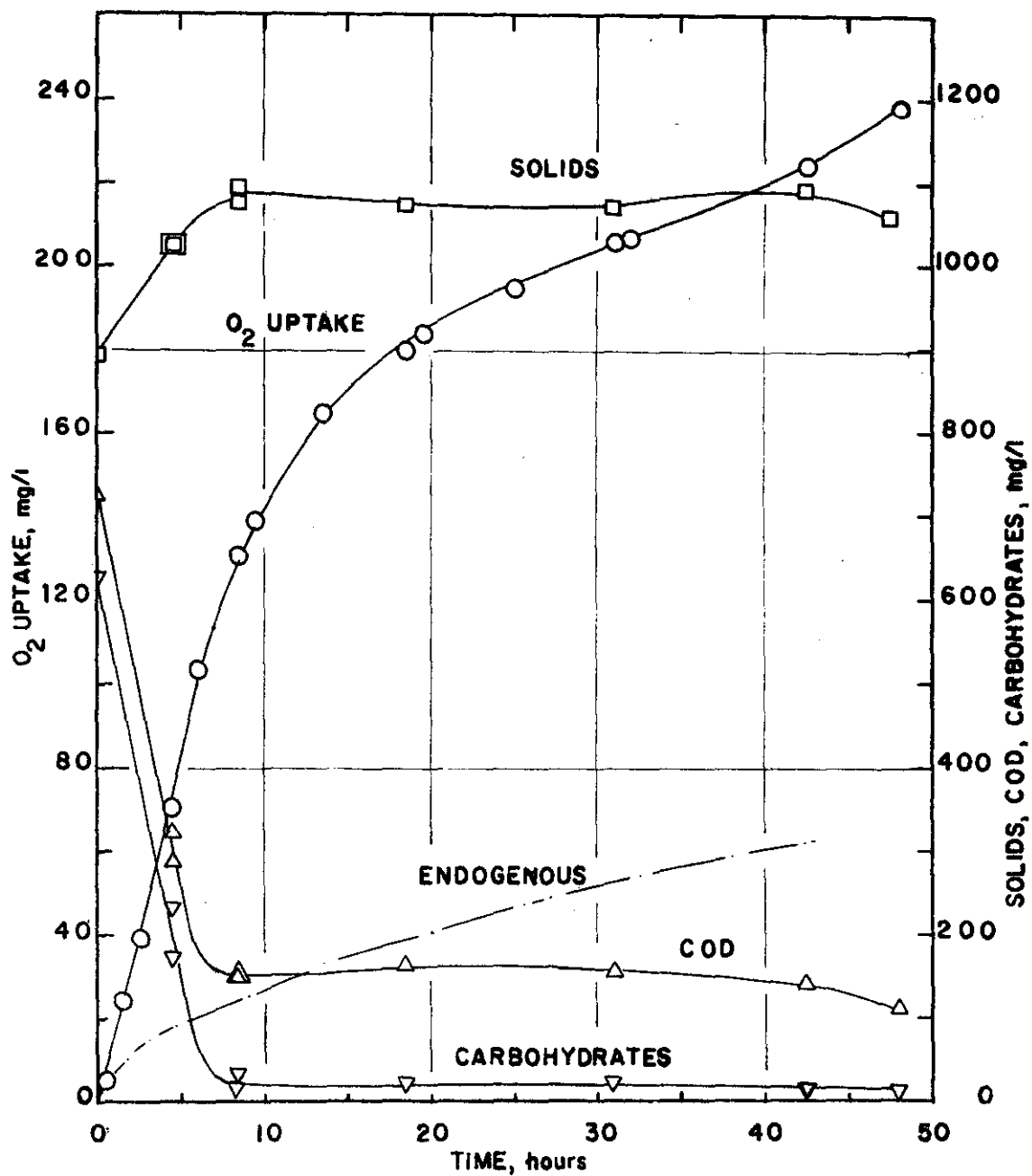


Figure 34 - Deoxygenation by photo-autotrophs;  
initial algal concentration 896 mg/l.

Figure 35 shows the response of a system with an initial algal concentration of 448 mg/l. The general pattern is the same as that for the previous system. The COD was removed in approximately eleven hours. The carbohydrate curve paralleled the COD curve, indicating no accumulation of metabolic products. The biological solids increased during the period of substrate removal, and then leveled off. The oxygen uptake curve indicated a slightly increasing rate for eleven hours, and a decreasing rate thereafter.

Figure 36 shows the response of a system with an initial algal concentration of 400 mg/l. The performance was very similar to the system described in Figure 35. The increasing trend in the oxygen uptake rate was more pronounced during the period of active substrate removal, and after removal of the COD the rate was linear.

Figure 37 shows the response of a system with an initial algal concentration of 215 mg/l. The active substrate removal period was twelve and one-half hours. There were no metabolic intermediates or end products during this active substrate removal, as indicated by comparison of carbohydrate and COD curves. The small residual COD and carryover COD were not due to carbohydrates. The biological solids concentration increased until the end of the COD removal period. Immediately after attainment of the maximum value, the solids concentration decreased slightly and thereafter rose slightly. The oxygen uptake curve indicates that a logarithmic growth phase was attained. After

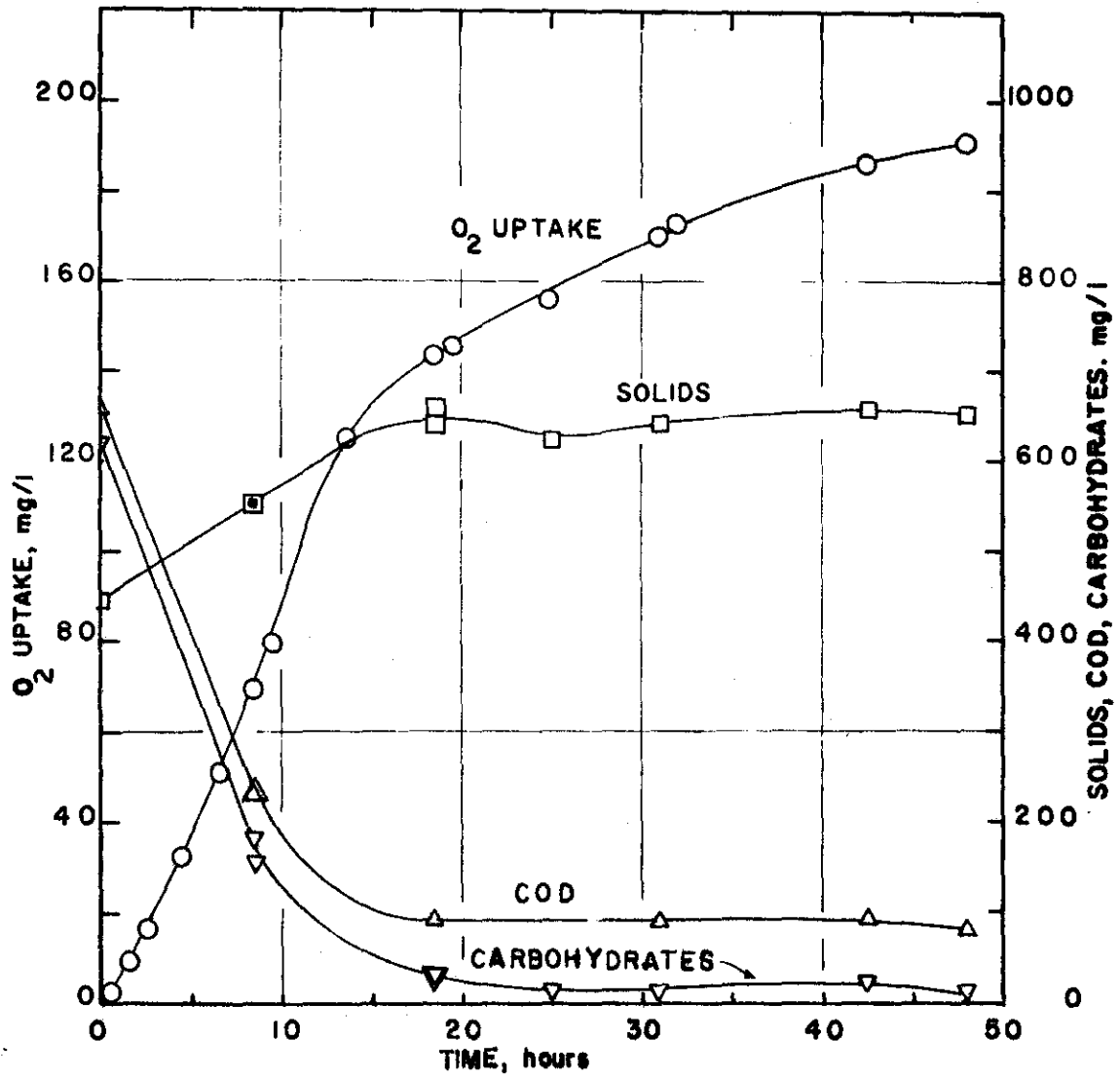


Figure 35 - Deoxygenation by photo-autotrophs; initial algal concentration 448 mg/l.

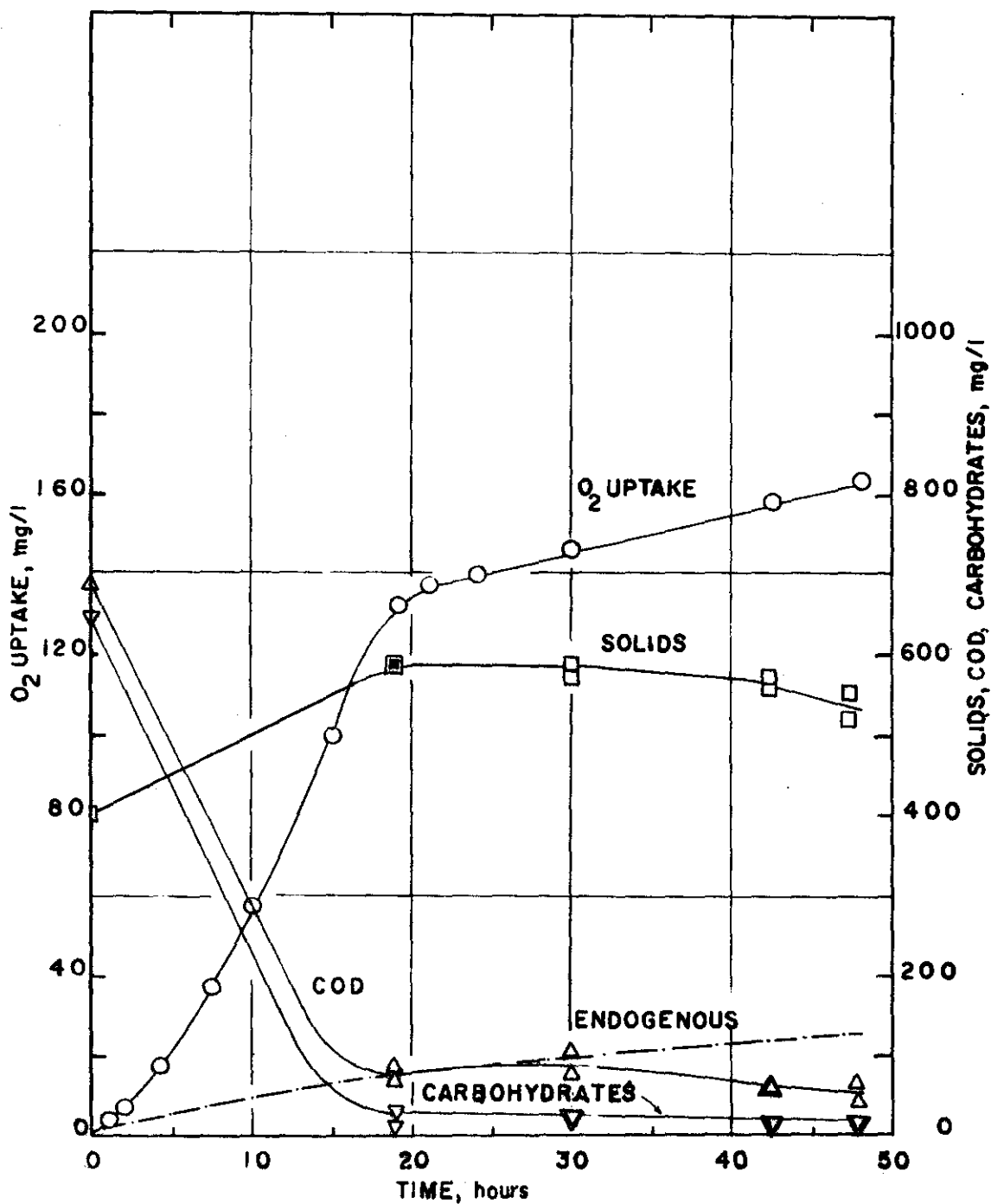


Figure 36 - Deoxygenation by photo-autotrophs; initial algal concentration 400 mg/l.

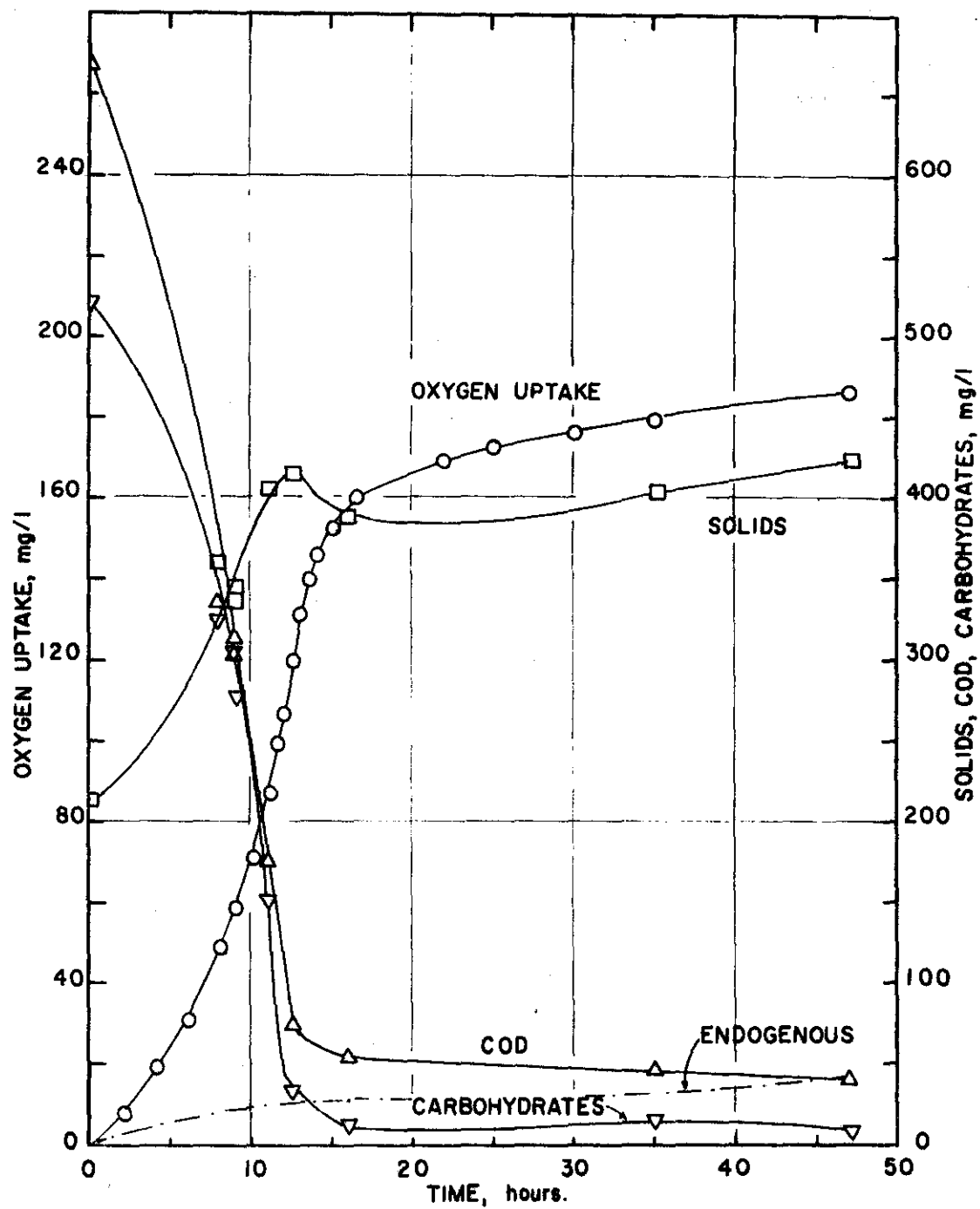


Figure 37 - Deoxygenation by photo-autotrophs; initial algal concentration 215 mg/l.



twenty-four hours the  $O_2$  uptake rate was more or less constant.

Figure 38 shows the response of a system with an initial algal concentration of 113 mg/l. The behavior was very similar to the system shown in Figure 37. The period of substrate removal and algal growth were prolonged to fifteen hours, compared to twelve and one-half hours in the previous system.

#### Endogenous Metabolism

Figures 39, 40, and 41 show the oxygen uptake and other related parameters for Chlorella pyrenoidosa during endogenous metabolism with various initial solid concentrations. Figure 39 shows the endogenous oxygen uptake, solids concentration and filtrate COD with respect to time for a system with an initial solids concentration of 1325 mg/l. Eight Warburg flasks were used in the beginning of the run. After 24, 48, and 96 hours, duplicate flasks were removed, and one of each was removed at 72 and at 120 hours for solids and COD determinations. The arrows on the oxygen uptake curve show the time of removal of flasks. The biological solids concentration gradually decreased from 1325 to 1164 mg/l, a decrease of 161 mg/l. The filtrate COD increased by approximately 20 mg/l.

The oxygen uptake in all flasks was uniform. For example, after an elapsed time of seventy hours, the cumulative oxygen uptakes in the four remaining flasks were 126, 120, 118, and 117 mg/l. The cumulative uptake in the

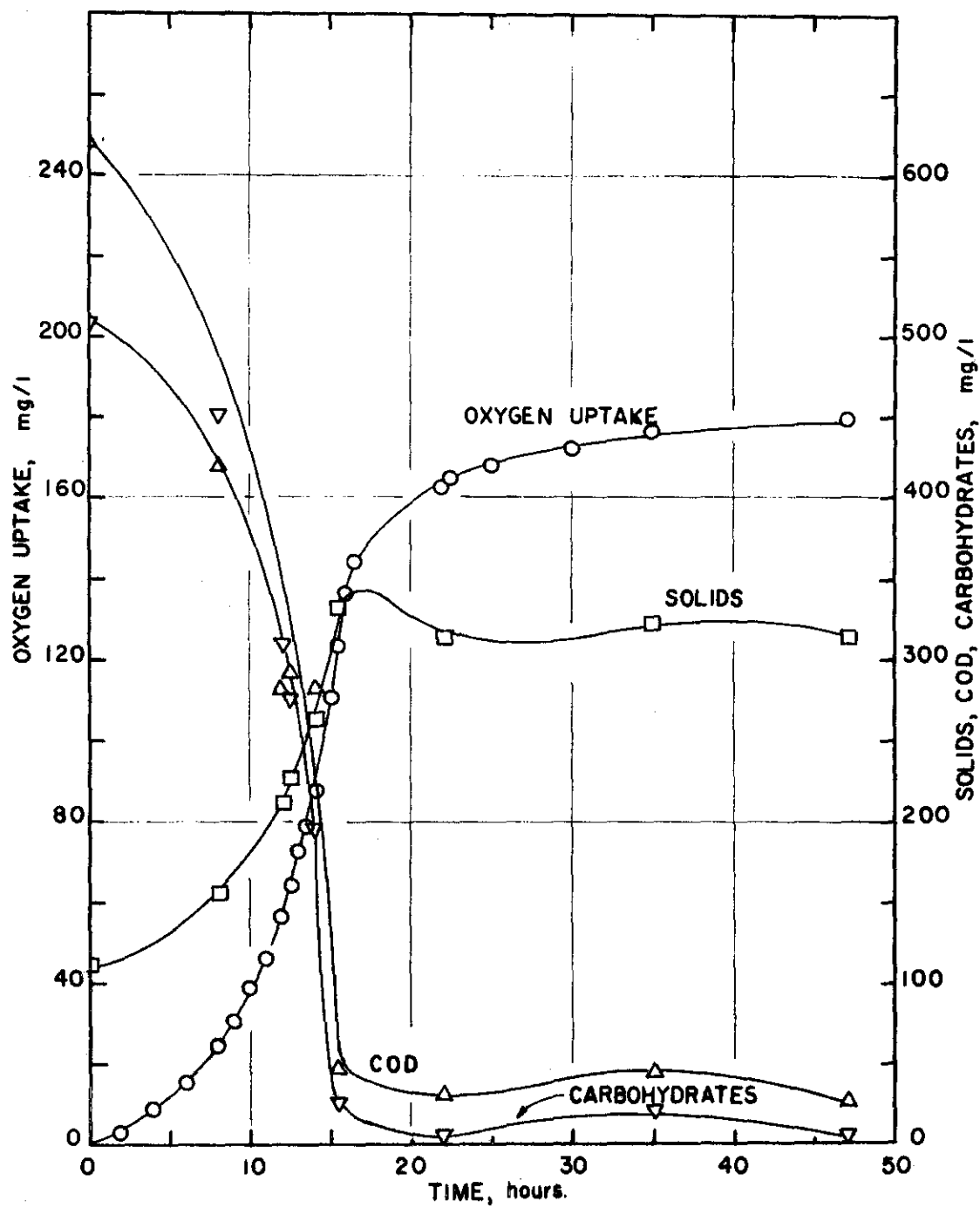


Figure 38 - Deoxygenation by photo-autotrophs; initial algal concentration 113 mg/l.

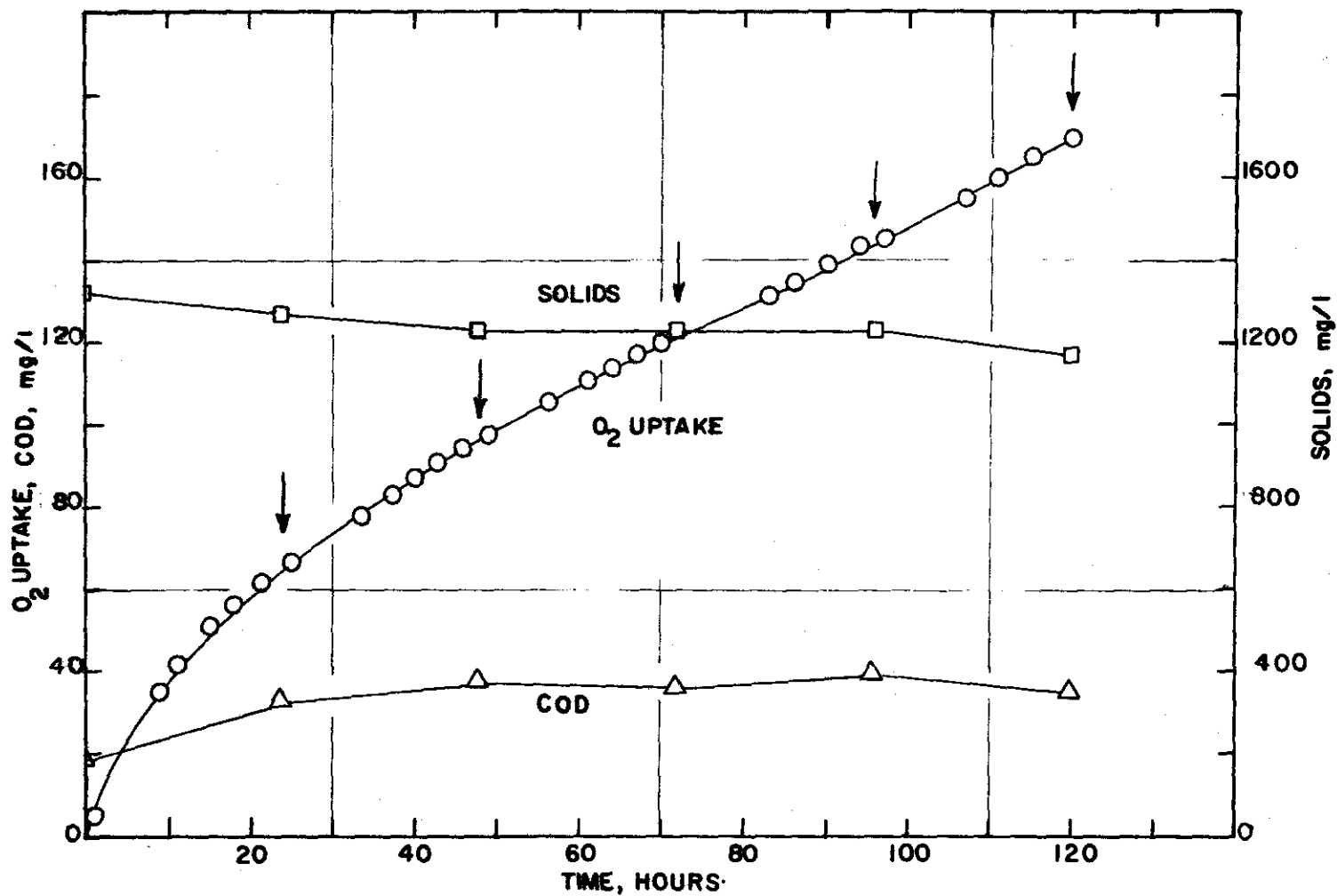


Figure 39 - Endogenous metabolism of *Chlorella pyrenoidosa*; initial algal concentration 1325 mg/l.

flask that remained at 120 hours was 170 mg/l. The oxygen uptake curve appears to follow a first order decreasing rate until eighty hours. Using the  $\alpha$  method described in detail elsewhere (81), the ultimate demand was calculated by selecting three points within the 80-hour range. On this basis the ultimate demand was 195 mg/l, and the rate constant was  $0.0056 \text{ hr}^{-1}$ . As a verification of the kinetic mode of the oxygen uptake curve, the observed  $\text{O}_2$  uptake values at various times were deducted from the ultimate demand of 195 mg/l, and were plotted on semi-logarithmic paper. The points, up to ninety hours, did fall on a straight line. The calculated slope of that line, the rate constant, was  $0.0054 \text{ hr}^{-1}$ .

Figure 40 shows the endogenous oxygen uptake for systems with final algal concentrations of 532, 316, and 220 mg/l. Five Warburg flasks were set up for each system. One flask in each system was removed at 60, 88, 120, 144, and 168 hours for determination of solids and filtrate COD. The oxygen uptakes were uniform in all flasks belonging to the same group. For the system with final solids of 532 mg/l, after 88 hours the cumulative oxygen uptake values for the remaining flasks were 73, 71, 75, and 74 mg/l. The cumulative uptakes in one week's time (168 hours) were 116, 45, and 21 mg/l, respectively, for the three systems. Here, also, the first portion of the curves follows a first order decreasing trend. Using the same method of calculation as in the previous case, the rate constants were calculated.

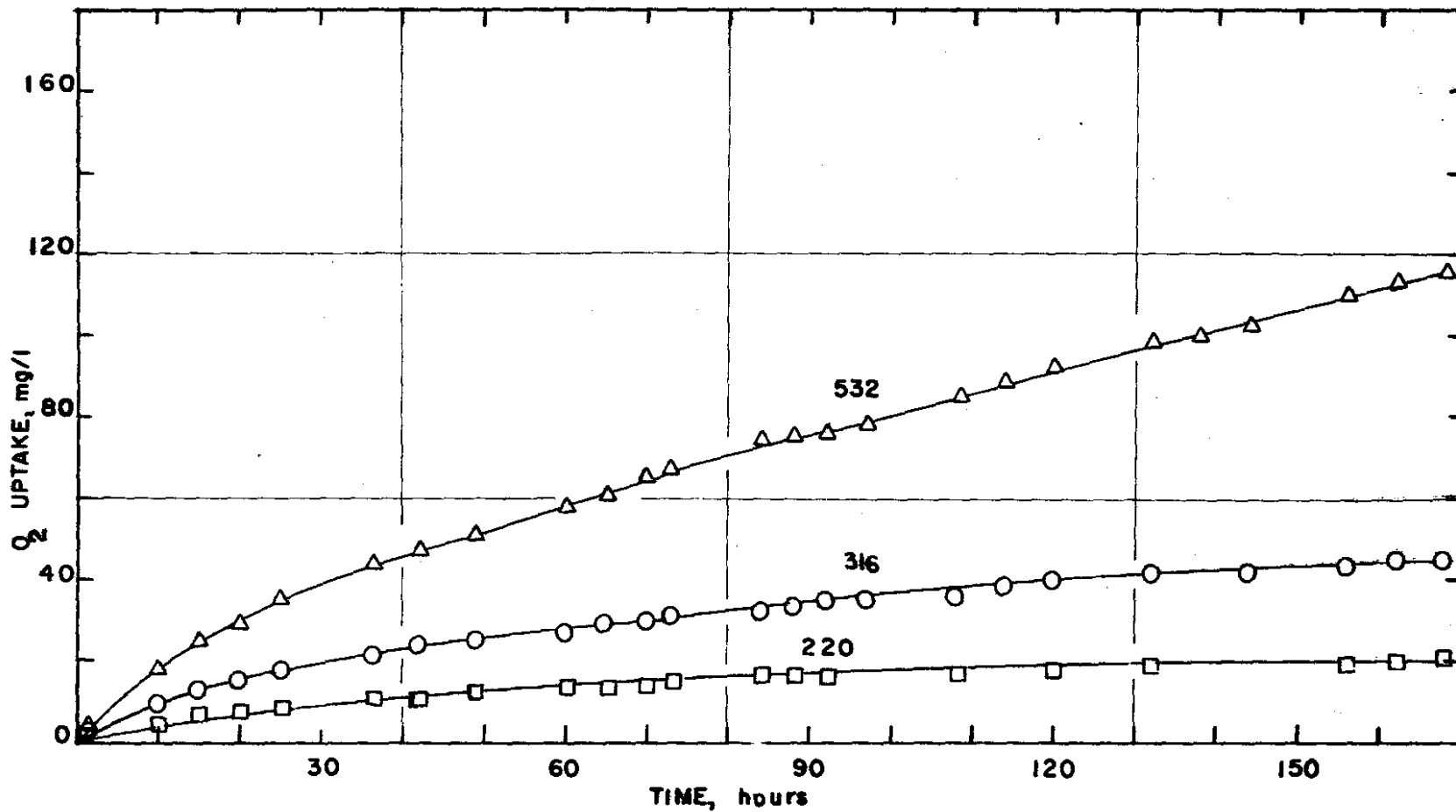


Figure 40 - Endogenous oxygen uptakes for indicated algal concentrations.

They were 0.0051, 0.0061, and 0.0063 hr<sup>-1</sup>, respectively.

Figure 41 shows the biological solids and filtrate COD values for the three systems. The changes in solids and COD concentrations were very small. However, the general pattern of solids shows a slight reduction for the 532 mg/l system, no change in the 316 mg/l system, and a slight increase in solids concentration for the 220 mg/l system. The filtrate COD increased slightly for the high solids system, whereas in the other two there was a small decrease in filtrate COD during the course of endogenous respiration.

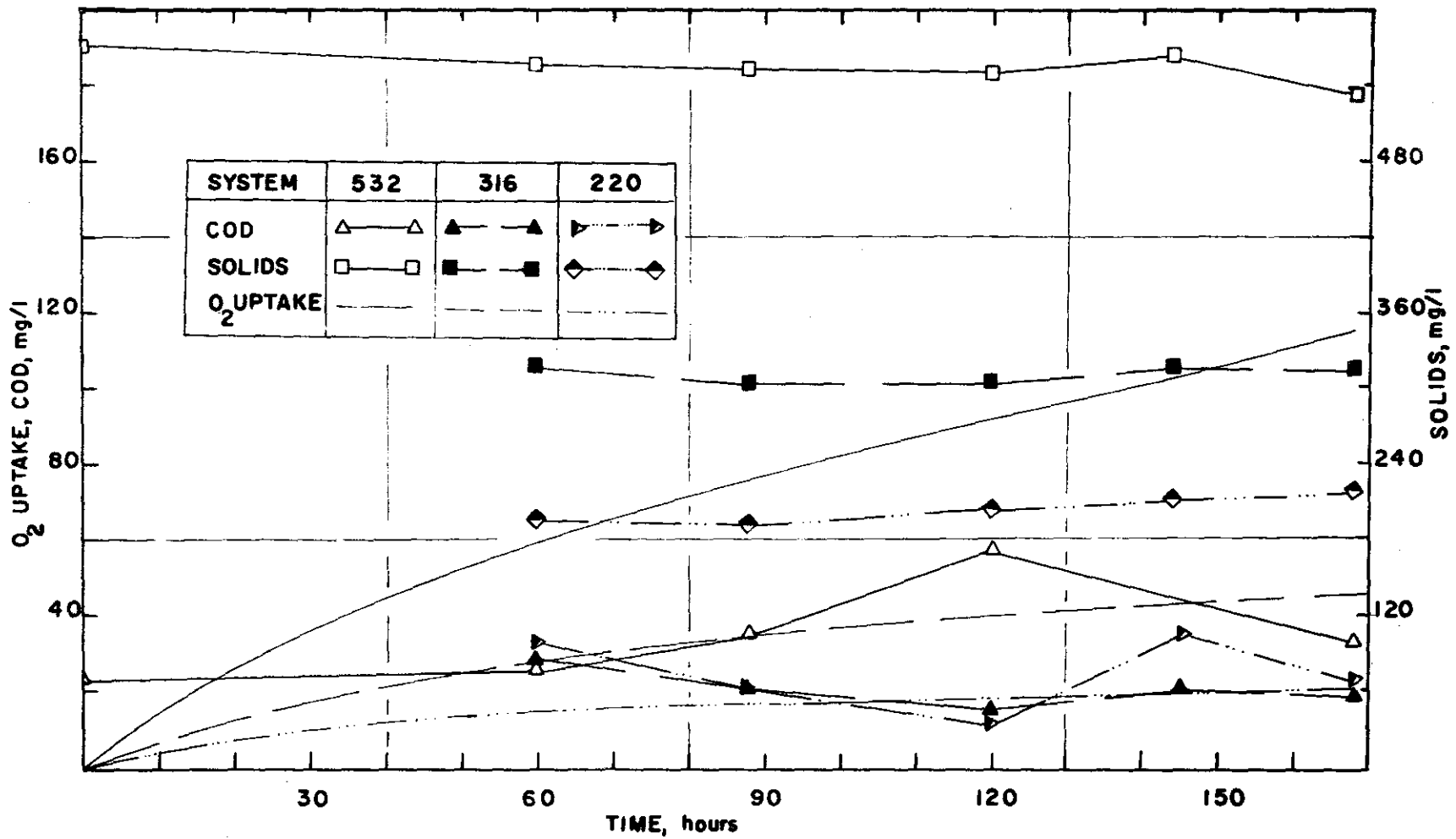


Figure 41 - Endogenous metabolism of *Chlorella pyrenoidosa* in systems with indicated algal concentrations.

## CHAPTER VI

### ANALYSIS OF RESULTS AND DISCUSSION

The effect of free gas transfer across the liquid surface interface of the pond on the performance of the experimental units can be evaluated by comparison of substrate removal efficiencies at the same loading levels when the free transfer was unobstructed and when it was totally prevented. For such a comparison, results of the experiments with feed concentrations of 100, 150, and 250 mg/l glucose in open and closed systems are considered.

The response of the open system to a loading of 100 mg/l glucose (Figure 7) showed two patterns. The dissolved oxygen levels at the end of light and dark periods, and the changes in DO were uniform during the first ten days of operation. Even though these parameters were also uniform in the last five days of operation, after the fifteenth day their values were quite different from those of the first ten days of operation. For example, the average DO change in the first ten days was approximately 7.5 mg/l, compared to 4.5 mg/l in the last five days. The biological solids and the oxidation reduction potential showed a steady decline from the eleventh day onward, and both reached minimum values at the end of the fourteenth day. The



decrease in solids might have been due to the buildup of autotoxic material (31) (48) (62) (63), or due to the constituents brought in by the tap water (71). Since there was a change in the color of the mixed liquor (darker to lighter green) there is some indication that both algal and bacterial solids were decreased. The decreased oxygen production in the last five days also indicated the possibility of a decrease in algal concentration. The increase in the biological solids after the fifteenth day might have been due mainly to bacteria, as the COD was reduced considerably during the period corresponding to the solids increase and since there was neither an increase in dissolved oxygen production nor an appreciable change in the intensity of the green color of the mixed liquor. The first ten days of operation were taken as the period of most "balanced operation," and the average for various parameters in that period are given in Table III.

In the open pond which received 150 mg/l glucose (Figure 8), all parameters were more or less steady except ORP. The ORP increased as the run progressed, indicating a decrease in the reductants and an increase in the oxidized materials (e.g., nitrate, although such analysis was not run). The last seven days of the run were taken as the most representative period of uniform operation, and the averages of those parameters are given in Table III. The open pond subjected to a loading of 250 mg/l glucose (Figure 9) behaved satisfactorily for five days, but after

TABLE III  
 AVERAGE VALUES OF PARAMETERS DURING BALANCED OPERATION  
 IN CONTINUOUS FLOW PONDS

Parameters Averaged	Open System			Closed System					
	100	150	250	100			150	250	
				14th to 23rd	24th to 36th	14th to 36th		0-9 days	Regenerated
Figures, #	7	8	9	11	12	11, 12	14	15	17
Maximum DO, mg/l	9.6	4.7	10.4	33.1	22.6	27.1	30.1	34.4	13.9
Minimum DO "	2.1	0.4	0	18.0	12.8	15.0	11.0	10.9	0.3
Daily Change "	7.5	4.3	10.4	15.1	9.8	12.1	14.1	23.5	13.5
Biological Solids "	91.9	91.5	50.0	57.0	50.0	53.0	59.0	73.0	73.0
Effluent COD "	114.1	90.5	64.5	48.0	45.0	46.6	65.0	40.0	56.0
COD Removal %	0	46.0	76.0	58.0	60.5	59.0	61.0	86.0	80.0

ten days the system failed with regard to oxygen production. The DO increase was more or less uniform in the first five days, and in the second five days it decreased progressively until it reached zero. The biological solids concentration was greater, whereas the pH level was lower in the second five days compared to the first five days of operation. The increase in solids in the latter half of the run was probably due to bacterial growth, since there was no corresponding DO increase in the light period, as would be expected from a greater algal population. This increase in bacterial cells could be expected to cause greater turbidity which, in turn, would further reduce the algal growth and oxygen production, finally bringing the DO level to zero. The reduced algal activity could have resulted in the slightly lower pH level in the second half of the operational period. It is suggested that the sequence of events described above is typical of the progressive deterioration of efficiency in oxidation ponds approaching overloaded conditions. The data of the first five days were taken as being representative of "balanced operation," and the average values are compared to those for the other systems in Table III.

The average DO change in the first nine days of operation for the closed pond at a loading of 100 mg/l glucose (Figures 10, 11, 12) was lower in comparison with the daily DO changes observed thereafter. Since there was no increase in the total biological solids concentration (in fact,

there was a slight reduction) during the latter part of the run, it is possible that the biomass might have consisted of more bacteria than algae in the initial stages. The capacity of bacteria to grow faster than the algae (e.g.,  $\mu = 0.18 - 0.15 \text{ hr}^{-1}$  for bacteria vs.  $0.09 \text{ hr}^{-1}$  for algae) might have caused this. As pointed out in the previous chapter, there were two periods of "balanced operation:" one for nine days, and the other for twelve days. The average values for a 22-day period consisting of these two "balanced operation" periods are given in Table III. The smaller changes in daily DO levels at the beginning of the run were also observed in the closed pond with the influent concentration of 150 mg/l (Figures 13, 14). "Balanced operation" was attained after ten days of operation until the end of the run. The behavior of the system on the thirteenth and fourteenth days was somewhat unique. During the light period of the thirteenth day the DO increase was small, almost half, compared to the increase for both the preceding and following days. This resulted in a lower DO level at the end of the succeeding dark period. The ORP also dropped considerably during the light period on the thirteenth day. For some (unknown) reason the system was thrown out of balance temporarily on that day. Possibly the algal metabolism might have been retarded on that particular day by certain metabolic products in the pond. The algal activity, indicated by the increase in DO during the light period, on the next (fourteenth) day was the maximum

attained - 18.2 mg/l against an average of 14.1 mg/l - during the period of "balanced operation." This was further reflected by a sharp increase in pH during the same period.

The closed pond which received 250 mg/l of glucose (System I, Figures 15 and 16) operated under "balanced conditions" for the first nine days. The dark green color of the reactor liquor became yellowish-brown after nine days, and the pond began to fail with respect to maintenance of oxygen level. The highest DO concentration, measured in this series of investigations, 38 mg/l, was recorded in this system, and the COD removal efficiency was 86 per cent. The observed decrease in DO levels and the subsequent failure of the system during the second half of the run was due to the reduced production of oxygen during the light rather than to any increased utilization of  $O_2$  in the dark. The "regenerated" system, which received the same load, 250 mg/l glucose (Figure 17) was light green in color and responded more or less in a uniform manner throughout the period of observation. The DO levels at the end of the dark period were close to zero (on two days zero readings were recorded) which indicated that the system was under maximum possible load for maintenance of aerobic conditions. The sharp decrease in ORP for short durations and the subsequent recovery to the original level may also be taken as an indication that the system was delicately balanced with respect to maintenance of aerobic conditions at this loading level. Average values of the operational parameters of

this system are given in Table III. A closed pond operated at an equivalent loading factor (twice the detention time and double the organic loading -- twenty days detention and 500 mg/l glucose) was completely anaerobic (Figure 18). The COD removal decreased from 88 per cent to 77 per cent during the period of observation. The maximum ORP was -50 millivolts at the end of the light period on the second day.

Comparison of parameters (Table III) for the experiments on the open system (Figures 7, 8, 9) would enable assessment of the effect of organic loading on the performance of the ponds. As the influent load was increased from 100 to 150 and 250 mg/l glucose in the three open pond systems, the intensity of the green color decreased. The biological solids level decreased surprisingly; values of 91.9, 91.5, and 50.0 mg/l, respectively, were recorded in the three systems. However, the decrease in solids level did not lead to a decrease in COD removal. The COD of the effluent filtrates were 114, 91, and 65 mg/l, corresponding to COD removals of 0, 46, and 76 per cent. It seems quite obvious from these results that at the lower organic loading more carbon was fixed than was removed. Whereas the carbon balances in these systems cannot be made, the two measured parameters, effluent COD, and biological solids, may be employed to obtain a rough idea of the sources and sinks of carbon.

Although the elemental compositions for different species are different and vary even within the species,

depending on growth environment, an average figure of 51 per cent of the weight of the biological solids may be attributed to carbon (31)(90). Since the value of the influent COD and the nature of the substrate were known, the influent organic carbon can be estimated fairly accurately by multiplying the average influent COD values by a factor 12/32 (192 mg of oxygen is required to oxidize 72 mg of carbon in glucose). However, determination of the organic carbon of the effluent may not be done accurately, because of the uncertainty of the nature of the effluent. Spot checks for glucose by the Glucostat test showed the absence of glucose. As seen in the literature review (55)(56)(57)(59)(60), the major excretory products of algae have been reported to be polysaccharides and organic acids of low molecular weights. Studies in this laboratory (91)(92)(93) showed that acetic acid was a major constituent in the intermediate products when heterogeneous bacteria metabolized glucose. Taking into account all of these points, a multiplication factor of 12/32 may be used for the estimation of effluent COD. (It might be noted that the carbon equivalent of acetic acid COD is also 12/32) Furthermore, since these calculations are for a comparison between systems under various loadings (for all of which the same method is adopted), any possible difference in the true value of organic carbon and the calculated values will apply to all systems and will

not significantly affect the comparison. On this basis the carbon contents in the solids of the three systems were 46.9, 46.6, and 25.5 mg/l. The carbon equivalents of the effluent COD were 42.8, 33.9, and 24.2 mg/l for systems with the influent loading of 100, 150, and 250 mg/l glucose, respectively. Addition of the carbon equivalents of effluent COD and solids yield 89.7, 80.5, and 49.7 mg/l output against an input of 42.8, 63, and 102 mg/l. These figures indicate a carbon addition of 46.9 and 17.5 mg/l in systems loaded with 100 and 150 mg/l glucose, and a carbon decrease of 52.3 mg/l in the system receiving glucose at 250 mg/l. The organic carbon addition could arise by  $\text{CO}_2$  removed from the carbonate system of the medium and by the fixation of  $\text{CO}_2$  from the atmosphere. The presence of 100 mg/l sodium bicarbonate in the feed could contribute carbon to a maximum amount of 14.3 mg/l ( $100 \times 12/72$ ). Under the conditions of the closed pond experiments (when the carbon exchange with the atmosphere could not take place and the only additional source other than that provided by the feed was the carbonate system), the only observed contribution from the carbonate system was 1.8 mg/l of carbon. Therefore, it may be discerned that the addition of carbon to the system at lower loadings was primarily due to the fixation of atmospheric carbon dioxide. Theoretical possibilities and indications in field observations (9) (31) (75) (76) (77) (78) to this effect were pointed out earlier. Such an effect occurs only in ponds subjected to low organic



loadings. As the complement of metabolically produced  $\text{CO}_2$  increases (as it would be expected to at higher loadings), the carbon addition effect is decreased (e.g., 150 mg/l system), and finally a carbon loss is registered (e.g., 250 mg/l system). From this discussion the evidence seems to indicate that atmospheric carbon dioxide is probably fixed by algae in the open ponds, and that this is more significant at lower loading levels, and this process decreases the overall efficiency of the ponds.

A scrutiny of the performance of the closed pond (Figures 10, 11, 12, 13, 14, 15, 16, 17) will serve to reinforce the analyses and tentative conclusions cited above. In the closed ponds the COD removal efficiencies were 59, 61, and 83 (average of 86 and 80 in two systems) percent in systems with influent loadings of 100, 150, and 250 mg/l glucose, respectively. The biological solids also exhibited an increasing trend; 53, 59, and 73 mg/l. Using the same method of calculation to account for the total carbon as in the previous section, the outgoing carbon concentrations were 44.6, 54.5, and 52.2 mg/l compared to incoming carbon of 42.8, 63, and 102 mg/l. These figures indicate that the carbon in the carbonate system was not needed for solids production except possibly at the lower loading (100 mg/l glucose). On the contrary, the reduction of organic carbon in the 150 and 250 mg/l systems (as well as in the 250 mg/l open pond system) indicate that metabolic  $\text{CO}_2$  would have increased the free  $\text{CO}_2$  in the

medium and the associated carbonates.

Considering the two systems, open and closed, which were subjected to the same organic loading, the effectiveness of closing the pond can be evaluated. In the open system with 100 mg/l glucose loading (Figure 7), the influent and effluent COD were the same, whereas 50 per cent COD removal was attained in the closed pond (Figures 11 and 12). The solids concentration in the open pond was 92 mg/l versus 53 mg/l in the closed pond. On this basis, the open system can be termed a "polluting system" rather than a treatment system, and the results augur well for the closed system. At a loading level of 150 mg/l, the COD removal efficiency of the closed system (Figure 14) was greater by 15 per cent, and the solids concentration in the open system (Figure 8) was greater by 55 per cent. Again, the advantage of a closed pond is apparent even though it is not so obvious as in the previous case. When the load was increased to 250 mg/l glucose, the difference in the COD removal was only seven per cent in favor of the closed pond (Figures 15, 17), and the solids were higher in the closed system by 46 per cent. This result seems reasonable, since some of the  $\text{CO}_2$  produced by bacterial metabolism in the open pond could have escaped to the atmosphere. The possibility that increased algal solids (as evidenced by the darker green color in the closed pond) might have been the reason for the small increase in the COD removal in the closed system seems reasonable in view of the greater daily changes in

the DO in the closed system than in the open system -- 13.5 (regenerated system) and 23.5 mg/l (System I) in the closed systems in comparison with 10.4 mg/l in the open system (Figure 9). There was no significant difference in allowable loading when expressed on a volumetric basis (mg/l); however, if the loadings were expressed on an areal basis (lb/acre/day), the allowable loading for the closed pond would increase by 25 per cent, because the operating volume was 50 liters in the closed pond while in the open pond it was 40 liters; the surface area of the ponds and detention times were the same in both cases.

The open pond which received 250 mg/l glucose (Figure 9) reached zero DO levels at the end of the dark periods in spite of the fact that the maximum DO levels at the end of the light periods were well above the saturation values. Any increase in the organic loading would have resulted in creating anaerobic conditions for a considerable time; therefore, for the ponds and experimental conditions herein employed, the maximum allowable loading which can be treated aerobically in a 10-day detention period could be taken as 250 mg/l. Wu (79) used somewhat similar ponds and concluded that 300 mg/l glucose should not be exceeded in order to avoid overloading ponds operating at a detention period of ten days.

In order to evaluate the contribution by algal photosynthesis to the overall oxygen balance of the ponds, the maximum allowable loading in ponds without algae was

estimated. The open ponds were subjected to increasing loads which eventually produced anaerobic conditions. The dissolved oxygen in the effluent of the open pond which received 50 mg/l glucose (Figure 19) was 3.7 mg/l. The DO at the center of the pond was close to 3.2 mg/l; the COD removal was 70 per cent. From these data it can be seen that the pond was not loaded to its maximum capacity. When the pond was subjected to 80 mg/l of glucose (Figure 20), the average DO levels were 3, 2, and 0.3 mg/l at the effluent, center, and the influent end, respectively. The DO at the influent end varied from a maximum of 0.6 mg/l to a trace amount on the third day of operation. This indicated that the pond was operated at maximum tolerable loading for total maintenance of aerobiosis. The COD removal was 76 per cent. The open pond became anaerobic when the influent feed concentration was 120 mg/l glucose (Figure 21). Even though the DO at the effluent end was approximately one mg/l, no DO was measurable in the first reach (inflow side to center) of the pond. The average COD removal was 81 per cent. From these results it could be concluded that the physical reaeration process alone may maintain aerobiosis in a pond of this configuration when the influent contains glucose at a concentration of up to 80 mg/l.

Comparing these systems without algae with open systems with algae, the relative contribution of photosynthetic oxygenation and physical aeration can be estimated. It was

seen previously that an open pond with algae could be operated aerobically up to a loading of 250 mg/l glucose (Figure 9). It is interesting to note that the COD removal efficiency in that system (open with algae) was 76 per cent, and the open pond without algae treating 80 mg/l glucose (Figure 20) also exhibited the same efficiency of 76 per cent. Under this criterion, i.e., maintenance of aerobic conditions, the additional aeration mechanism (photosynthesis) allowed the system to accommodate a three-fold increase in organic loading. Roughly, two-thirds of the aeration could then be attributed to the photosynthetic process, and one-third to the physical aeration process. In other words, the contribution by biological oxygenation is twice that of physical aeration. However, it should be noted that the samples taken for the determination of COD in the two systems were obtained in a different manner. In the ponds containing algae, COD determinations were made on the filtered effluent, whereas the unfiltered effluents were used for the pond devoid of algae. In the pond devoid of algae, the solids production was low due to the lower loading and the small amounts of solids which were produced settled to the bottom of the tank and the effluent was clear. In brief, even though different sampling procedures were used, the effluent from the pond devoid of algae was for all practical purposes equivalent to the filtrate.

There was probably a difference in the degree of quiescence in the dark and light ponds which was caused by

alternation of light and dark periods in the lighted ponds. As may be recalled, the system containing algae was operated on a light cycle of 12-hours-on and 12-hours off. The lighting increased the temperature of the mixed liquor by approximately  $4^{\circ}\text{C}$ , and during the dark periods the temperature dropped  $4^{\circ}\text{C}$ . This alternation in the temperature could be expected to cause convection currents which would have accelerated the physical gas transfer process as well as providing better mixing of the reaction liquor. Also, evidence for "phototaxis" was observed, i.e., the algae tended to migrate toward the water surface during the light period. This movement of the population, in addition to the temperature change, tended to improve mixing, and it can be said that the pond containing algae (like ponds in the field) constitutes a semiquiescent body of water, whereas the totally dark pond might be termed quiescent. These differences (although worthy of note) seem somewhat small, and it is felt that they do not militate against the validity of the comparisons which were made between totally dark and light-dark ponds.

In field ponds, wave action created by wind (which was absent in the laboratory ponds) might exert an influence by increasing the area of the air-liquid interface and thus would enhance mixing. Under these conditions more oxygen would be absorbed during subsaturation periods, and more oxygen would be stripped during supersaturation periods compared to the laboratory ponds. Even though the light

intensity in the field would be higher than that used in the laboratory (approximately 8000 ft-c in the field versus 450 ft-c in the laboratory study), this may not be of any additional use for the photosynthetic process, since the higher intensity is well above the "saturating" light. On the contrary, while light was available to the laboratory units uniformly at all the light periods, field ponds would not get sufficient lighting on overcast days. This factor might be expected to lower the photosynthetic oxygenation. However, there is another factor which enhances the biological aeration, i.e., the depth of light penetration. In the laboratory ponds the light intensity at the surface was 450 ft-c, and at the bottom would be approximately 90 ft-c. Photosynthesis occurs in the one-foot depth of the pond at a maximum rate near the surface and at a light-limited rate near the bottom, and the variation is logarithmic. In the field ponds, assuming an average intensity of 6000 ft-c at the surface, the intensity at about 1.75 ft would be approximately 450 ft-c. Photosynthesis will occur at the maximum rate at this 1.75 ft depth. In addition, in the next one-foot depth, 1.75 to 2.75 feet, algal oxygenation will take place which alone will be equal to that of the laboratory ponds. So, in photosynthetic oxygenation also the factors encountered in field ponds are opposite in effect and tend to cancel each other; scum formations would reduce the effectiveness of both the aeration mechanisms. In view of these considerations, the relative contributions by physical

reaeration and algal oxygenation in the field ponds also could be expected to be in the ratio of 1:2. Another point to be considered in the extrapolation of the laboratory results is the greater depth of field ponds compared to the depth of the laboratory units. Since both the aeration mechanisms depend upon the surface area of the pond -- physical aeration on the air-liquid interfacial area and photosynthesis on the area of the lighted surface -- an increase in depth or in effect a decrease in surface area would reduce the overall capacity of the pond. The comparatively greater depths might not affect the relative contribution of the two aeration processes, but could lower the maximum allowable loading of the ponds. Furthermore, the operation in the laboratory was a controlled one, whereas in the field such controlled conditions do not exist.

#### Physical Gas Transfer Characteristics of the Ponds

The physical oxygenation and deoxygenation characteristics of both ponds used for continuous flow studies (Figures 22 to 25) are given in Table IV. It can be seen that the provision of baffles in one of the ponds did not change the aeration characteristics, and both the processes of deaeration and reaeration exhibited essentially the same first order velocity constant. The observed and computed saturation values were expressed as percentages of the values given in Standard Methods (corrected for temperature only); the average of those percentages was 92.61. In a



TABLE IV

## PHYSICAL DEOXYGENATION AND OXYGENATION CHARACTERISTICS OF EXPERIMENTAL PONDS

#	Characteristics	Deoxygenation		Oxygenation	
		Pond D	Pond C	Pond D	Pond C
1	Saturation value observed, mg/l	8.27	8.32	8.18	8.15
2	(1) as % of St. Method value, %	93.13	93.48	93.38	93.25
3	Saturation value, computed, mg/l	8.21	7.81	8.14	8.26
4	(3) as % of St. Method value, %	92.45	87.75	92.92	94.51
5	$K_2$ , day <sup>-1</sup> based on (1) and base 10	0.288	0.305	0.308	0.298
6	$K_2$ , hr <sup>-1</sup> based on (1) and base 10	0.0120	0.0127	0.0128	0.0124
7	$K_2$ , day <sup>-1</sup> based on (1) and base e	0.662	0.702	0.708	0.685
8	$K_2$ , hr <sup>-1</sup> based on (1) and base e	0.0276	0.0292	0.0294	0.0285
9	$K_2$ , day <sup>-1</sup> based on (3) and base 10	0.282	0.292	0.316	0.290
10	$K_2$ , hr <sup>-1</sup> based on (3) and base 10	0.0118	0.0122	0.0132	0.0121
11	$K_2$ , day <sup>-1</sup> based on (3) and base e	0.649	0.672	0.726	0.667
12	$K_2$ , hr <sup>-1</sup> based on (3) and base e	0.0271	0.0281	0.0304	0.0278

previous study in this laboratory (94) the average of such values for forty experiments was 92.06.

The rates of absorption and stripping of  $O_2$  being the same, the total quantity of gases lost or gained depends upon the magnitude of the driving force, namely, deficits or excesses, and the duration of each operation. In systems where the period of operation above saturation and below saturation are equal, the mass of  $O_2$  transferred depends only on the difference in DO concentrations. For such cases where the maximum DO levels exceed 16 mg/l, the oxygen lost to the atmosphere would be more than the gain from the atmosphere. It can be seen from Table III that all closed systems except the "regenerated" system would have experienced a loss of oxygen to the atmosphere had they not been closed, thus obviating some of the benefits of photosynthetic oxygenation.

#### Biological Deoxygenation by Organotrophs

The logarithmic oxygen uptake rates (increasing first order,  $K_1$ ,  $hr^{-1}$ ) for various loadings in both the ponds and BOD bottle systems (Figures 26 to 33) are shown in Table V. In the last column the average  $K_1$  values of duplicate experiments are given. These  $K_1$  values, individual and average, for the various substrate concentrations are plotted in Figure 42.

The range of substrate concentration employed in these studies was from 40 to 100 mg/l glucose. Referring to Table III, it is noted that the effluent COD under "balanced

TABLE V  
 DEOXYGENATION CONSTANTS DUE TO ORGANOTROPHS FOR  
 VARIOUS SUBSTRATE CONCENTRATIONS

Glucose mg/l	System	Expt. shown in Figure #	$K_1, \text{hr}^{-1}$	Average of Duplicates
40	Pond	26	0.104)	0.108
40	Pond	27	) - 0.113)	
40	BOD Bottle	26	0.082	0.082
60	Pond	28	0.128)	0.123
60	Pond	29	) - 0.117)	
60	BOD Bottle	28	0.134)	0.105
60	BOD Bottle	29	) - 0.076)	
80	Pond	30	0.162)	0.139
80	Pond	31	) - 0.115)	
80	BOD Bottle	30	0.116)	0.109
80	BOD Bottle	31	) - 0.102)	
100	Pond	32	0.161)	0.148
100	Pond	33	) - 0.135)	
100	BOD Bottle	32	0.123)	0.104
100	BOD Bottle	33	) - 0.084)	

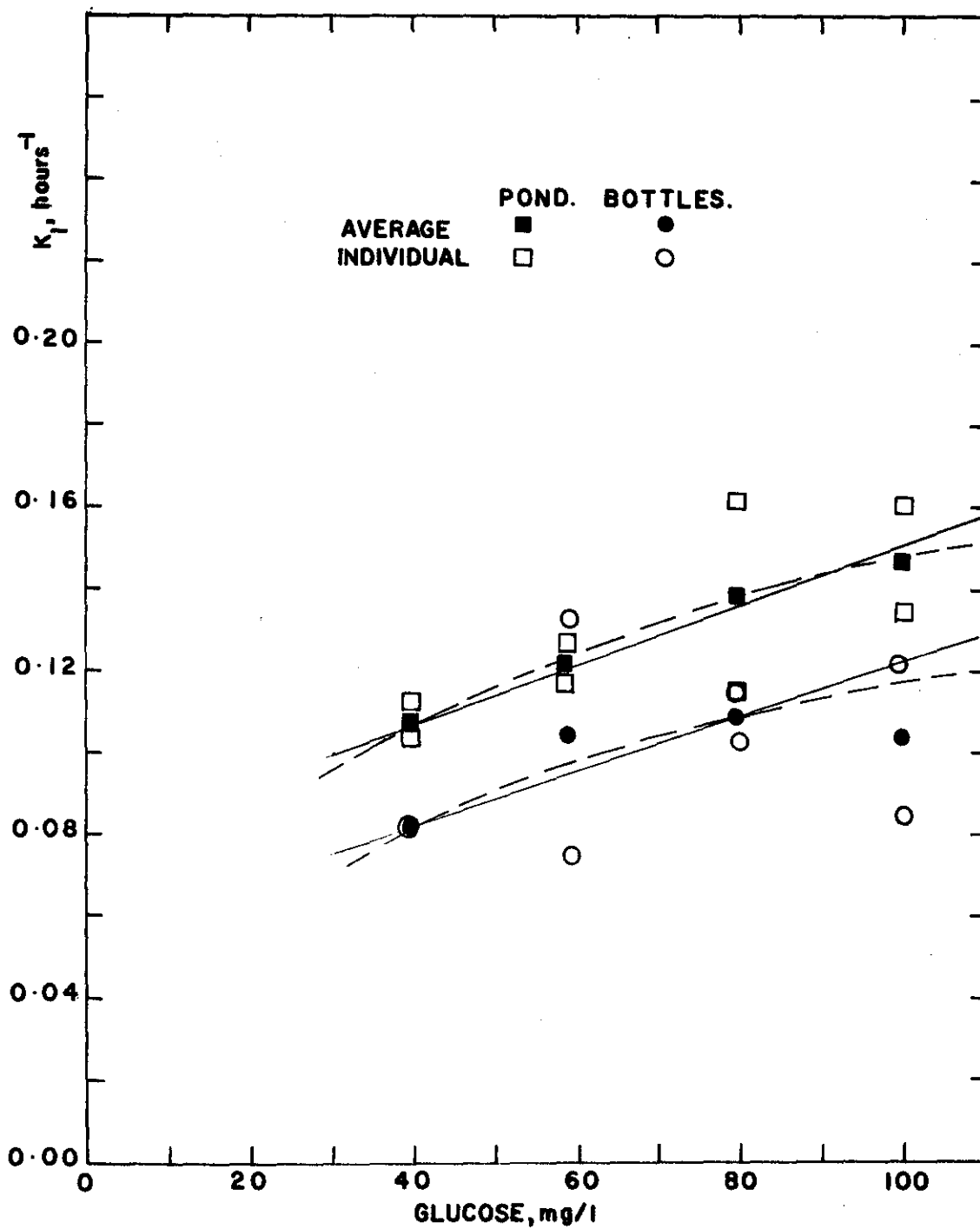


Figure 42 - Relation between substrate concentration and deoxygenation constants due to organotrophs.

operation" of the various systems, both open and closed, was in the range between 40 and 114 mg/l. Therefore, since it has been fairly well established that biological growth is dependent upon substrate concentration, the range of substrate concentrations used in the batch deoxygenation studies might be expected to yield a fairly reliable estimate of the organotrophic deoxygenation rate in the ponds. In general practice, deoxygenation studies are conducted in standard BOD bottles. The applicability of the results obtained in such fashion have been questioned by various investigators (95)(96). Gaudy, et al. (97) quoted Monod to the effect that "he was sometimes accused of feeling that if it (a certain phenomenon) happened in Escherichia coli, it also happened in elephants." To answer the somewhat analogous question "If it happens in BOD bottles should it happen in the pond?", the deoxygenation studies were conducted in the experimental ponds as well as in BOD bottles. It should be recalled that under "balanced operation" in the experimental oxidation ponds the solids concentration in the effluent was fairly constant. At a given dilution rate, steady biological solids concentration could occur only if the cells were in a logarithmic growth phase. Therefore, in these batch deoxygenation studies, increasing first order rates in the logarithmic growth phase were calculated.

As can be seen from Table V and Figure 42, the  $K_1$  values increased with increasing substrate concentration in both the pond and BOD bottle system. This indicates that

under conditions prevailing in the "balanced operation," biological deoxygenation was proportional to the substrate concentration, and even at the highest concentration the growth rate, as reflected in the  $O_2$  uptake, was substrate-limited.

From the plot of oxygen uptake rates for various substrate concentrations shown in Figure 42, it cannot be determined whether the increase in  $O_2$  uptake rate with increasing glucose concentration follows the hyperbolic relationship of Monod, since the data could also be fitted to a straight line. However, this observation cannot be taken as a disproof of the Monod relationship, because the range of substrate concentrations was very small (40 to 100 mg/l). It has been found that for heterogeneous populations, the relation between growth rate,  $\mu$ , and substrate concentration is in accord with Monod's equation and approximately 500 mg/l of substrate is required to attain the maximum growth rate (at temperatures comparable to those employed in this study). It therefore seems that the four substrate concentrations would encompass a narrow degree of curvature on a hyperbolic curve. It is generally assumed that the  $O_2$  uptake curve reflects the population curve during logarithmic growth; therefore, one might expect that the type of relationship exhibited on the basis of growth rate would also hold true for  $O_2$  uptake rate. Ramanathan (98) has shown in preliminary experiments that there is an apparent linear relationship between  $O_2$  uptake rate and

growth rate for heterogeneous populations of sewage origin grown on Glucose. Further work along this line would be useful.

The  $K_1$  rates calculated from the BOD bottle data were on the average 25 per cent lower than the rate constants calculated from the data obtained in the ponds. Nejedly (99), in his studies on the rate of  $O_2$  uptake in BOD bottles as compared to rivers, observed lower rates in the BOD bottles. He attributed this to the fact that the river comprised a turbulent system which provided better mixing which, in turn, caused a higher rate of  $O_2$  uptake. This explanation is not applicable to the present study, because both the ponds and the BOD bottles were maintained under quiescent conditions. It is difficult to envision how the mere difference in volume of reaction fluid could affect the results, but enough data were obtained to indicate that a real difference did exist in  $O_2$  uptake rate, and this aspect could prove to be a fruitful channel for further investigation.

#### Biological Deoxygenation by Photo-autotrophs

The results of deoxygenation studies using photo-autotrophs were presented in Figures 34 to 41. Glucose at a concentration of 500 mg/l was used as substrate in all heterotrophic experiments, since it was felt that at this concentration growth would not be substrate-limited (or at any rate, severely limited). Gaudy and co-workers (100)(101) have proved conclusively that the mechanisms as well as the

kinetic order of substrate removal were different when low and higher initial bacterial solids were employed in laboratory scale activated sludge process, and they showed that less oxygen was consumed at the point of substrate removal with high initial solids concentrations.

Pearsall and Bengry (39) cultured Chlorella in the dark on glucose and found that the growth rate was at first exponential; then as the cell number reached approximately 600 cells/cu mm ( $6 \times 10^6$  cell/ml), the growth curve became linear. The linear phase did not depict the endogenous phase, since only 20 per cent of the substrate was used even after six days of linear growth. To determine whether a difference in the kinetics of oxygen uptake existed at different initial algal concentrations and to determine the range of concentrations at which the two different modes of oxygen uptake exist, experiments were conducted with varying initial algal concentrations from 113 mg/l to 896 mg/l.

As can be seen from the figures (34 to 38), the  $O_2$  uptake curve of the system with an initial algal concentration of 896 mg/l (Figure 34) was linear during the active substrate removal period, whereas in the systems with initial algal concentrations of 215 (Figure 37) and 113 mg/l (Figure 38) the oxygen uptake curves were more characteristic of logarithmic growth. The systems with 448 (Figure 35) and 400 mg/l (Figure 36) algae showed kinetics which suggested that logarithmic growth phase was approached



during the active substrate removal period.

The kinetic order and the rate constants for all of the experiments are summarized in Table VI. Also shown are the percentages of the theoretical oxygen demand exerted at the time of substrate removal (for both glucose and total COD). Before discussing the relation between the values shown in Table VI for Chlorella pyrenoidosa and the findings of other workers for heterogeneous microbial populations, it is appropriate to discuss the validity of the results from which the information for the table was obtained.

In an actively growing heterogeneous microbial population, the substrate utilized by the organisms is channeled into synthesis of new cell mass and into respiration ( $O_2$  uptake) for the production of energy for various metabolic activities. All of the substrate removed can be reasonably accounted for as the sum of cell synthesis and respiration. Gaudy, et al. (102) have reported four methods by which a material balance can be made, and they calculated percentage recovery close to 100 per cent for their experimental data. To the author's knowledge, this type of balance has not yet been applied to algal cultures grown heterotrophically.

A materials balance for the present study was made (on the basis of carbon) (102) using the COD removed, solids produced, and the oxygen uptake at the point of substrate removal. In all of the systems the percent recovery was only  $66 \pm 6$  per cent. While no proven reason for this

TABLE VI  
 KINETIC CONSTANTS AND PERCENT THEORETICAL OXYGEN  
 UPTAKE FOR SYSTEMS WITH VARIOUS INITIAL  
 SOLIDS CONCENTRATIONS

Initial Solids mg/l	Figure #	Kinetic Order	First Order hr <sup>-1</sup>	Zero Order mg/l O <sub>2</sub> /hr	Percent Theoretical Oxygen Demand Exerted at the Time of	
					COD Removal	Glucose Removal
896	34	0	-	15.73	17.3	17.0
448	35	0	-	9.15	19.5	20.0
400	36	0*	-	7.29	18.0	17.9
215	37	1	0.089	-	18.8	26.3
113	38	1	0.095	-	19.8	26.7

\* Approaching First Order

apparent low recovery can be advanced, the following explanation seems to present a reasonable possibility for the observed discrepancy. The method for the determination of COD is based upon the assumption that all organic materials in the sample are oxidized under the conditions of the test. While this assumption is true for many organic substances, there are some organics (e.g., aromatic hydrocarbons) which are not registered by the COD test (87). If such organic substances were produced by the algae and were present at the point of indicated substrate removal, the indicated COD would not provide a means of estimating the actual concentration of the remaining carbon resource. Thus a greater removal of carbon source than actually occurred would be registered and the percentage recovery would be low.

The carbon balances for the systems at various times after the completion of the substrate removal were also calculated; the percentage recovery increased as the run progressed. On the average, the maximum percent recovery was 75.5 per cent, approximately 10 per cent more than the values obtained at the point of substrate removal:

Initial Solids mg/l	Percent Recovery at the Time of COD Removal	Maximum Percent Recovery near End of Run
896	67.5	81.5
448	63.8	79.6
400	60.3	64.2
215	64.9	73.2
113	72.0	78.8

The improvement in the balance was due to a significant amount of oxygen uptake after the apparent exhaustion of the carbon source, as indicated by the COD test. The increased  $O_2$  uptake could be attributed to endogenous uptake or to the utilization of carbon source not registered by the COD test. However, the major part of the increased  $O_2$  uptake can be attributed to the latter cause, since the endogenous  $O_2$  uptake was lower than the increased demand after the exhaustion of COD.

The percentages of theoretical oxygen demand exerted at the time of glucose removal (as measured by the anthrone test) at the time of COD removal are given in Table VI. It can be seen from Table VI that the percent theoretical  $O_2$  demand exerted at the point of substrate removal, whether expressed as COD or glucose, increased as the initial solids concentration decreased. The decrease is more pronounced when the analysis is based on the anthrone test. The difference in the decrease of percent theoretical oxygen demand using the glucose and total COD removal supports the premise made previously that some nonregisterable COD, probably produced during substrate removal, existed in the system. The limited (three solids concentrations) investigation of McWhorter and Heukelekian (103) and the exhaustive work by Gaudy and co-workers (100) (101) with bacterial populations showed the same phenomenon of decreased percent theoretical oxygen demand with increasing initial solids concentration at the point of COD removal. Comparing

values obtained by McWhorter and Heukelekian with those obtained in this research, it can be noted that there was a small difference of four per cent at the higher levels of solids (21 per cent at 1010 mg/l [103] and 17.3 per cent at 906 mg/l algae). This difference increased to 10 per cent at low solids levels (30 per cent at 0.5 per cent settled sewage seed [103] and 19.8 per cent at 113 mg/l algae). These differences can be expected in the light of the conclusion of Rao and Gaudy (100) that  $O_2$  utilization, although dependent upon the initial concentration of biological solids, can vary with each specific sludge.

Concerning the percentage theoretical  $O_2$  demand exerted at the point of glucose (anthrone) removal, the data of this research do not support the conclusion of McWhorter and Heukelekian that a constant 18 per cent was exerted. The decreasing trend in percent theoretical oxygen demand at the time of glucose (anthrone) removal observed in the present investigation confirms the findings of Rao and Gaudy. In addition, the values obtained are in the same range as their values. In Figure 43 the values obtained in this investigation are plotted along with the values obtained by Rao and Gaudy (see Figure 8 [100]). It seems reasonable to conclude that, under heterotrophic metabolic conditions, Chlorella pyrenoidosa exerted percentage theoretical oxygen demands at the point of substrate removal which were inversely proportional to the initial solids concentrations in the range tested. Thus, for the

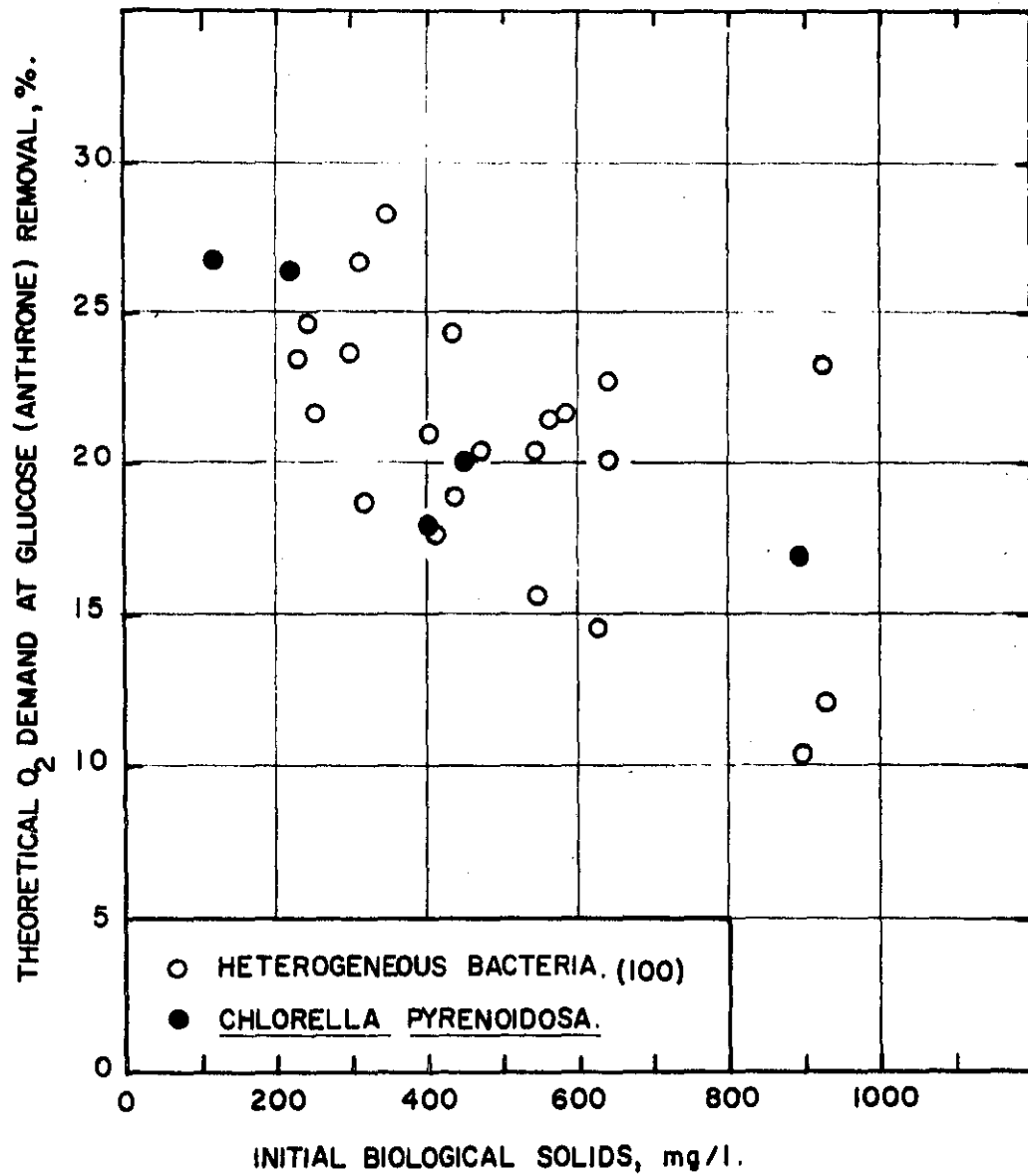


Figure 43 - Effect of initial biological solids on percentage of theoretical oxygen demand exerted at time of glucose (anthrone) removal.

algae as well as bacteria it would appear that the initial biological solids concentration affect both the kinetics of the system (zero order at high solids, first order at low solids) and the amount of oxygen required for removal of the original exogenous carbon source. Also, it would appear that the point at which the observable shift in kinetic mode occurs can be defined by the food/micro-organisms ratio and the value is approximately unity.

## CHAPTER VII

### SUMMARY AND CONCLUSIONS

It was seen that the experimental oxidation ponds could be operated under "balanced conditions" wherein parameters such as maximum DO, minimum DO, biological solids, and effluent COD were (more or less) constant. In some systems there were two such periods of 'balanced conditions' with respect to the daily DO changes (closed systems at 100 mg/l and 250 mg/l), and even in these cases the biological solids and the effluent COD were approximately constant. At times the 'balanced operation' was disturbed temporarily (closed pond at 150 mg/l) and in two cases disrupted permanently (open pond at 250 mg/l, closed pond at 250 mg/l, system I). In some cases the algal activity, and hence the daily DO change, was less at the beginning of the operation of the ponds.

The data indicated that atmospheric CO<sub>2</sub> was fixed by the algal population of the open pond and that this effect made the open pond at the low loading (100 mg/l) into a polluting pond rather than a waste treating pond, since approximately 100 per cent more carbon was present in the effluent than was present in the influent. In the 150 mg/l system the carbon content was increased by approximately



25 per cent, whereas there was a 50 per cent decrease in carbon content in the 250 mg/l system. Because of the gain in carbon from the atmosphere, closing the ponds to the atmosphere increased significantly the purification efficiency at low loadings, and moderately at higher loadings. Though the provision of gas-impermeable, light-transmitting covers for field ponds (which would prevent entry of  $\text{CO}_2$  and escape of oxygen) is not impossible from an engineering point of view, it should be thoroughly investigated before such a recommendation is made. Since more atmospheric  $\text{O}_2$  would be expected to enter the pond liquor near the effluent end of the pond, it seems apparent that long detention times should not significantly improve carbon removal; indeed, they may tend to obviate the purpose for which the pond was installed. Oxidation ponds are not designed (at any rate not at present) as reactors for growing algae, but as reactors for removing organic matter in waste waters.

The data indicated that the maximum allowable loading in an open pond without algae was 80 mg/l glucose, whereas the load could be increased to approximately 250 mg/l glucose with an algal population operated with the same detention period. Thus it could be concluded that the net contribution from photosynthesis was approximately twice that from oxygenation by physical transfer from the atmosphere. The maximum allowable loading (for maintenance of aerobic conditions) of 250 mg/l in the open ponds, with algae, if expressed as an areal loading amounted to 63 lb COD/acre/day.

Wu (79) used a 36-liter pond with 30 liters of waste liquor and a surface area of 1387 cm<sup>2</sup> compared to the 40-liter volume and 1500 cm<sup>2</sup> pond in the present study, and showed that 61.7 lb COD/acre/day could be satisfactorily treated.

In the last subsection of the literature review it was pointed out that the substrate removal efficiencies of oxidation ponds are expressed by two means--percent COD removal and percent BOD removal--and the percent COD removal was always lower than the percent BOD removal. Determination of the purification efficiency in field ponds (74) (75) (76) have been made on both bases. The COD removal efficiencies attained in the present research when the ponds were loaded to their maximum capacity to maintain aerobic conditions were 76 per cent in the open system and 83 per cent in the closed system. If these organic loading removal efficiencies had been expressed on a BOD basis, they would be well over 90 per cent. Therefore, the purification efficiency (for filtrate) of the laboratory pond approximated the field pond efficiencies based on pond effluents. Various factors which come into play when laboratory data are extrapolated to the field conditions were pointed out previously, and it was seen how these factors tended to counterbalance one another, thus indicating that the relative contributions of the two aeration processes (physical transfer and photosynthetic oxygenation) found in the present laboratory studies could also be expected to exist

in field ponds.

In general, oxidation ponds seem to present a "paradox." They are attractive in certain localities because of their low cost of construction and low operation and maintenance costs. If low loadings are employed in order to maintain aerobic conditions, the photo-autotrophic organisms fix atmospheric carbon dioxide, thereby militating against the aim of waste water treatment. The situation is so severe at low loadings that even when solids separation is not taken into consideration, oxidation ponds add more pollution (organic carbon) than they remove. To avoid this undesirable aspect, oxidation ponds should be operated at near maximum loadings, but if they were operated in such a manner they would tend to become anaerobic when any possible shock loads were added to the system.

#### CONCLUSIONS

Based on the results and the author's interpretation of them, the following conclusions seem warranted:

1. Oxidation ponds operated at low loadings with a detention period of ten days are not really in balance biologically, because bacteria do not supply all of the required carbon for the algae, and this leads to a case where the effluent can contain more carbon than the influent.
2. In general, due to physical oxygen exchange processes across the air-liquid interface of the

pond, more oxygen is lost to the atmosphere than is gained from it.

3. The adverse operational aspects brought out in conclusions 1 and 2 may be eliminated by closing the ponds to the atmosphere without shielding light, i.e., use of transparent, gas-impermeable cover. For example, a "Saran"-type membrane attached to a floating grid could be tried.

4. Closed ponds increased the purification efficiency significantly at lower organic loadings, and moderately at higher loadings.

5. For satisfactory performance on the basis of maintenance of aerobic conditions, the maximum allowable loading in an open pond was 250 mg/l glucose at 10-day detention periods or 63 lb COD/acre/day.

6. An open pond devoid of algae in which physical reaeration was the only aeration mechanism responded satisfactorily to an organic loading of 80 mg/l glucose.

7. Based upon the laboratory studies, it seems reasonable to predict that in field installations photosynthetic oxygenation contributes twice as much to the oxygen resource of the pond as does physical aeration.

8. The similarity of the results obtained by Rao and Gaudy (100) using organotrophic mixed microbial populations and those in the present study using Chlorella pyrenoidosa under organotrophic growth conditions suggests that biological solids concentration exerts a general effect upon the kinetics and mechanisms of heterotrophic metabolism.

## CHAPTER VIII

### SUGGESTIONS FOR FUTURE WORK

Analysis of the results of this investigation suggested the following items for possible future investigation:

1. Experimentation with a closed pond in a field scale pilot plant might be usefully undertaken. The suitability of various transparent membranous materials and means of holding them in place should be explored.

2. Further investigations pertaining to the relation between organic removal efficiency and detention period seem warranted, especially for detention periods of less than ten days.

3. The effect of sludge deposition on the oxygen resource of oxidation ponds needs further study.

4. If further laboratory work using the experimental ponds is undertaken, the work should include the following aspects:

- (a) Separate determination of algal and bacterial population in the ponds.

- (b) Determination of complete carbon balance, i.e., organic carbon, free  $\text{CO}_2$ , carbonates in the influent and effluent, and the carbon component of the biological solids.

- (c) Correlation of BOD and COD on influent and effluent samples.
- (d) Determination of the major organic compounds in the effluent (filtrate).

5. Further work to confirm the effect of initial algal concentration on the kinetics and mechanism of substrate removal during heterotrophic metabolism which was observed in the present study would be of considerable value.

(Further experimentation along these lines has already been designed.)

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VITA

O. V. NATARAJAN

Candidate for the Degree of  
Doctor of Philosophy

Thesis: STUDIES ON FACTORS AFFECTING DISSOLVED OXYGEN  
CONCENTRATIONS IN AEROBIC OXIDATION PONDS

Major Field: Engineering

Biographical:

Personal Data: Born on November 16, 1935, in  
Odaiyakulam, Tamilnad (Madras), India, the son of  
Velusamy and Palaniammal.

Education: Graduated from Board High School,  
Udumalpet, India, in 1951; attended  
Pachaiyappa's College, Madras, India, passed the  
intermediate examination in 1953; graduated from  
P.S.G. College of Technology, University of  
Madras, in 1957, received Bachelor of Engineering  
degree in Civil Engineering. Received Master of  
Science degree in Public Health Engineering from  
Annamalai University, India, in 1963. Completed  
requirements for the degree of Doctor of  
Philosophy at Oklahoma State University in May,  
1969.

Professional Experience: Worked as Section Officer in  
Public Works Department of Madras State (India)  
Government, from June, 1957, to September, 1957;  
served as Lecturer in Nachimuthu Polytechnic,  
Pollachi, India, from 1957 to 1960; Lecturer and  
Personal Assistant to the Principal, Thiagarajar  
College of Engineering, Madurai, India, from  
1960 to 1964; Teaching Assistant and then  
Research Assistant, Oklahoma State University,  
1964 to 1968.

Membership in Professional Societies: Water Pollution  
Control Federation, American Water Works  
Association.