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

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EFFECT OF ORGANIC LOADING ON REAERATION IN SEMI-QUIESCENT WATERS

Thesis Approved:

Courtney F. Sawyer Jr.
Thesis Adviser

Raymond A. Mill

D. D. Durham
Dean of the Graduate College

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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:

$$(CH_2O)_Y + O_2 \longrightarrow CO_2 + H_2O + \text{new bacterial cells.}$$

In the above equation $(CH_2O)_Y$ 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 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:

$$CO_2 + 2H_2O \longrightarrow \text{algae } (CH_2O) + H_2O + O_2$$

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).

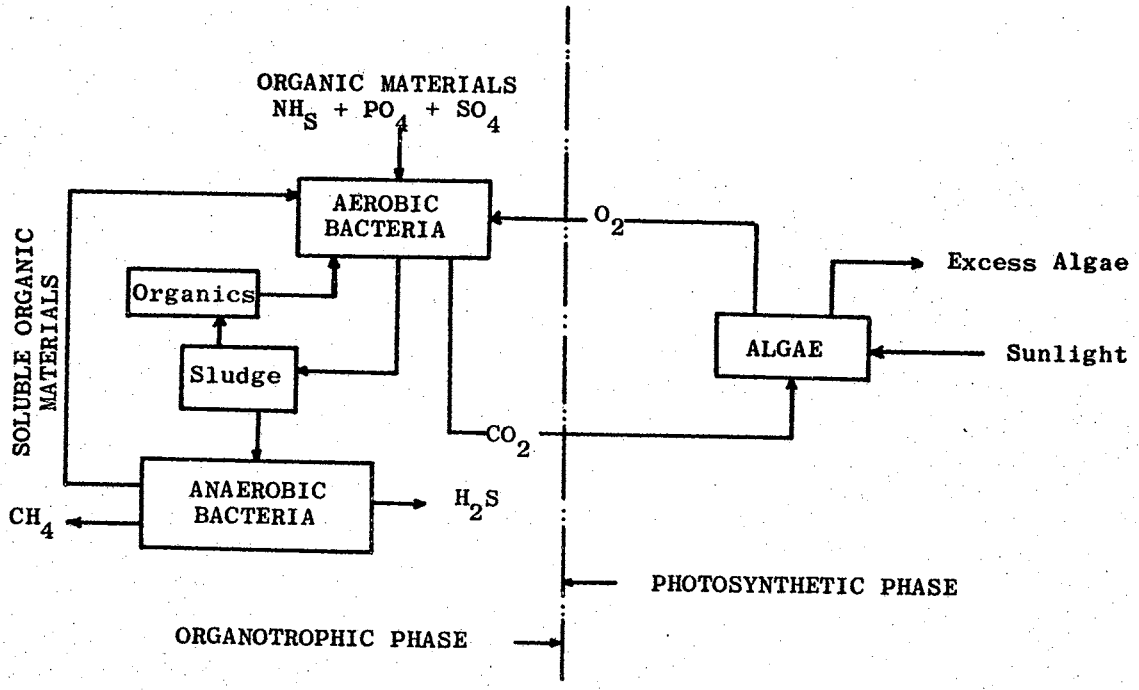

Figure 1. Schematic Representation of Major Biological Cycles Which may occur in Oxidation Pond.

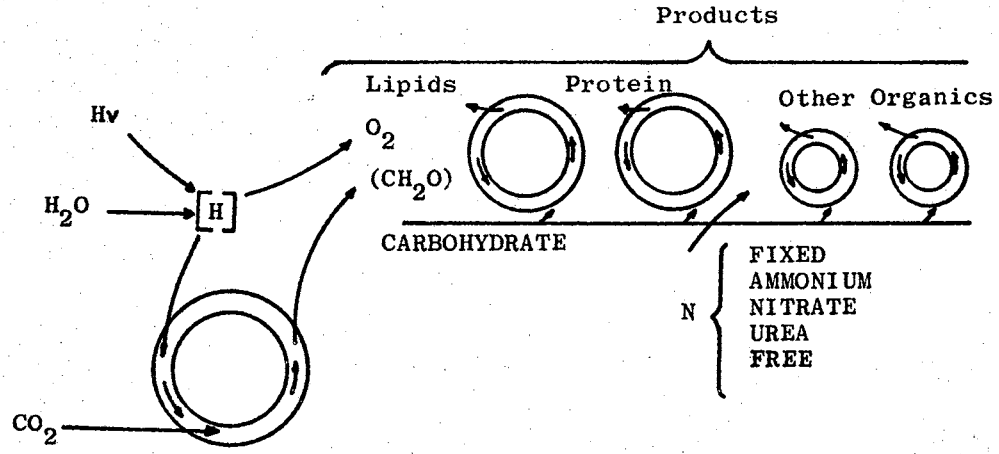

Figure 2. Simplified Diagram of Algal Cell Synthesis.

B. The Path of Carbon Photosynthesis

The pathway of carbon fixation in photosynthesis may be considered as a series of reactions starting with CO_2 and ending with the fixing of the carbon in CO_2 into a carbohydrate storage compound (24). In its most simple form, the reaction can be given as follows:

$$CO_2 + 4(H) \longrightarrow (CH_2O) + H_2O$$

where (CH_2O) represents the carbohydrate and (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 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

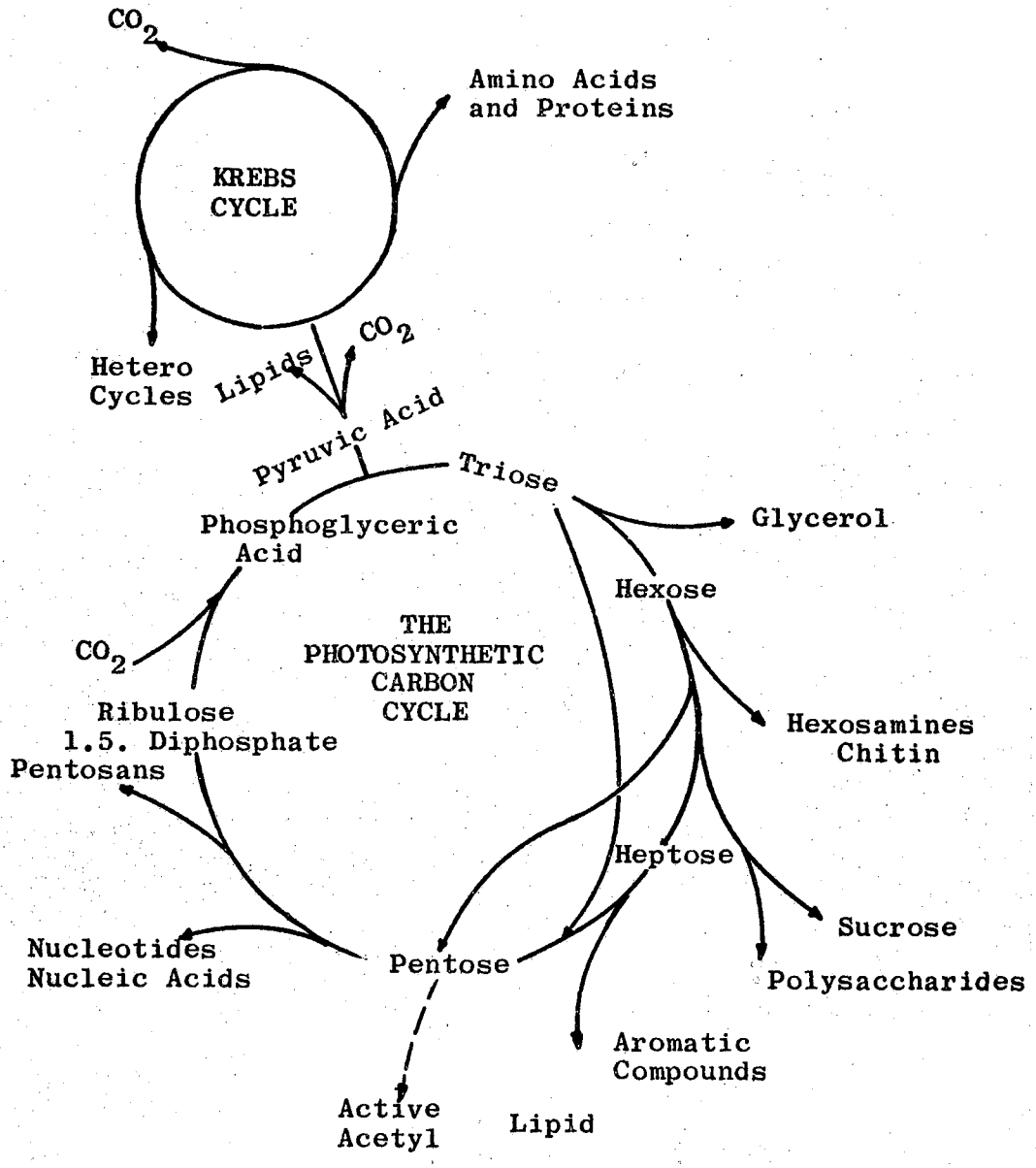

Figure 3. Carbon Compound Synthesis from Carbon Dioxide After the Path of Carbon In Photosynthesis By J. A. Bassham, 1957.

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

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^= + 2H^+$$

In the first reaction, 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 $\xrightarrow{CO_2}$ 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 $NADPH_2$ 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 ($NADPH_2$) 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 $NADPH_2$) 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:

$$HOH + \underset{\text{(oxidant)}}{A} \xrightarrow[\text{chloroplast}]{\text{light}} AH_2 + O_2$$

In the above equation AH_2 represents reducing power which the algae use to fix CO_2 in accordance with the following reaction:

$$CO_2 + 2AH_2 \longrightarrow (CH_2O) + H_2O + 2A$$

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_2 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_2 concentration does not affect the rate of photosynthesis; however, if a higher light intensity is used, a higher CO_2 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^{o}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^2

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-

tles 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

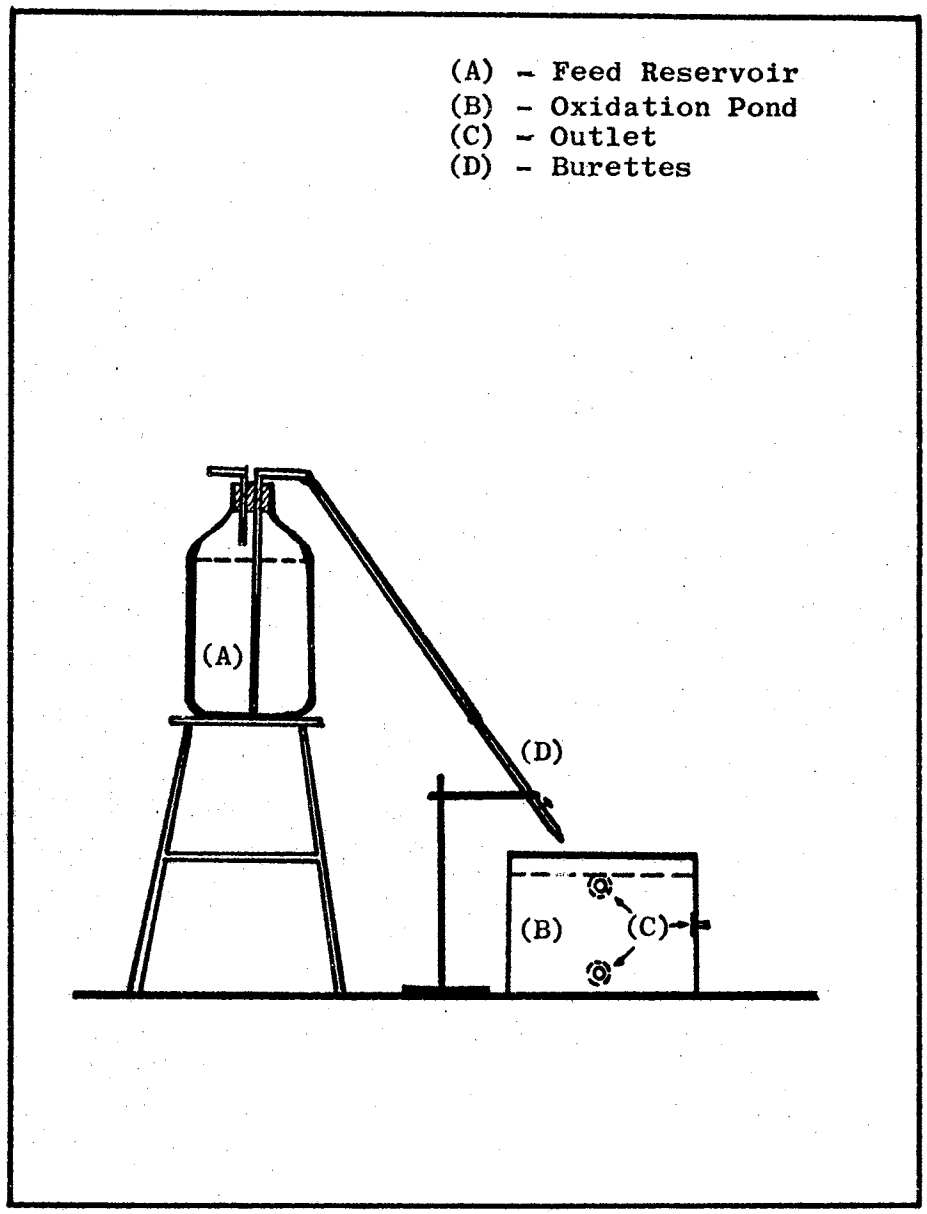

Figure 4. Schematic Representation of the Continuous Flow Oxidation Pond.

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
$(NH_4)_2SO_4$	500 mg/l
$Mg(SO_4) \cdot 7H_2O$	100 mg/l
$FeCl_3 \cdot 6H_2O$	0.5 mg/l
$MnSO_4 \cdot H_2O$	10 mg/l
$CaCl_2 \cdot 2H_2O$	7.5 mg/l
Trace Elements (Tap Water)	100 ml/l
*1.0M potassium phosphate (pH 6.8)	40 ml/l

*K_2HPO_4 : 107 grs/0.5 l
KH_2PO_4 : 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[37]

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[37]

Constituent	Concentration
Dipotassium Phosphate	24.0 gm/l
Sodium Chloride	22.5 gm/l
Magnesium Sulfate (hydrate)	12.5 gm/l
Calcium Phosphate, $CaHPO_4 \cdot 2H_2O$	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 μ (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 biolgical 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°C. ± 1°.

(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°C. ± 1°.

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:

$$2Na_2SO_3 + O_2 \longrightarrow 2Na_2SO_4$$

$$252 \quad + 32 \longrightarrow 284$$

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	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	7.35	4.64	2.71	4.53	2.82
25	7.56	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	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	0.67	7.05	0.63
107	7.68	7.05	0.63	7.10	0.58
143	7.46	7.45	0.01	7.46	0.00

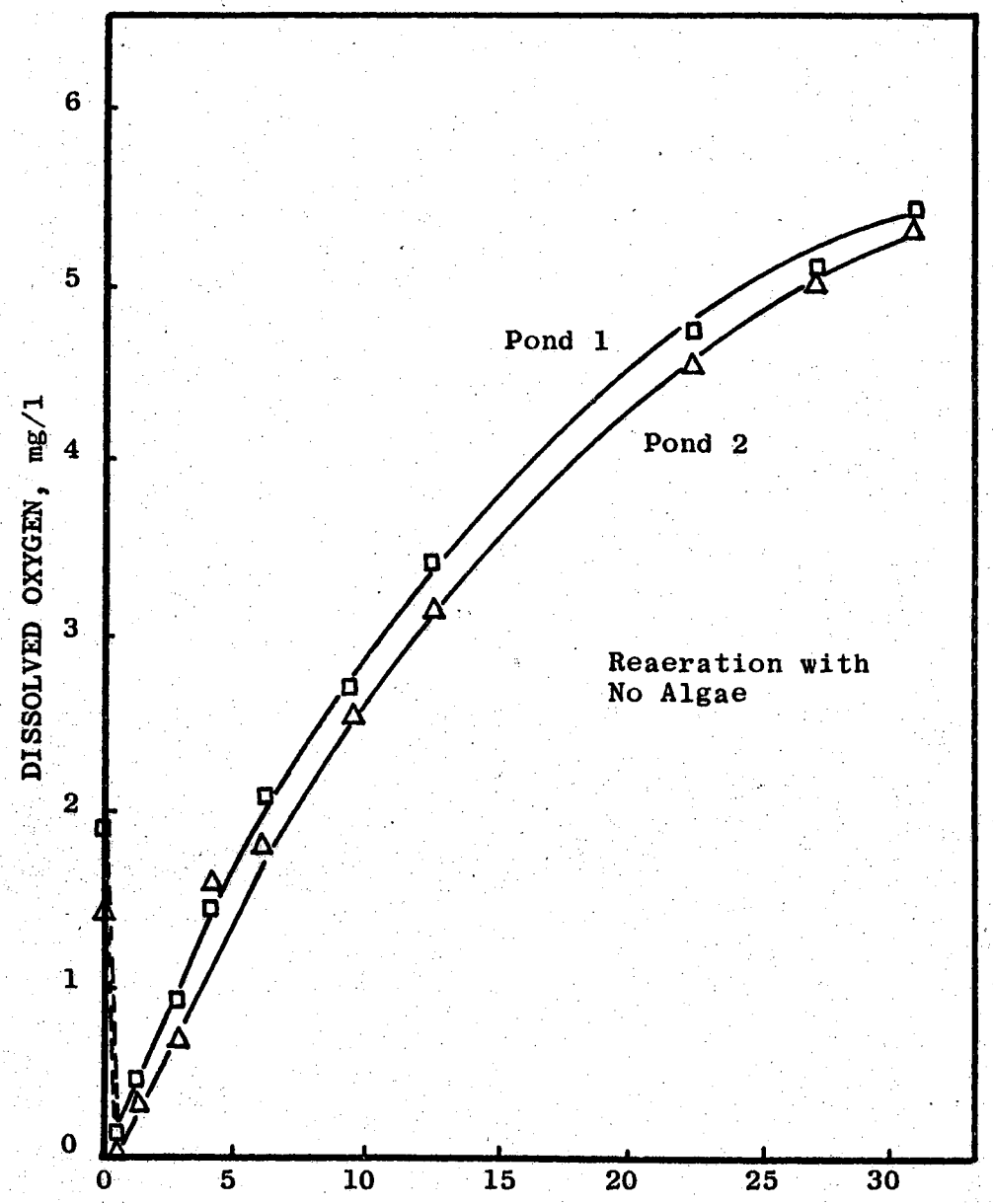

Figure 5. Physical Reaeration in Experimental Pond.

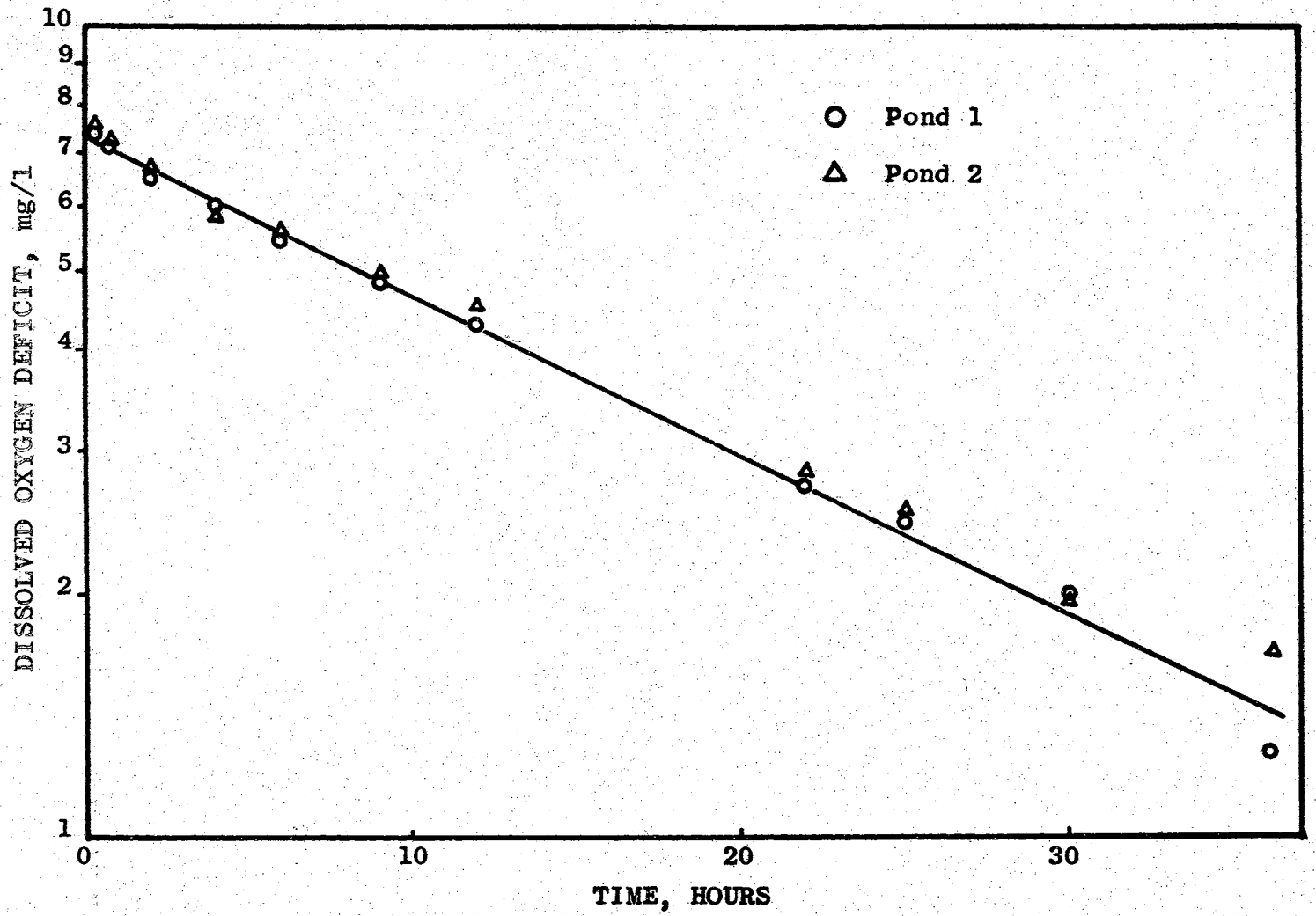

Figure 6. Semi-logarithmic Plot of Dissolved Oxygen vs. Time for Physical Reaeration in Experimental Pond.

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 Sodium Bicarbonate DO mg/l	Pond 2 100 mg/l Sodium Bicarbonate DO mg/l	Pond 3 300 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 = $22^{\circ}C. \pm 1^{\circ}$

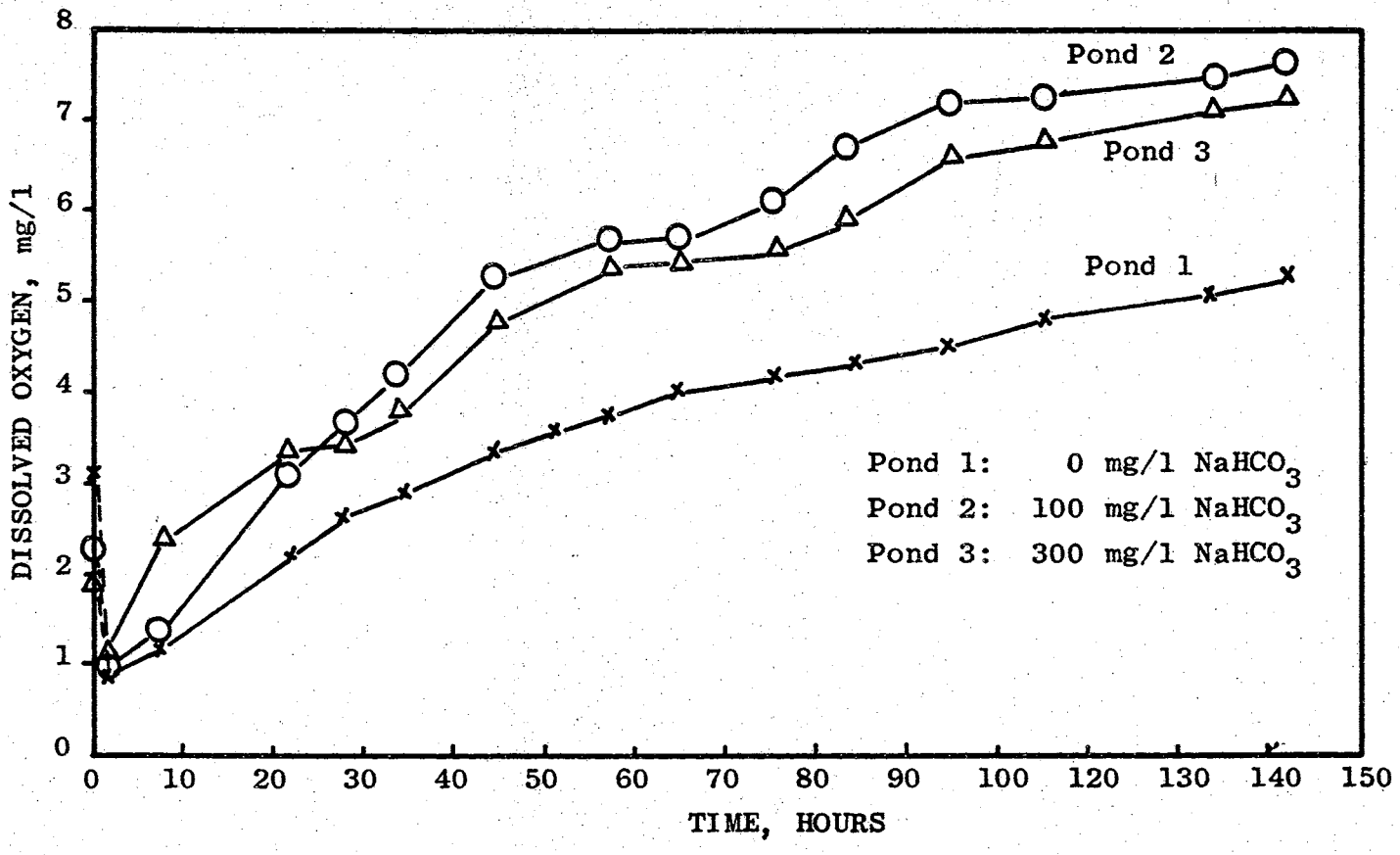

Figure 7. Combined Physical and Algal Reaeration in Experimental Oxidation Pond.

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

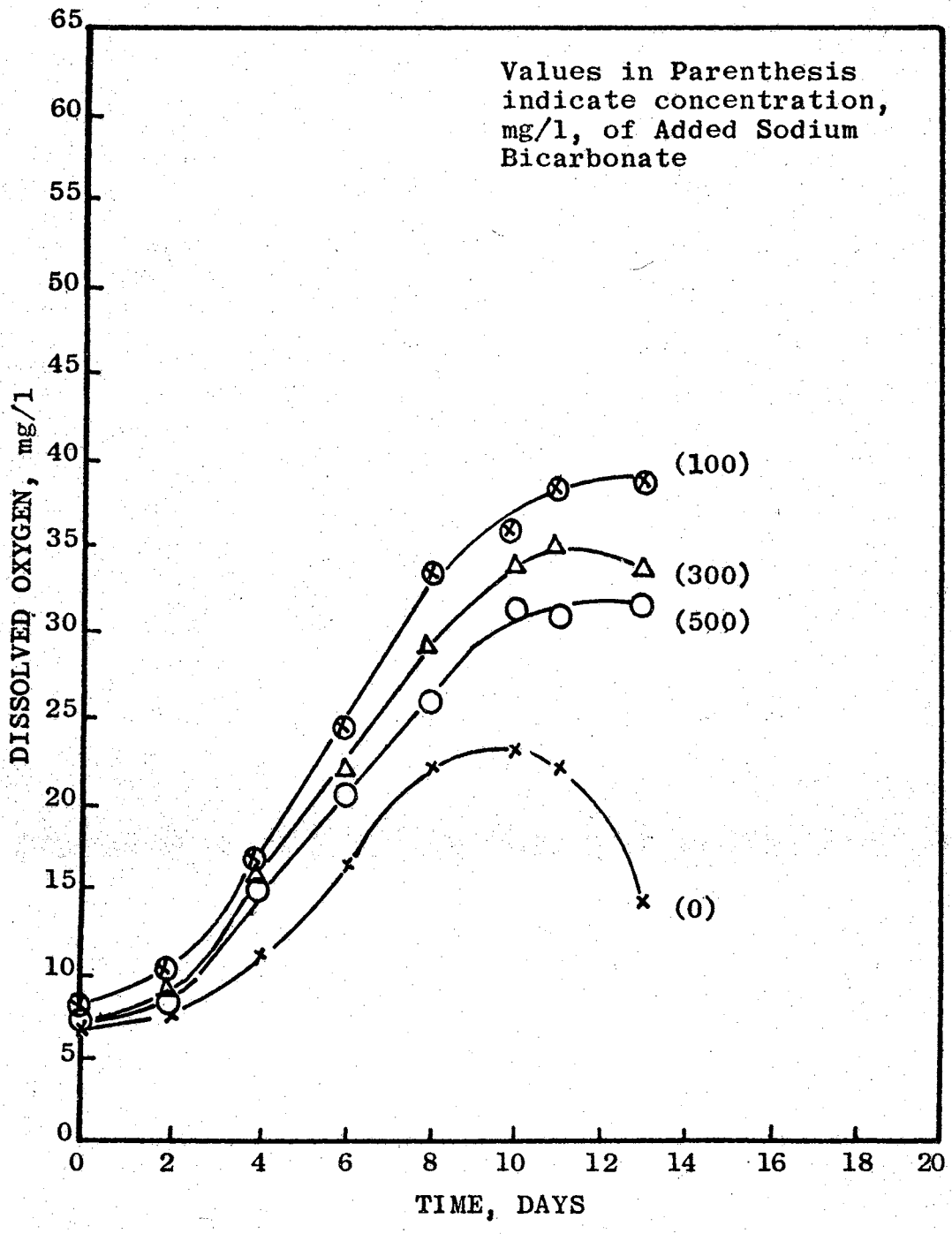

Figure 8. Effect of Sodium Bicarbonate Concentration on Algal Production of Oxygen - No Sodium Sulfite Added.

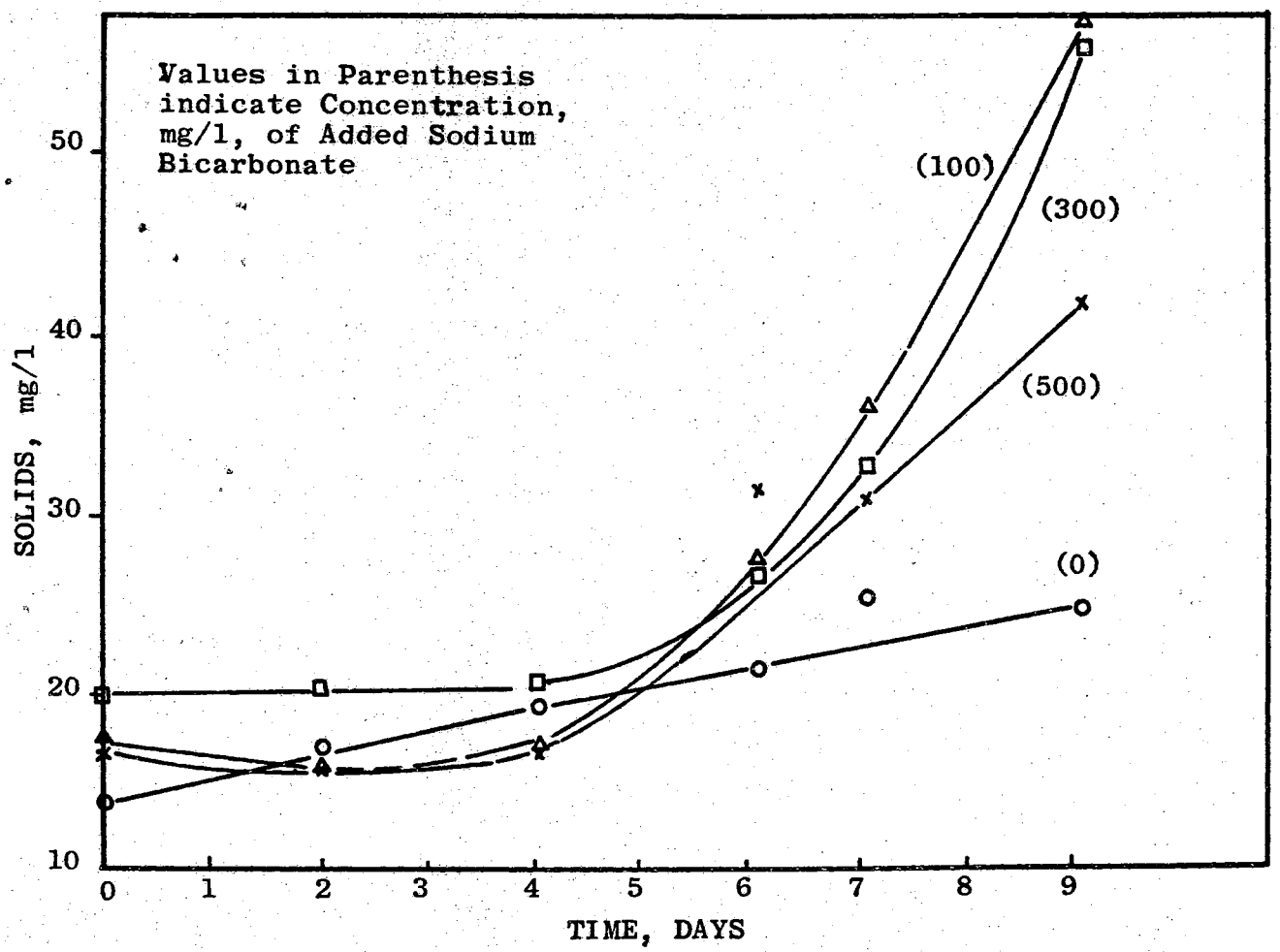

Figure 9. Effect of Sodium Bicarbonate Concentration on Production of Algal Solids

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

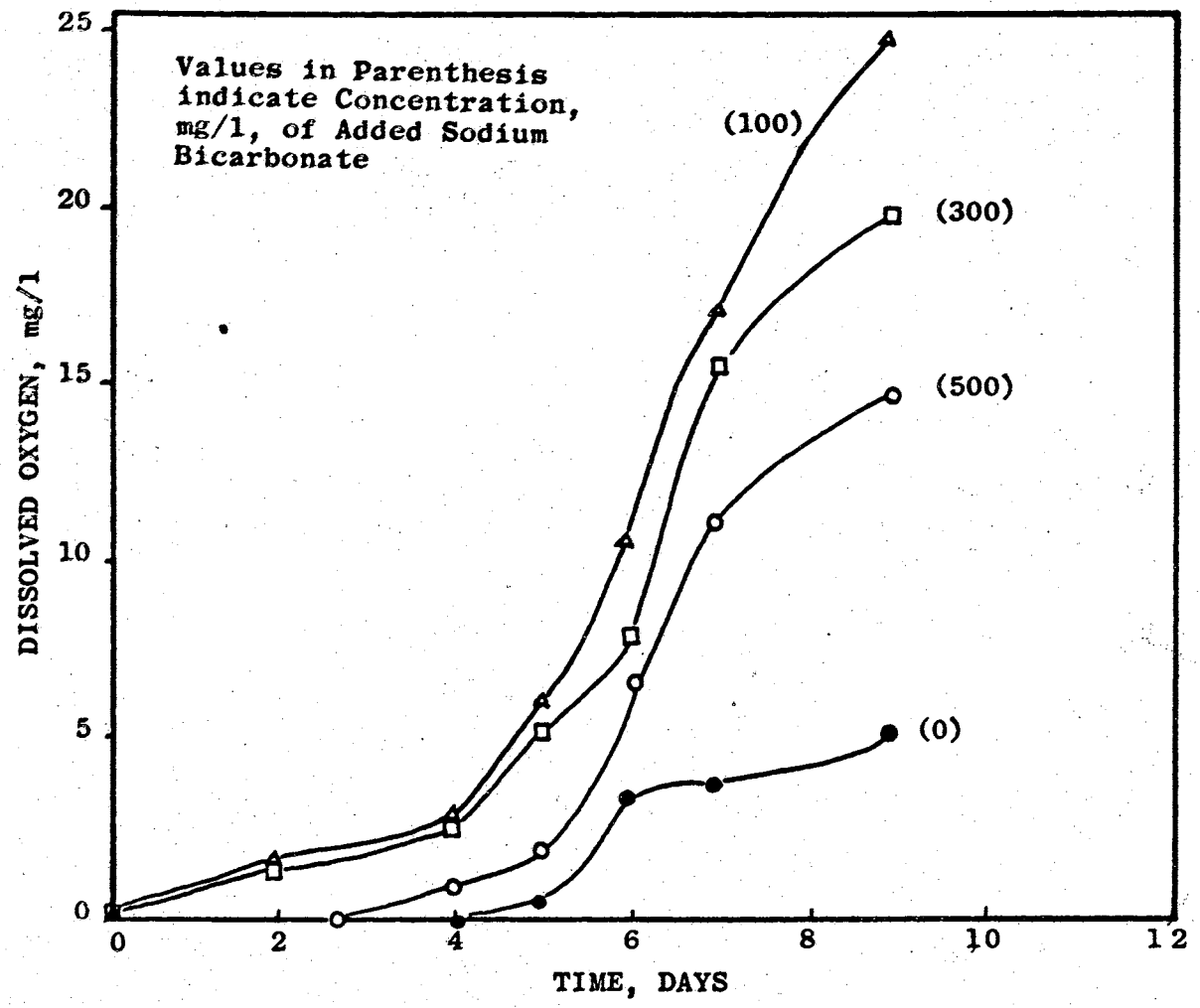

Figure 10. Effect of Sodium Bicarbonate Concentration on Algal Production of Oxygen - Sodium Sulfite Added.

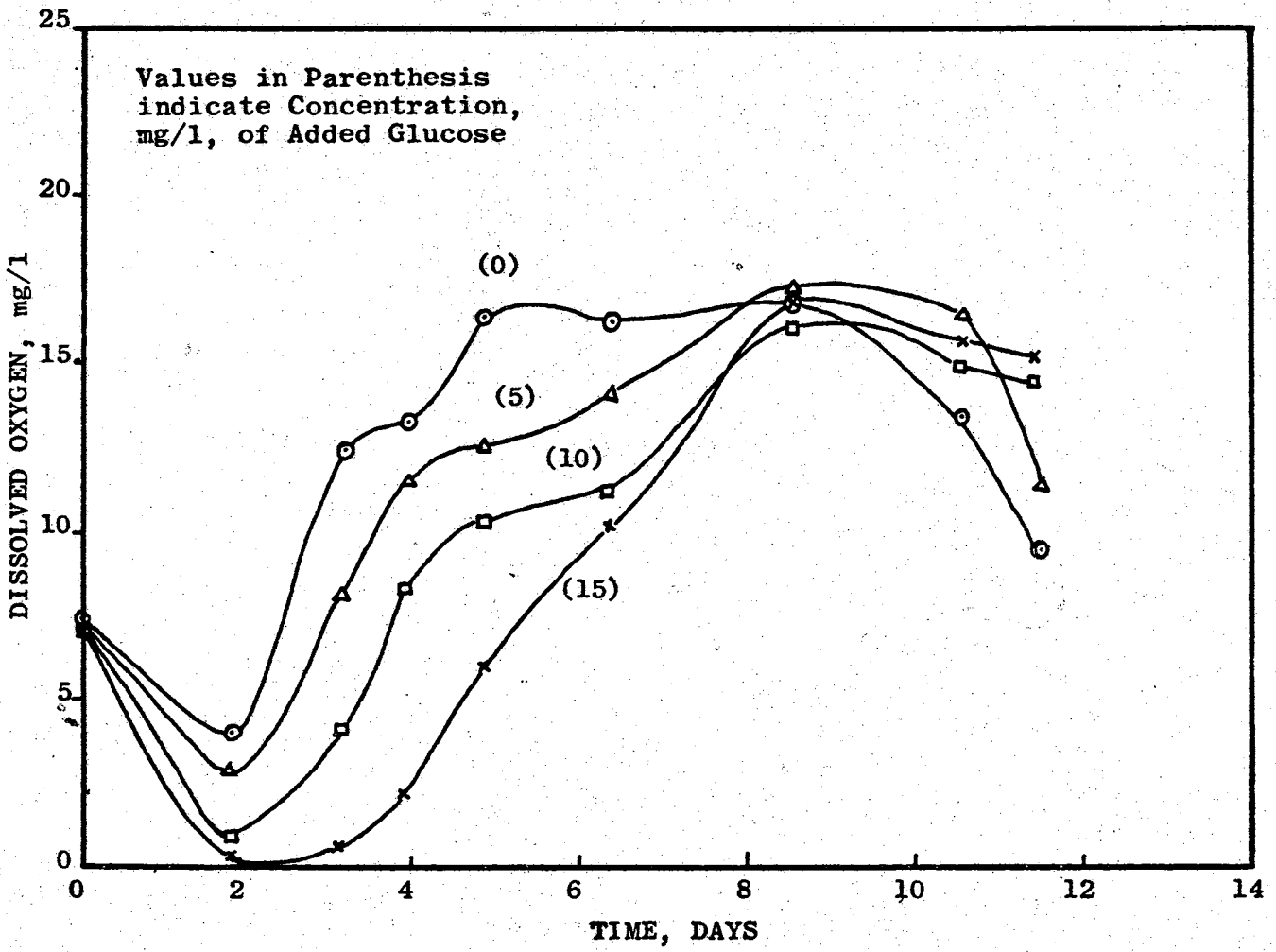

Figure 11. Effect of Various Organic Loadings on Oxygen Production in Closed Systems Containing Heterotrophic Bacteria and Algae.

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

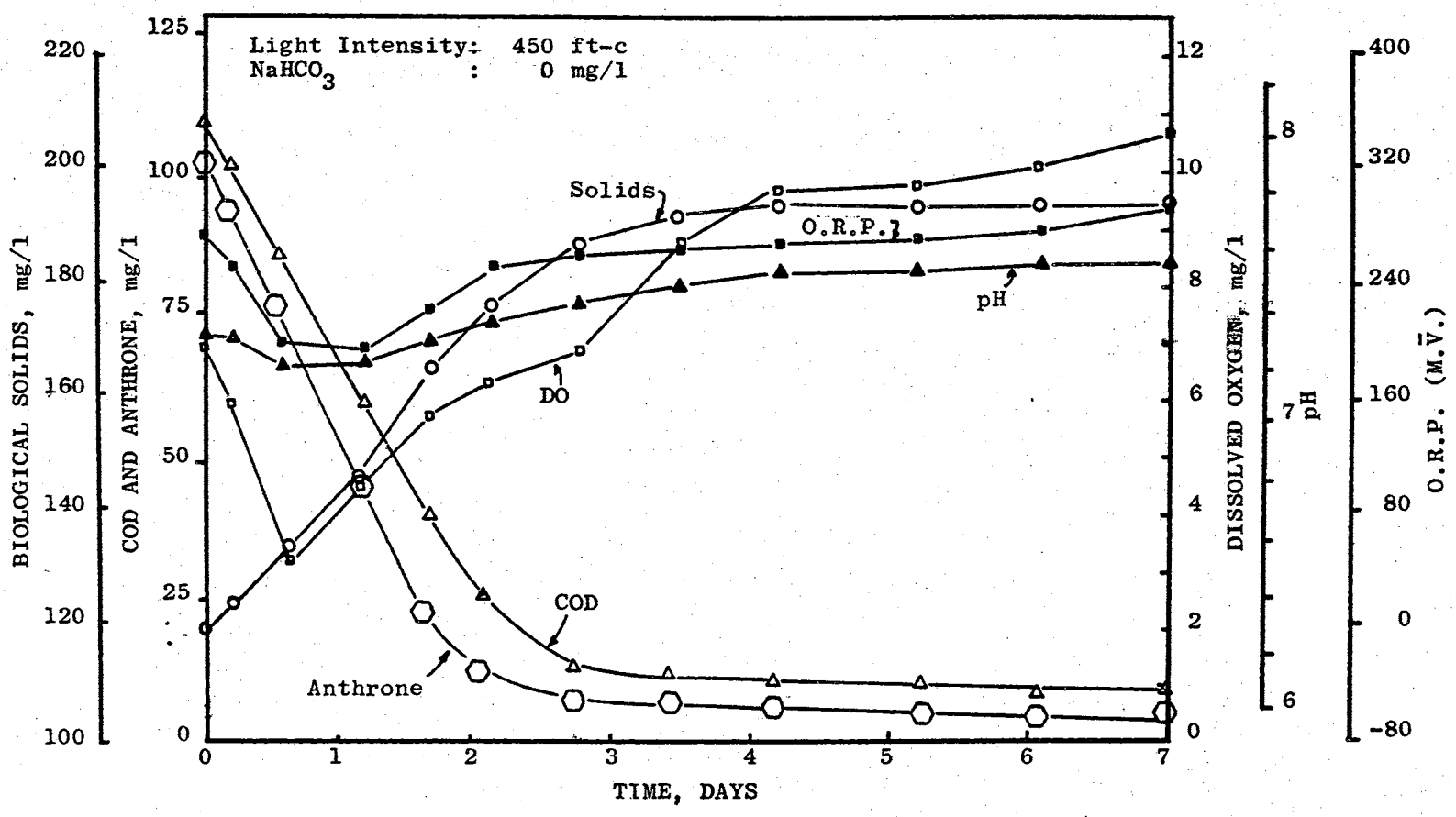

Figure 12. Purification of 100 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

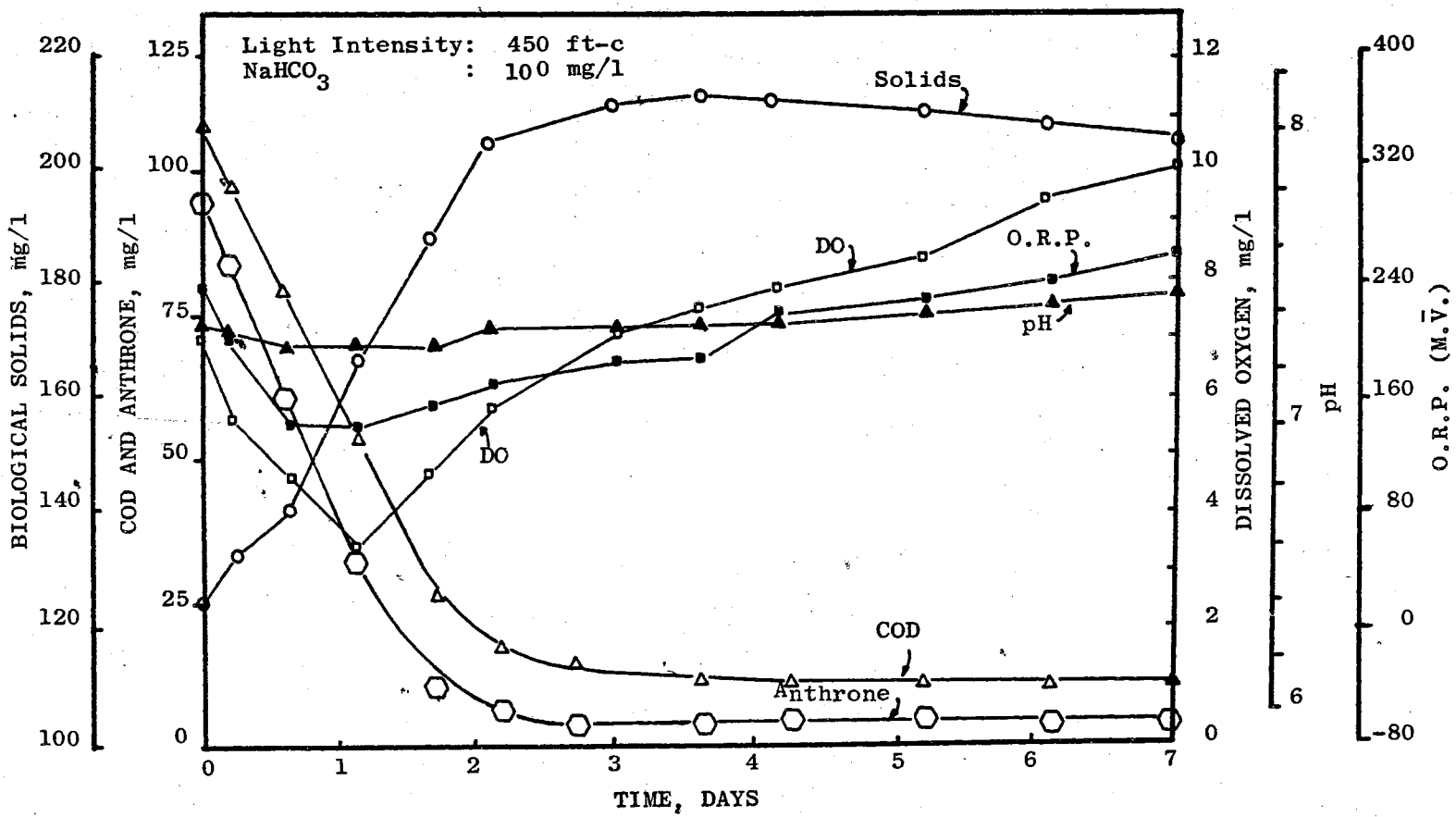

Figure 13. Purification of 100 mg/l Glucose in a Batch Oxidation Period with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

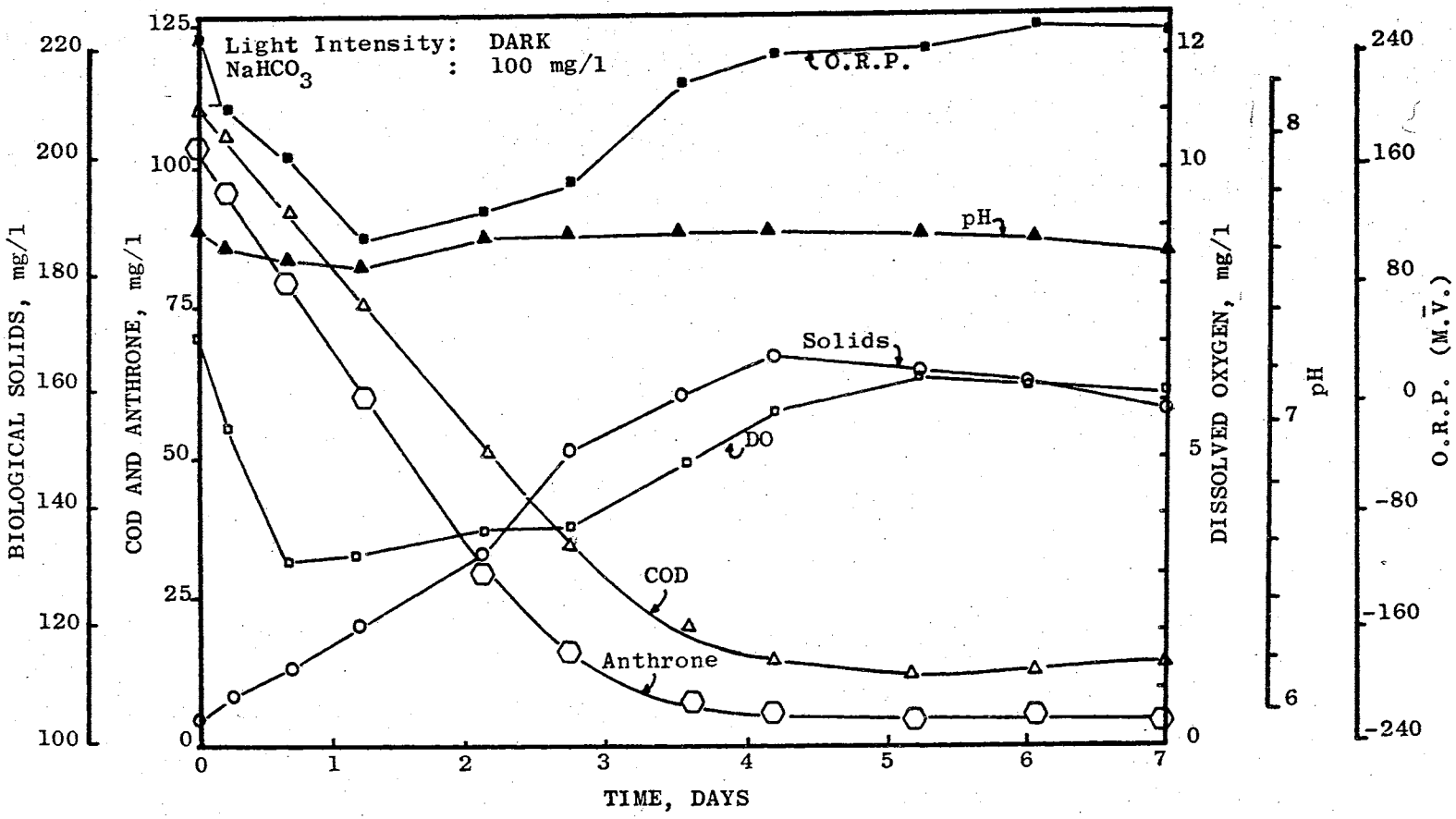

Figure 14. Purification of 100 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

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

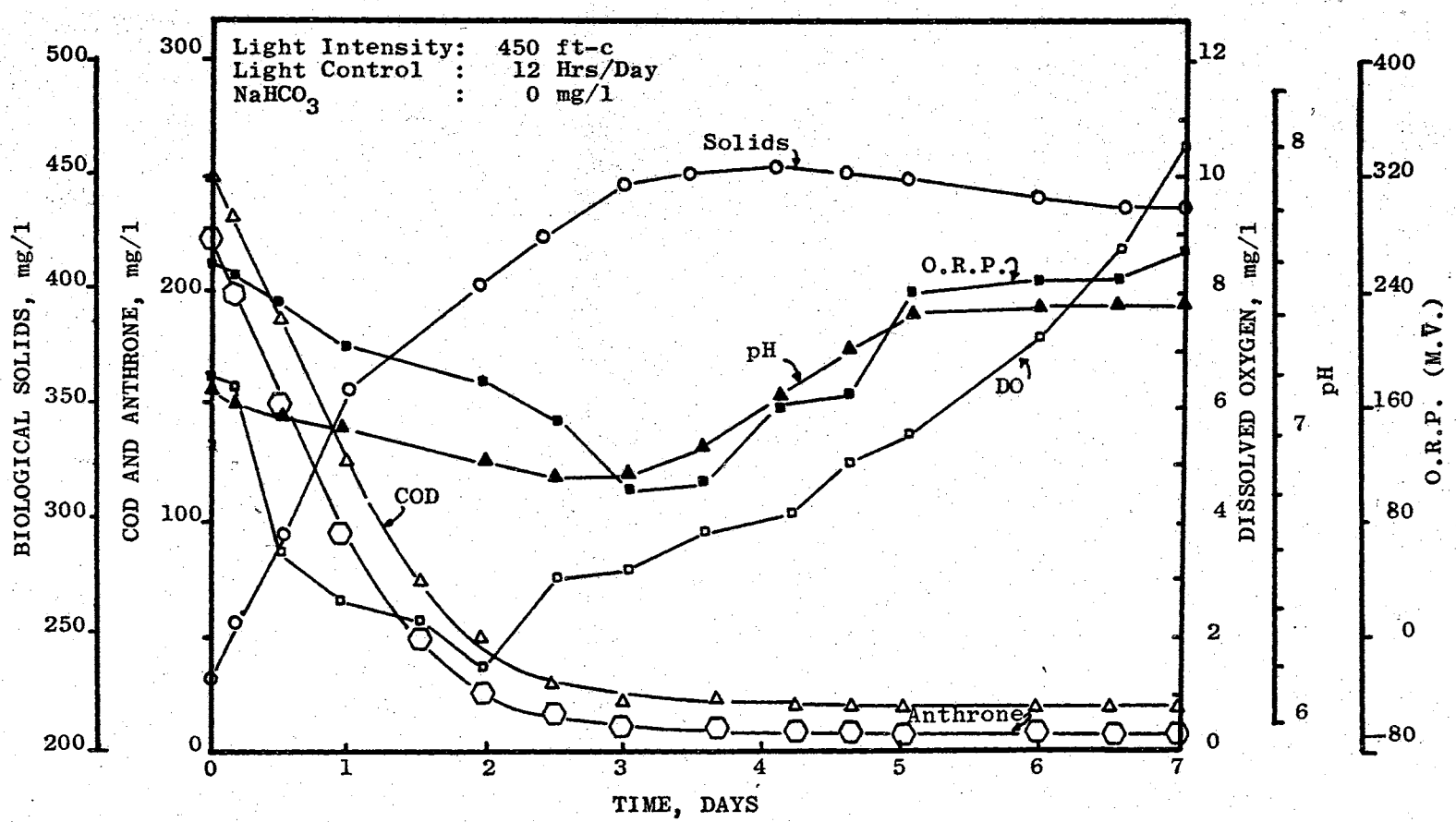

Figure 15. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

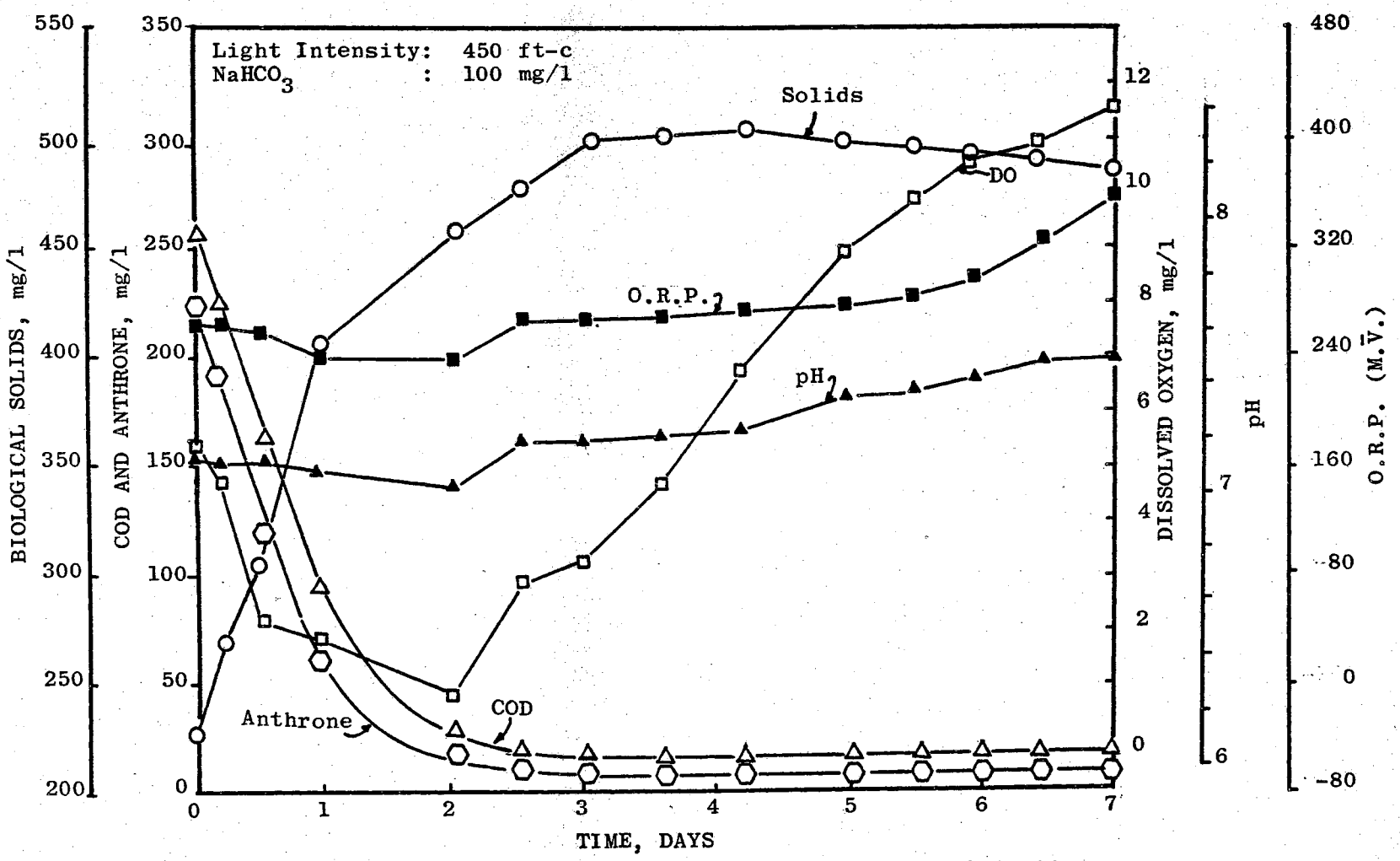

Figure 16. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l of Sodium Bicarbonate (Light Period - 12 Hrs/Day).

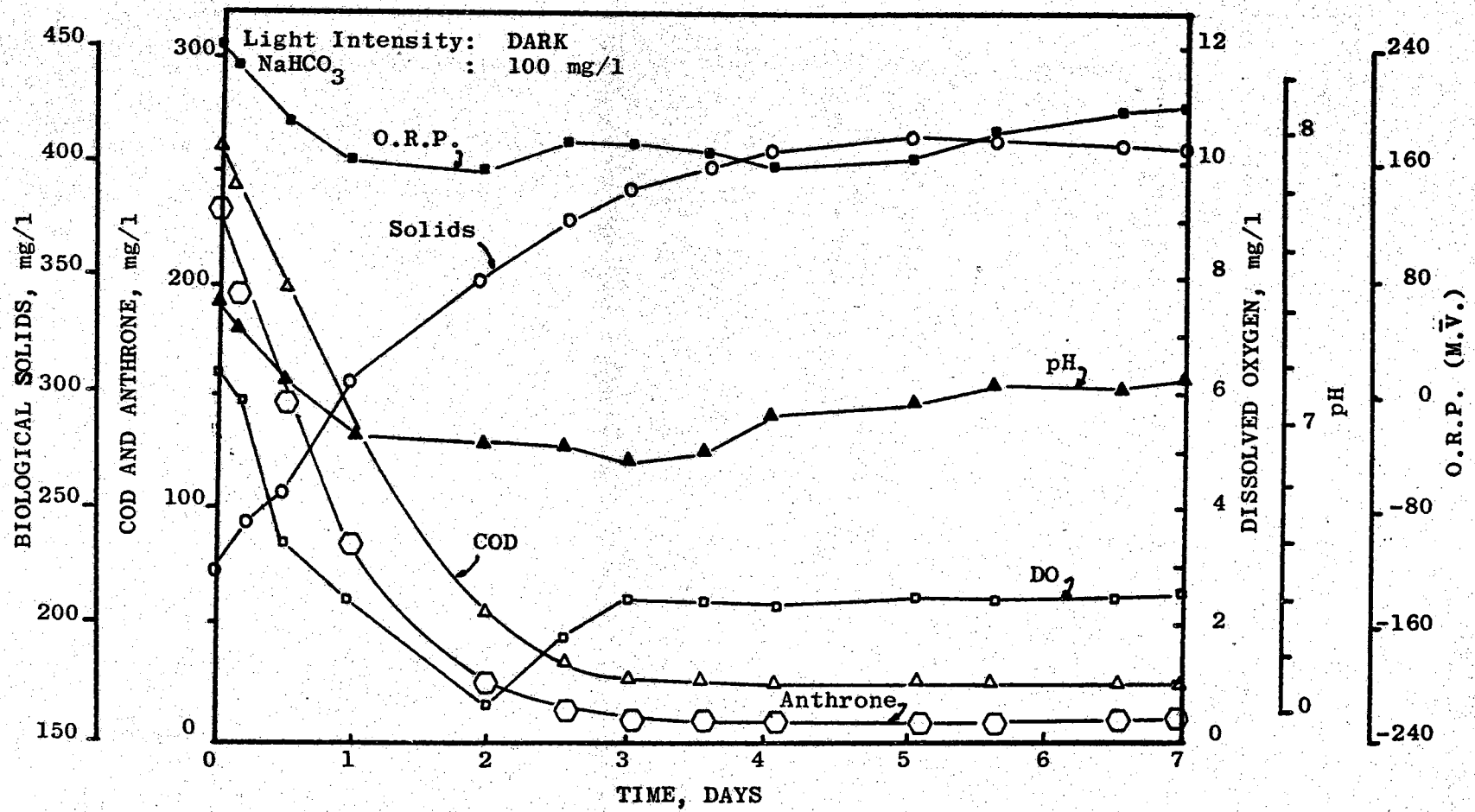

Figure 17. Purification of 200 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition used under Condition of No Lighting.

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

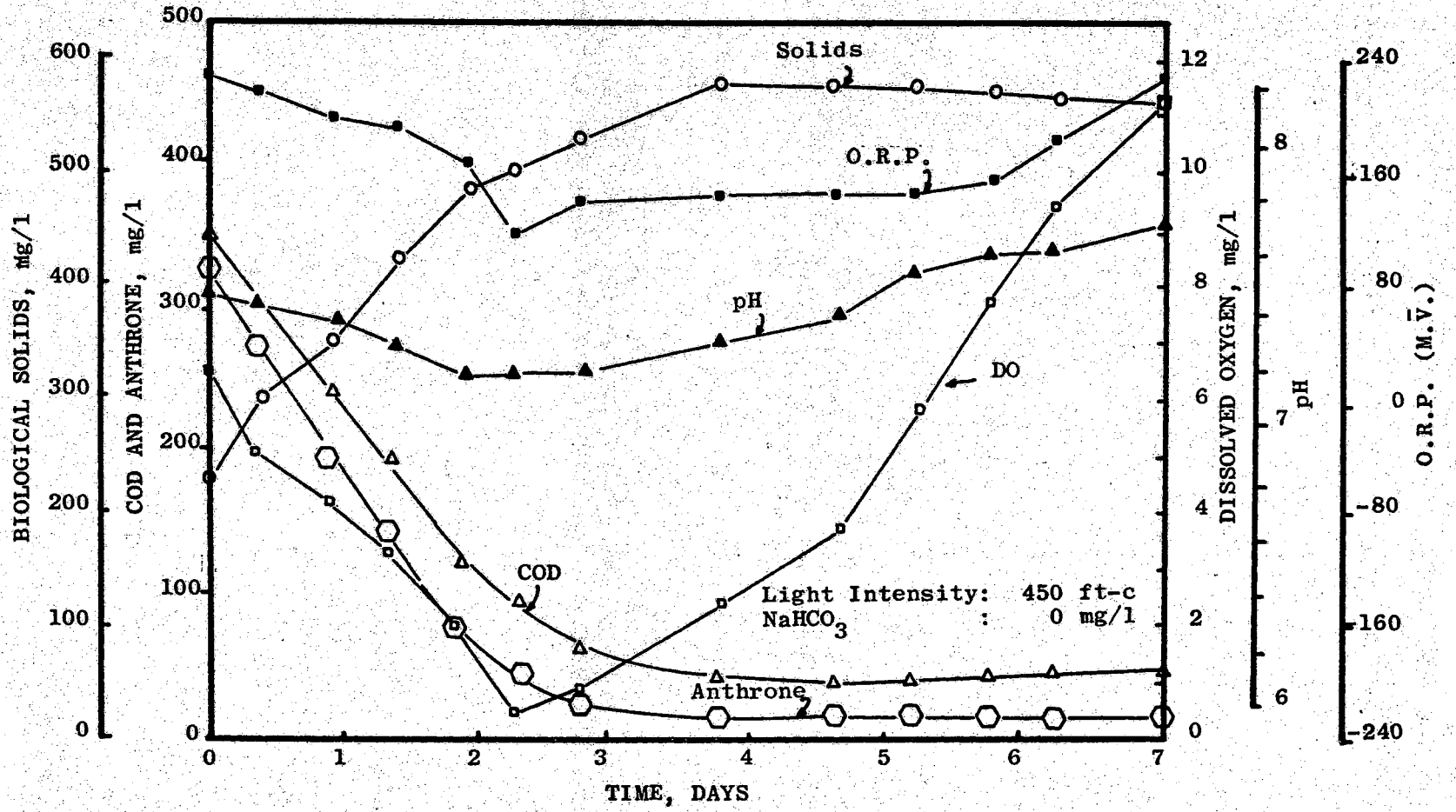

Figure 18. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

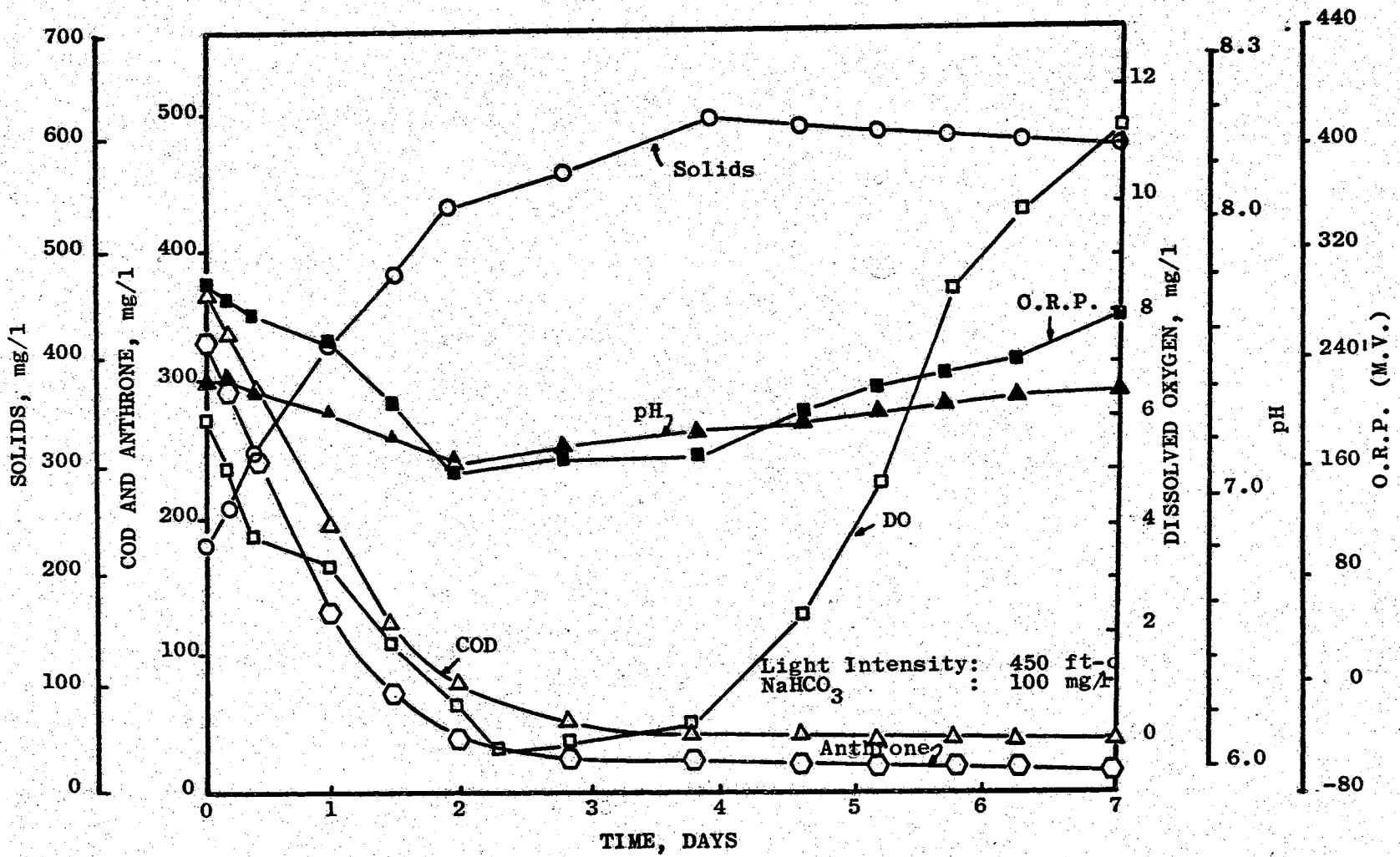

Figure 19. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l of Sodium Bicarbonate (Light Period - 12 Hrs/Day).

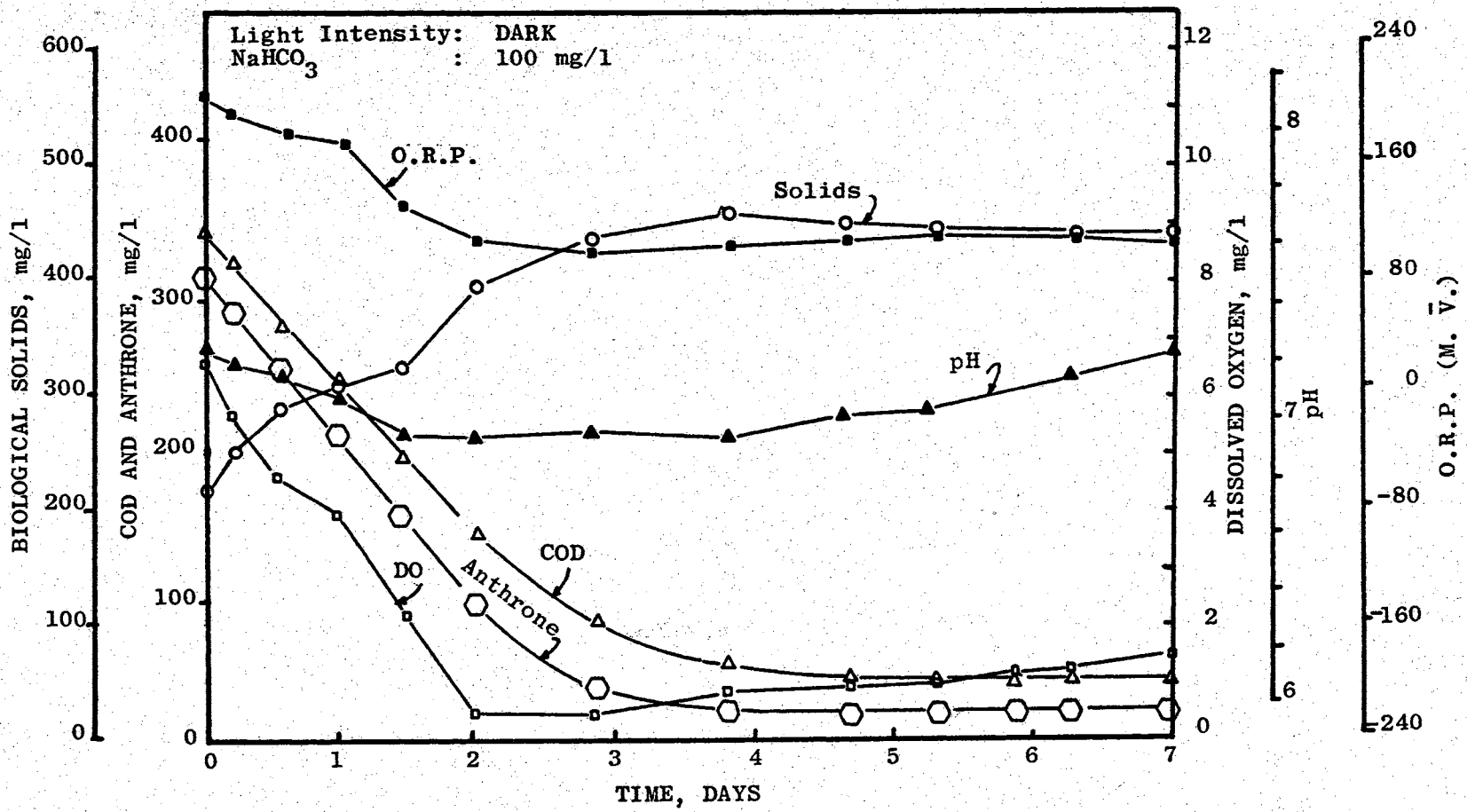

Figure 20. Purification of 300 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

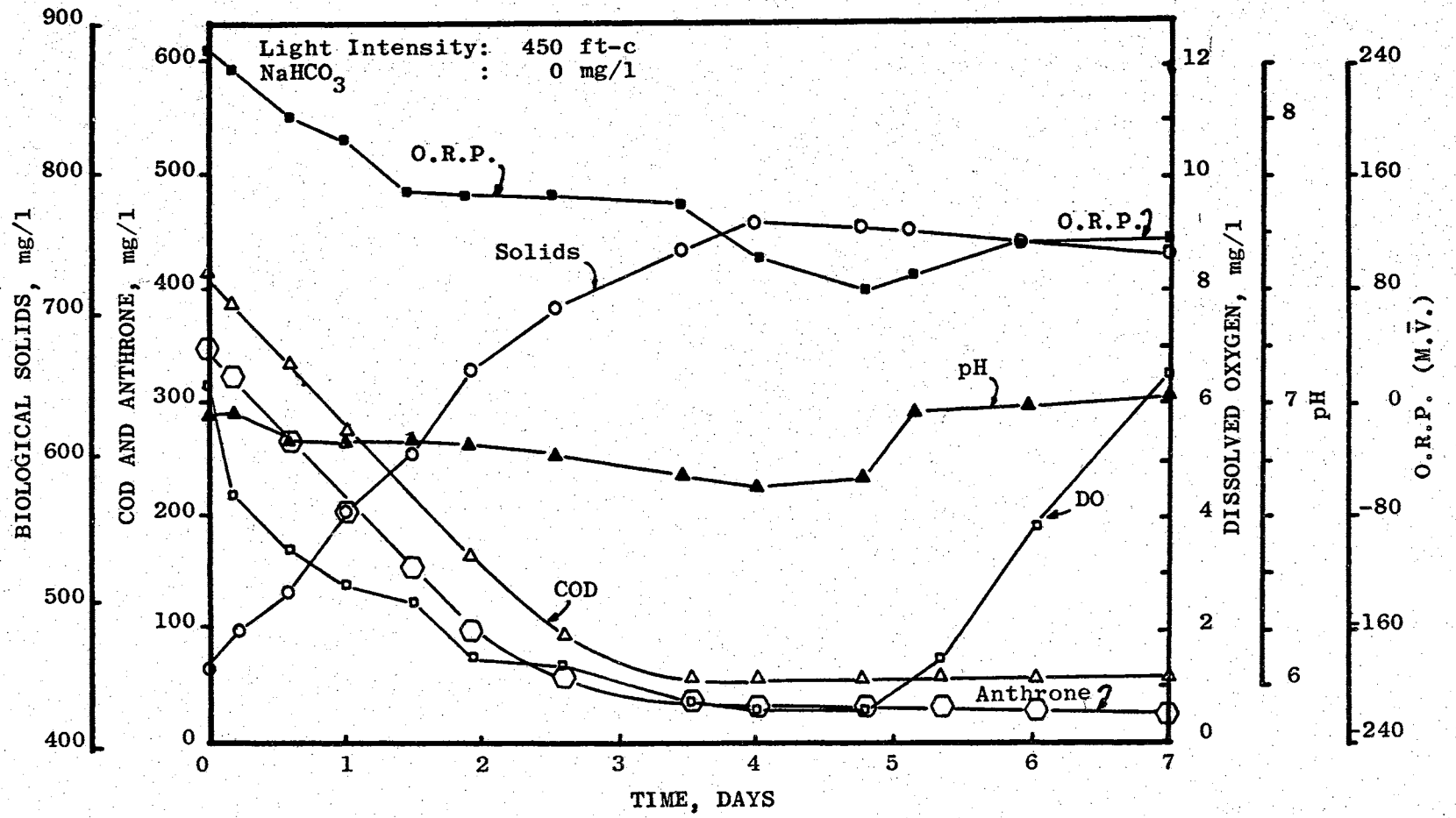

Figure 21. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

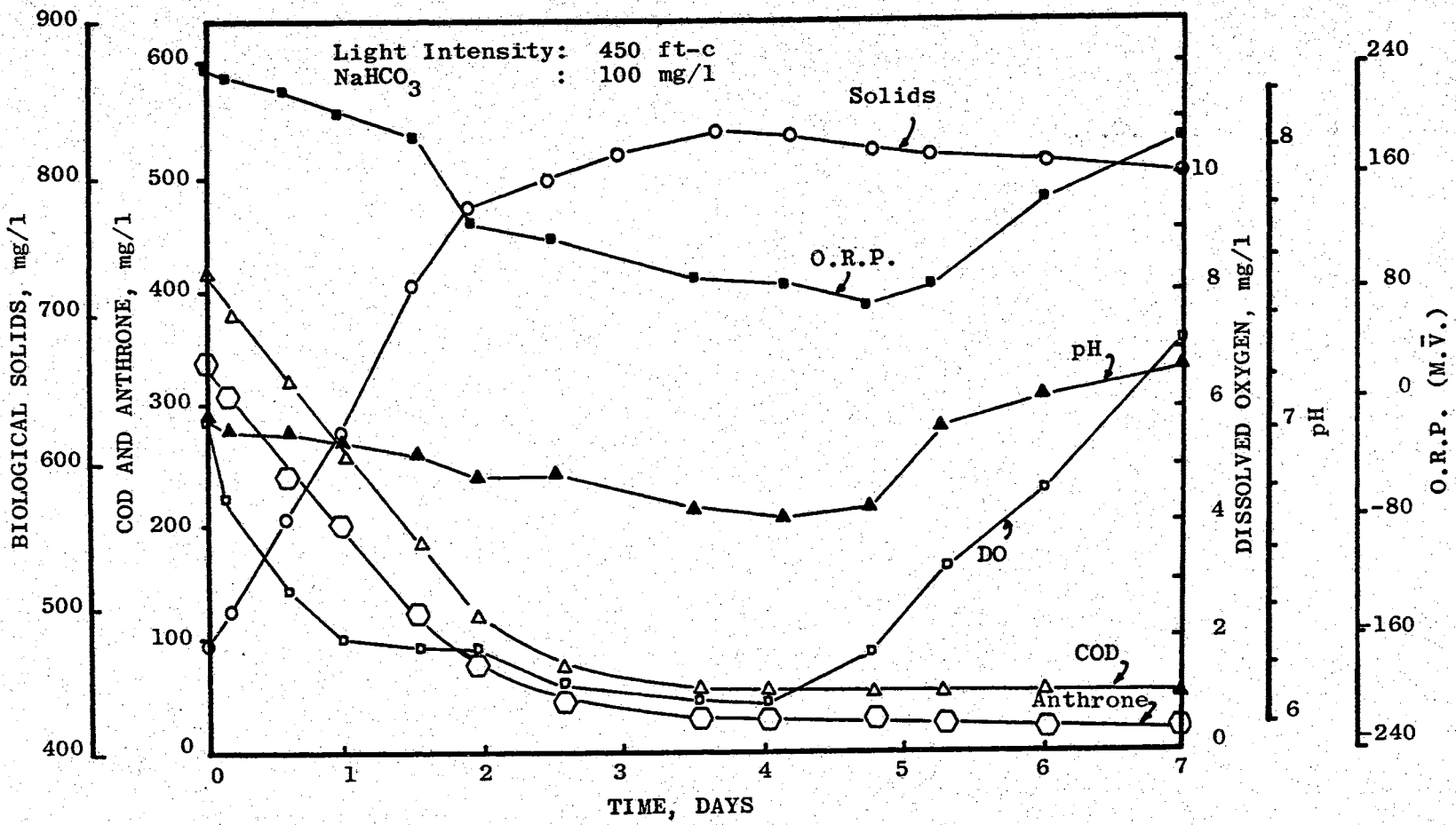

Figure 22. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

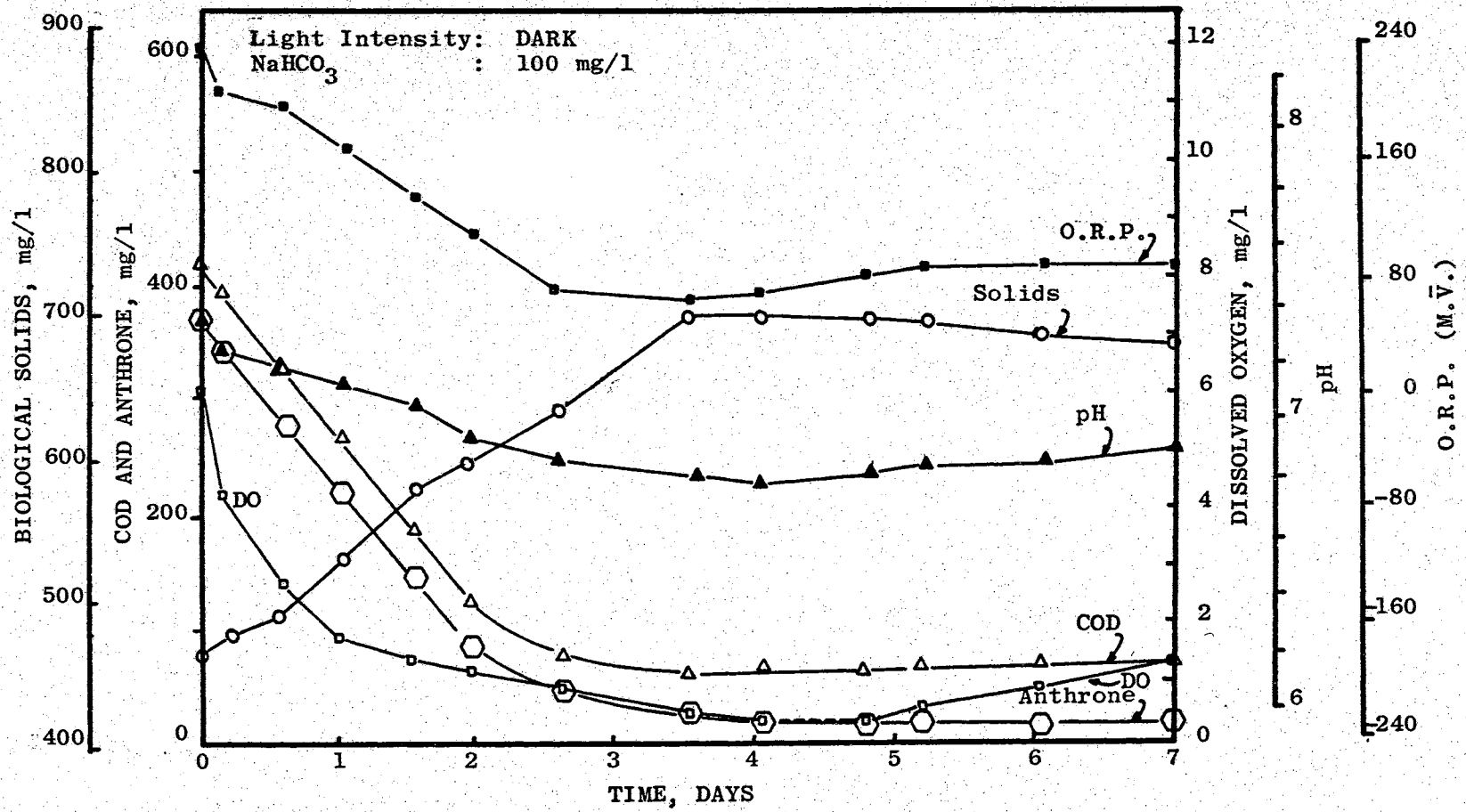

Figure 23. Purification of 400 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

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

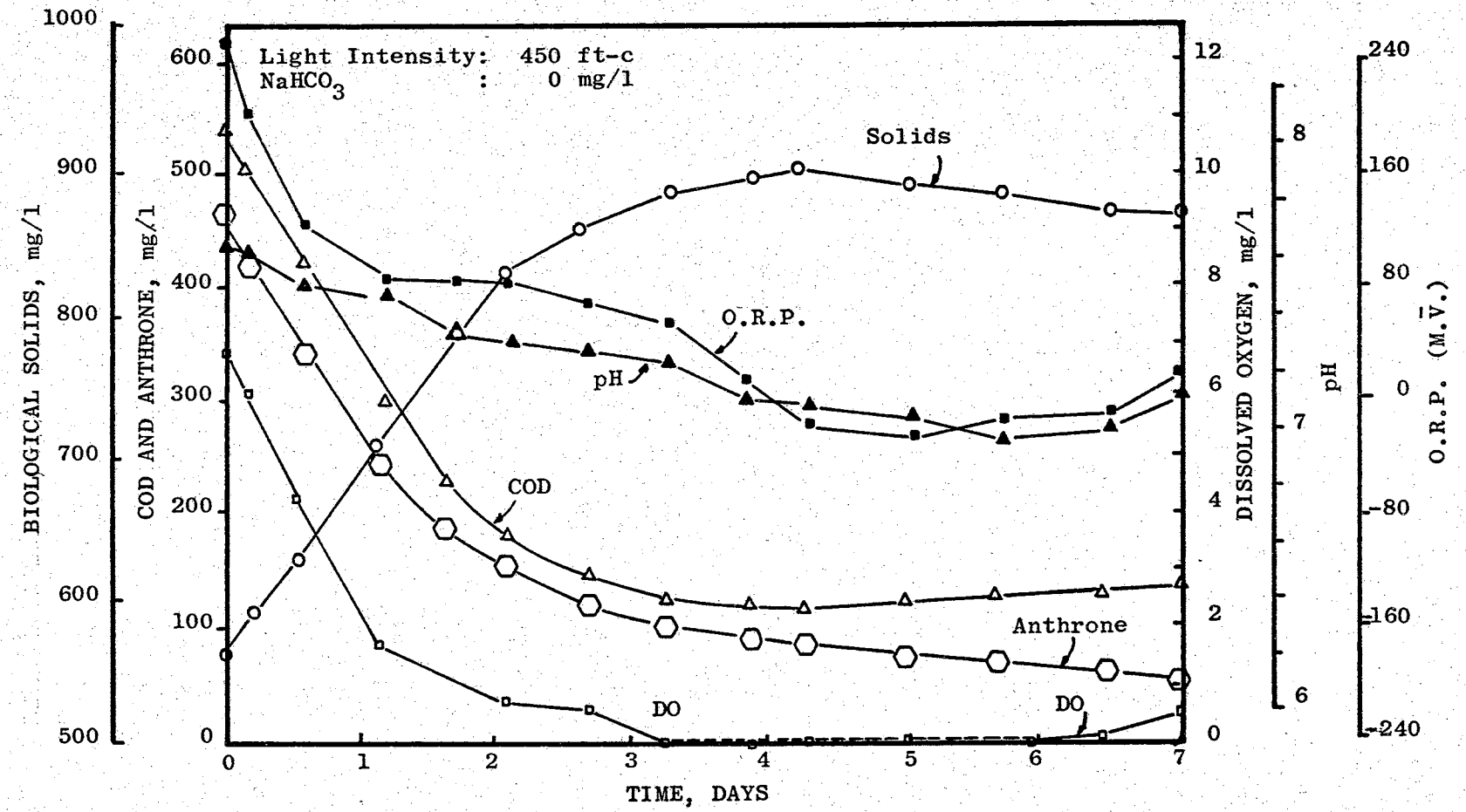

Figure 24. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

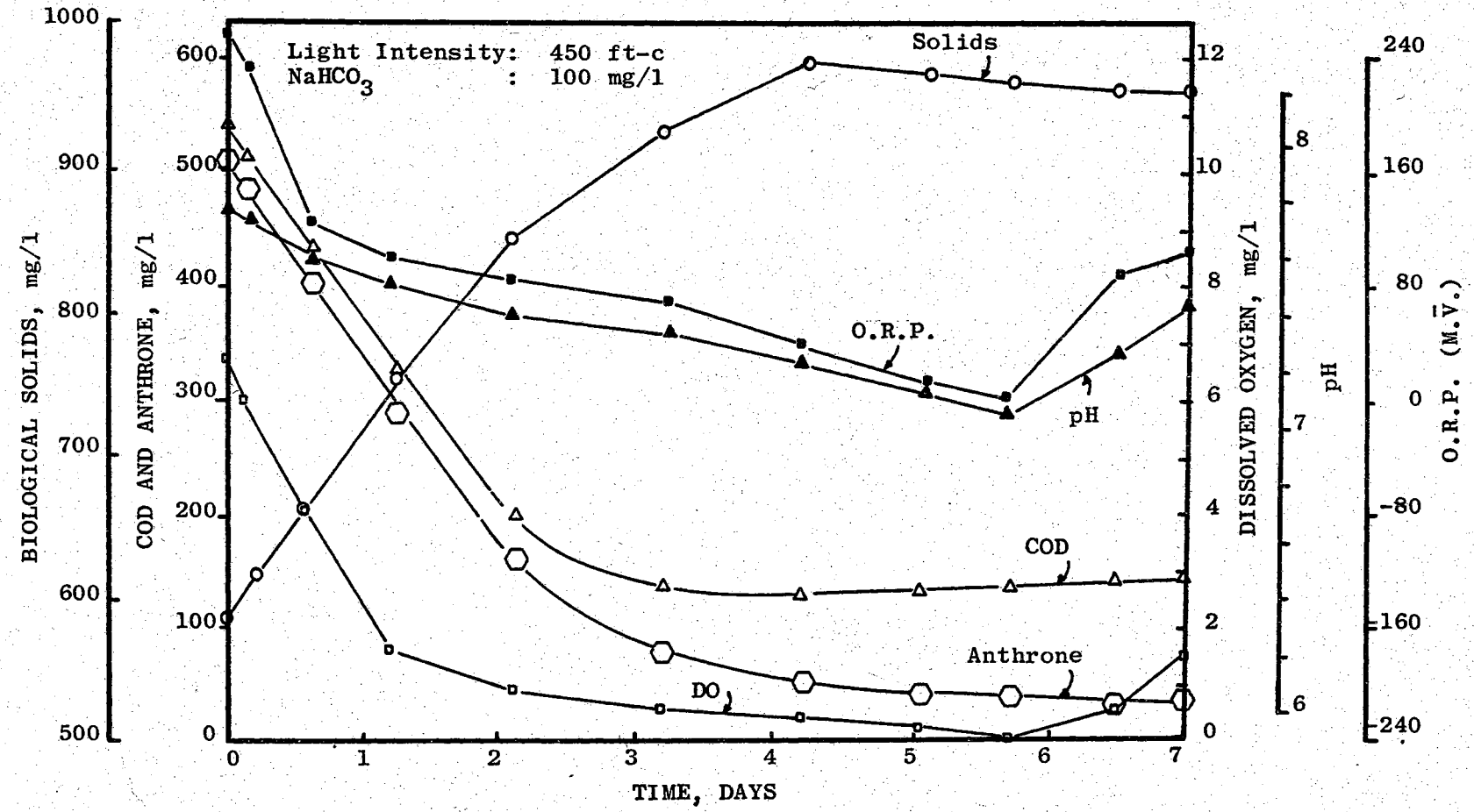

Figure 25. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

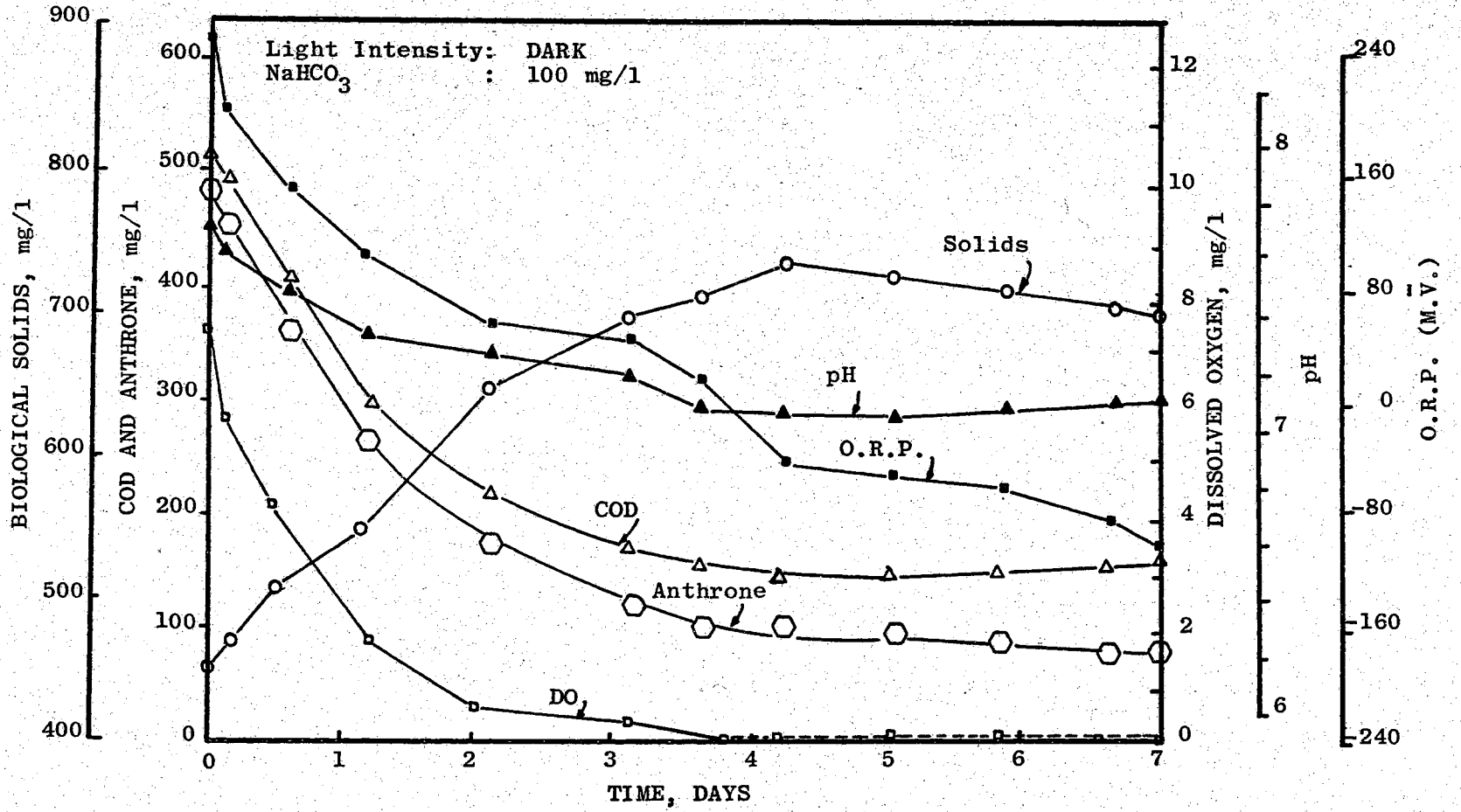

Figure 26. Purification of 500 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

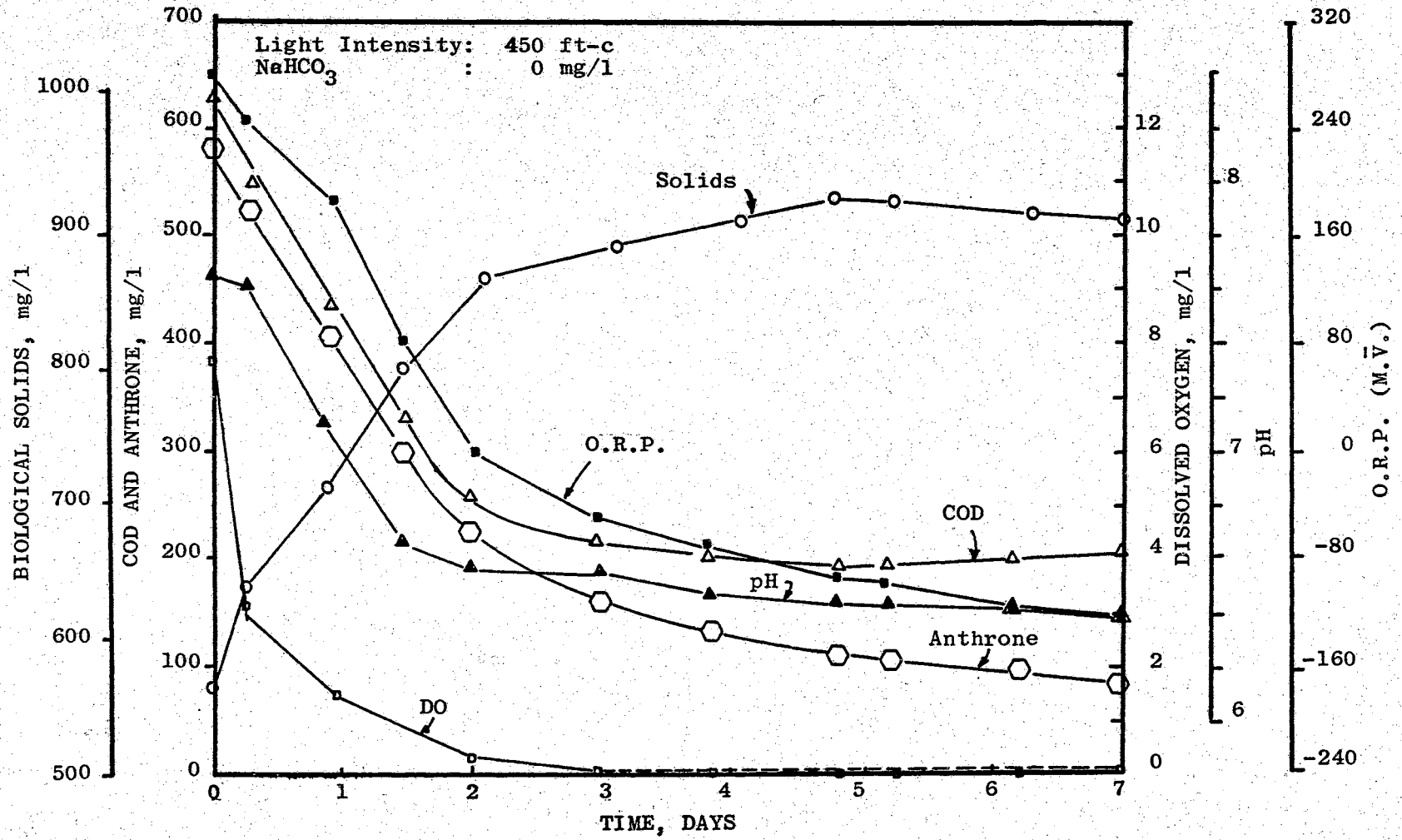

Figure 27. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with No Sodium Bicarbonate added (Light Period - 12 Hrs/Day).

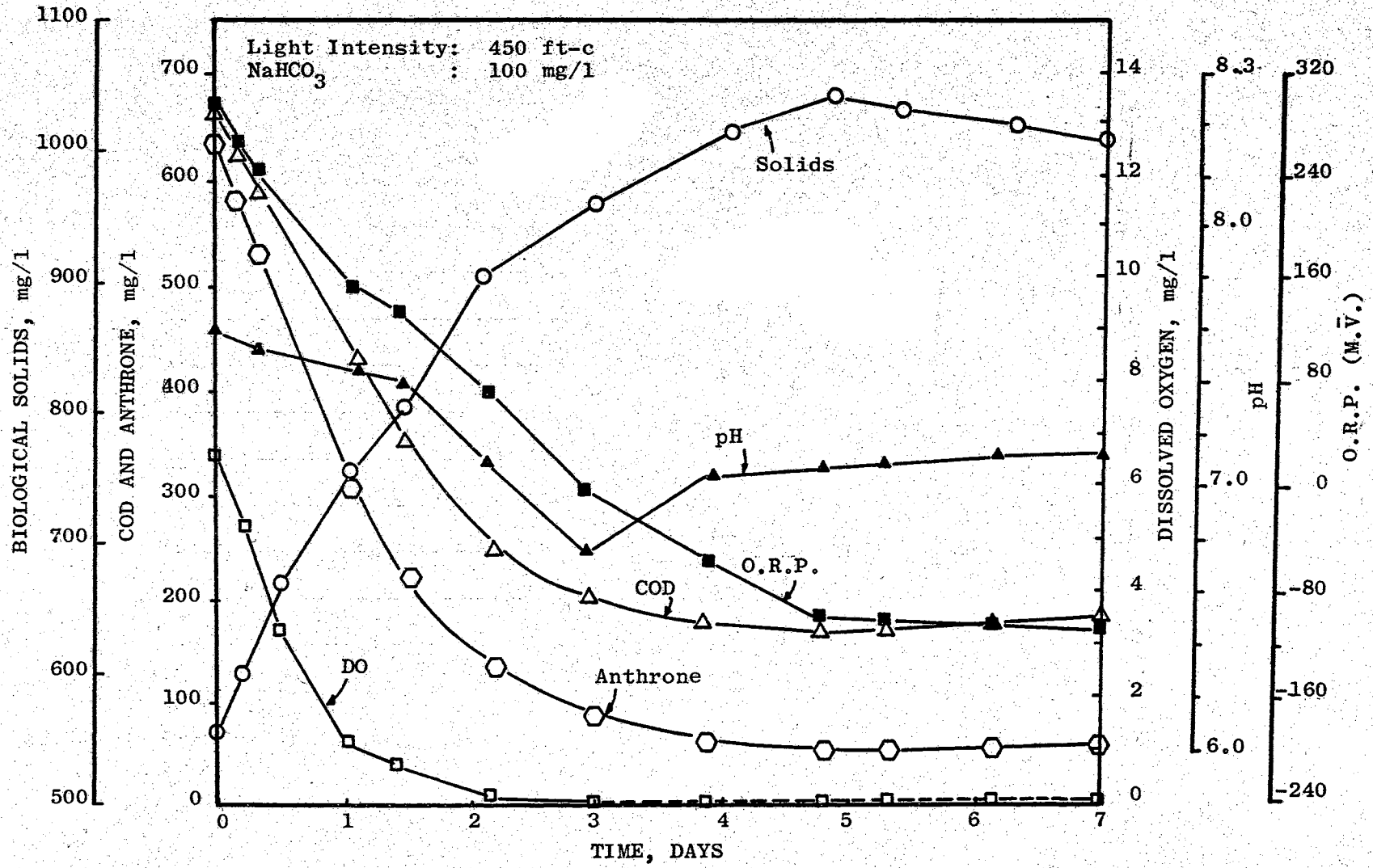

Figure 28. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

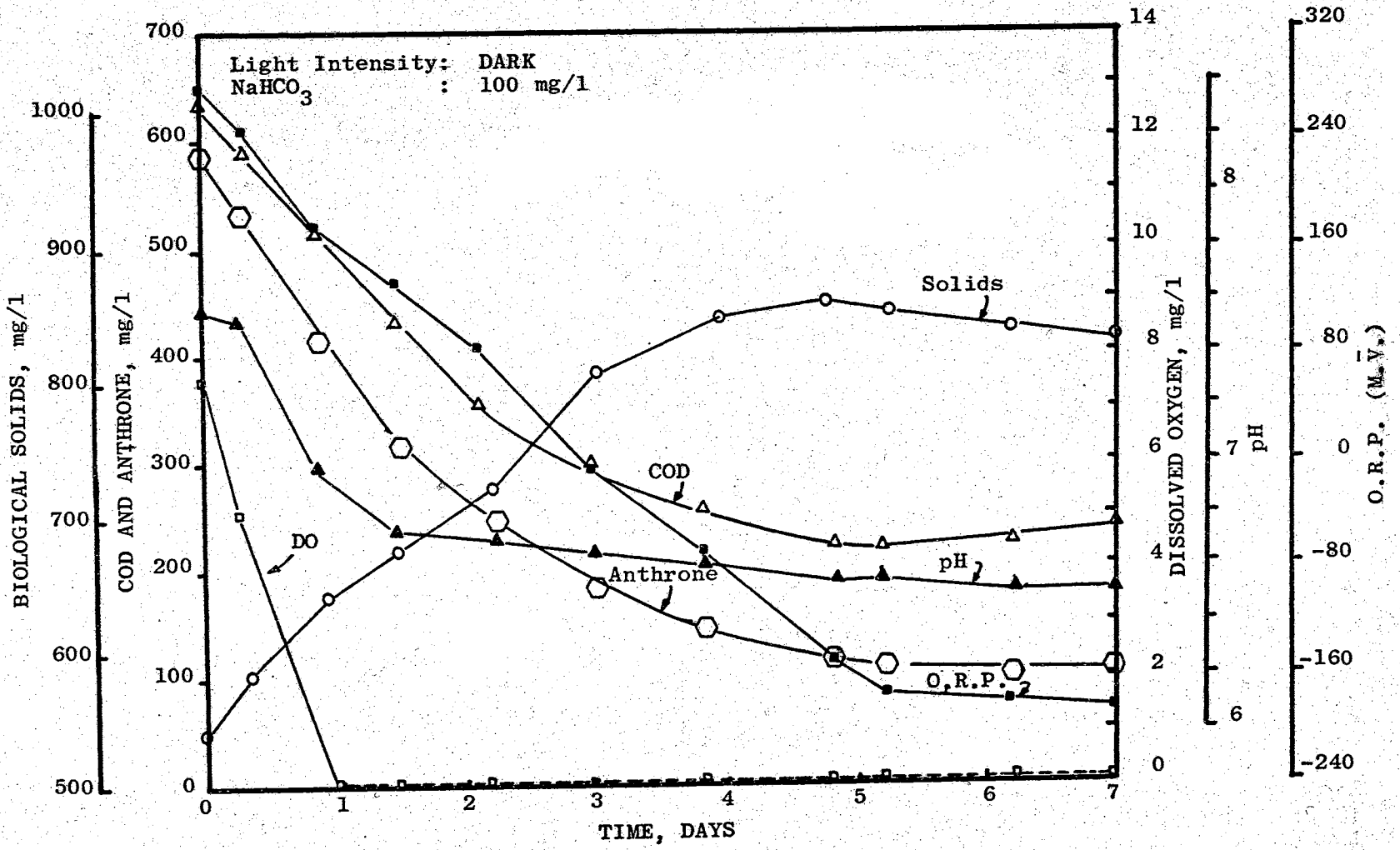

Figure 29. Purification of 600 mg/l Glucose in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

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

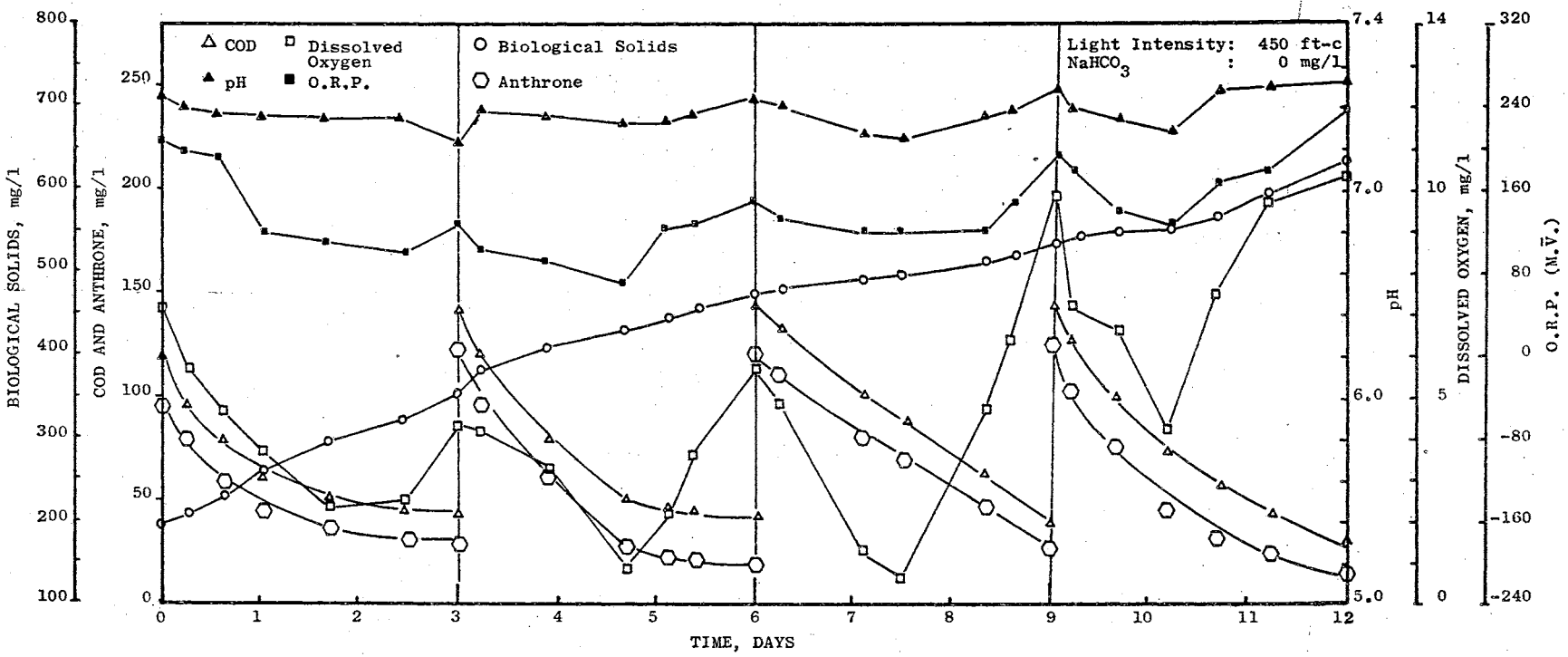

Figure 30. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

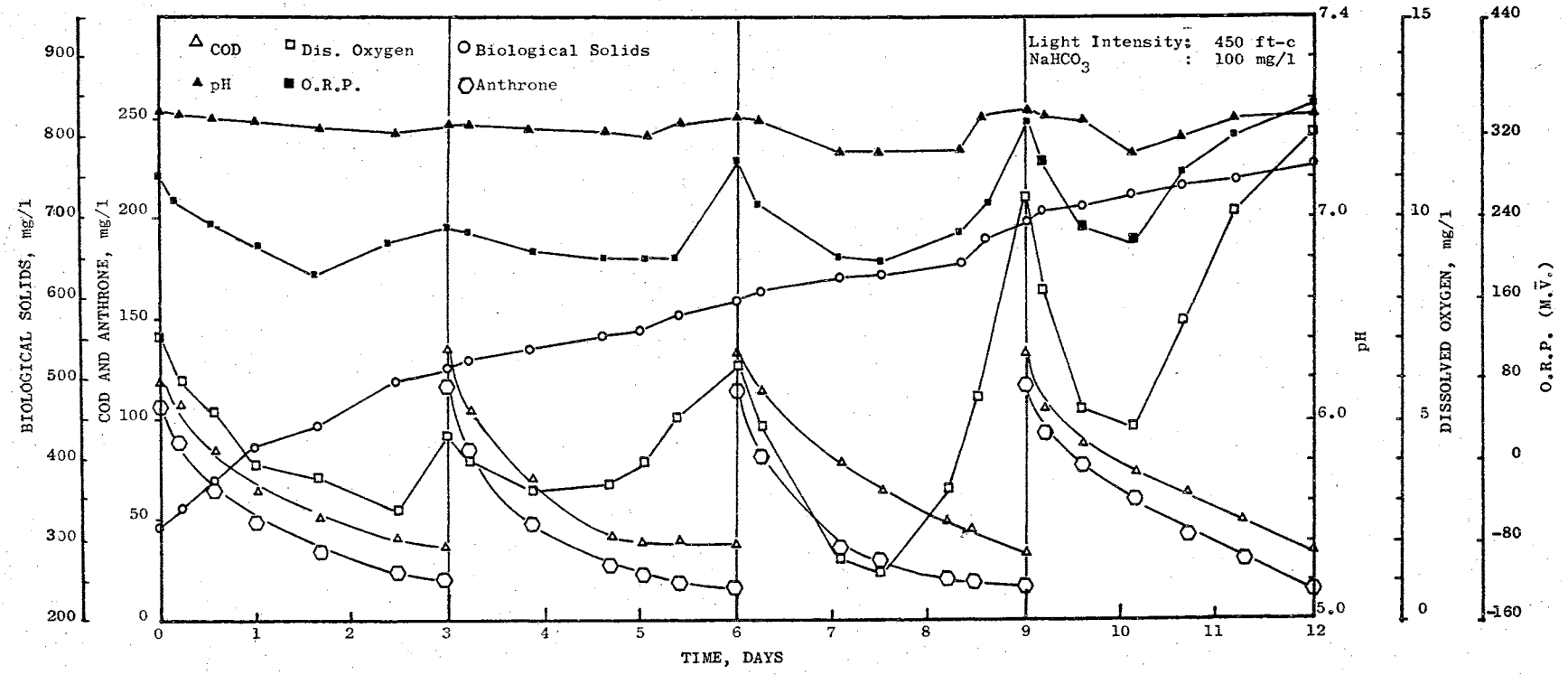

Figure 31. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

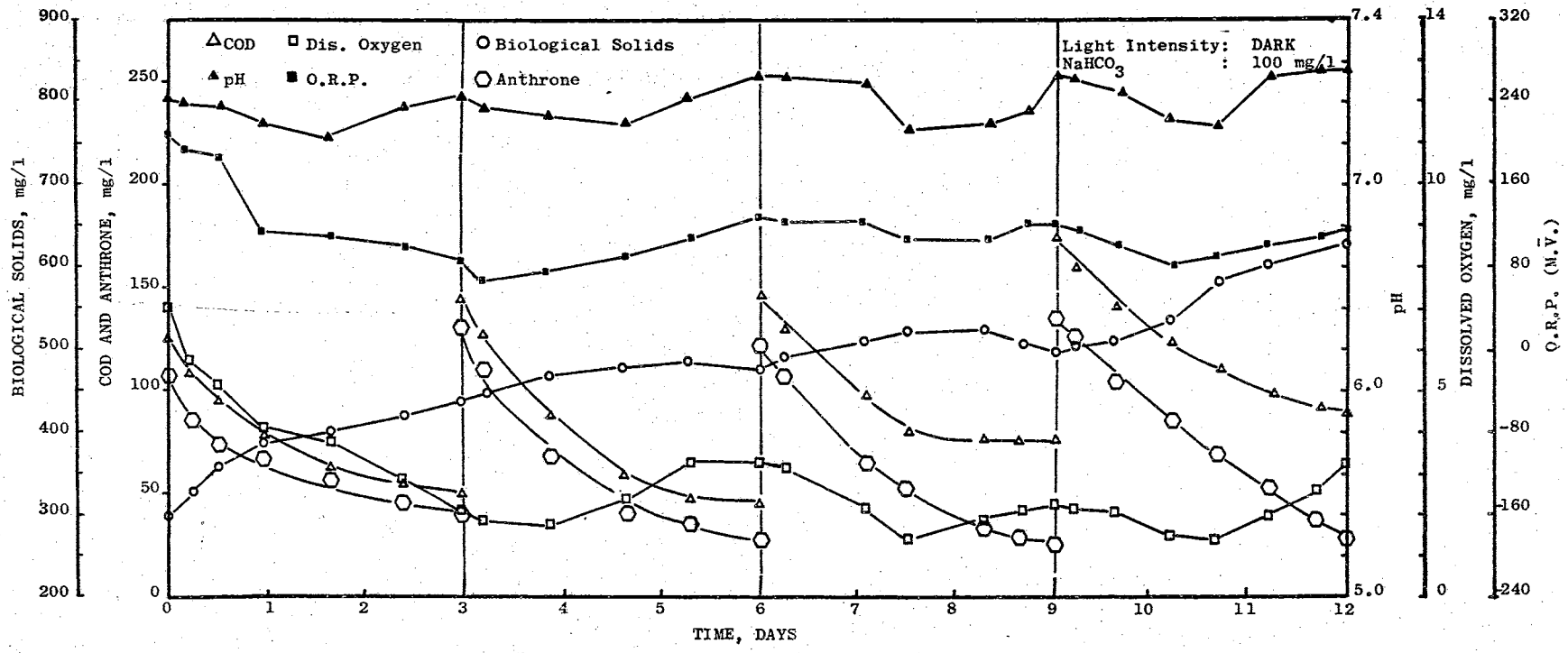

Figure 32. Purification of 100 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

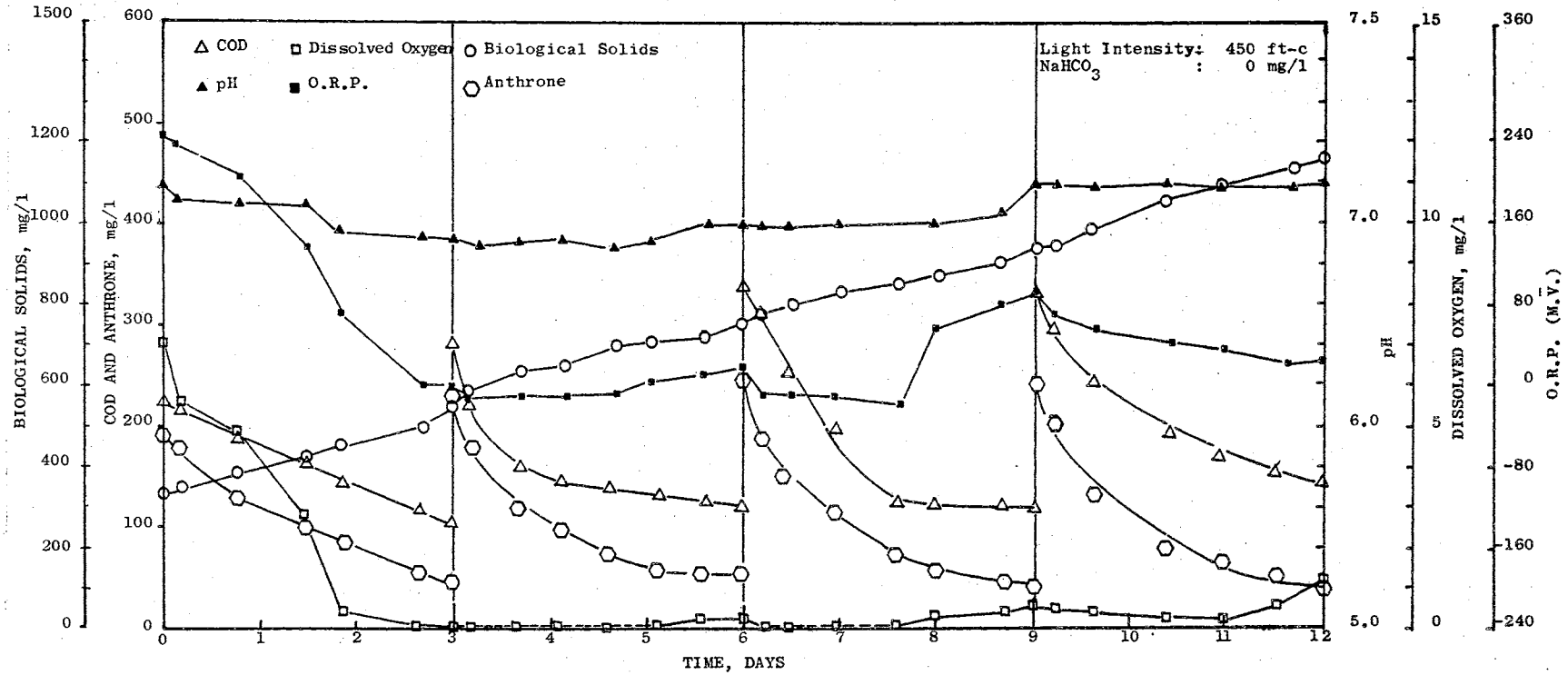

Figure 33. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

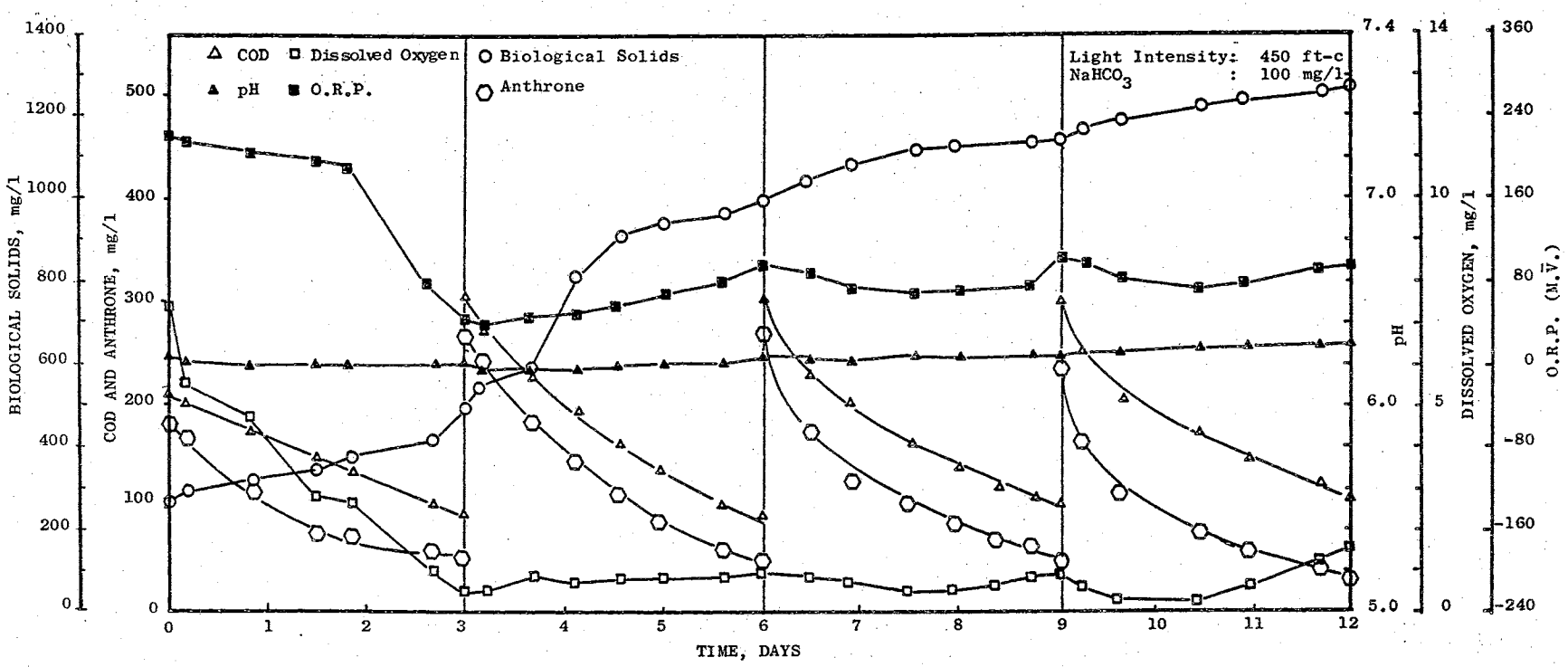

Figure 34. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

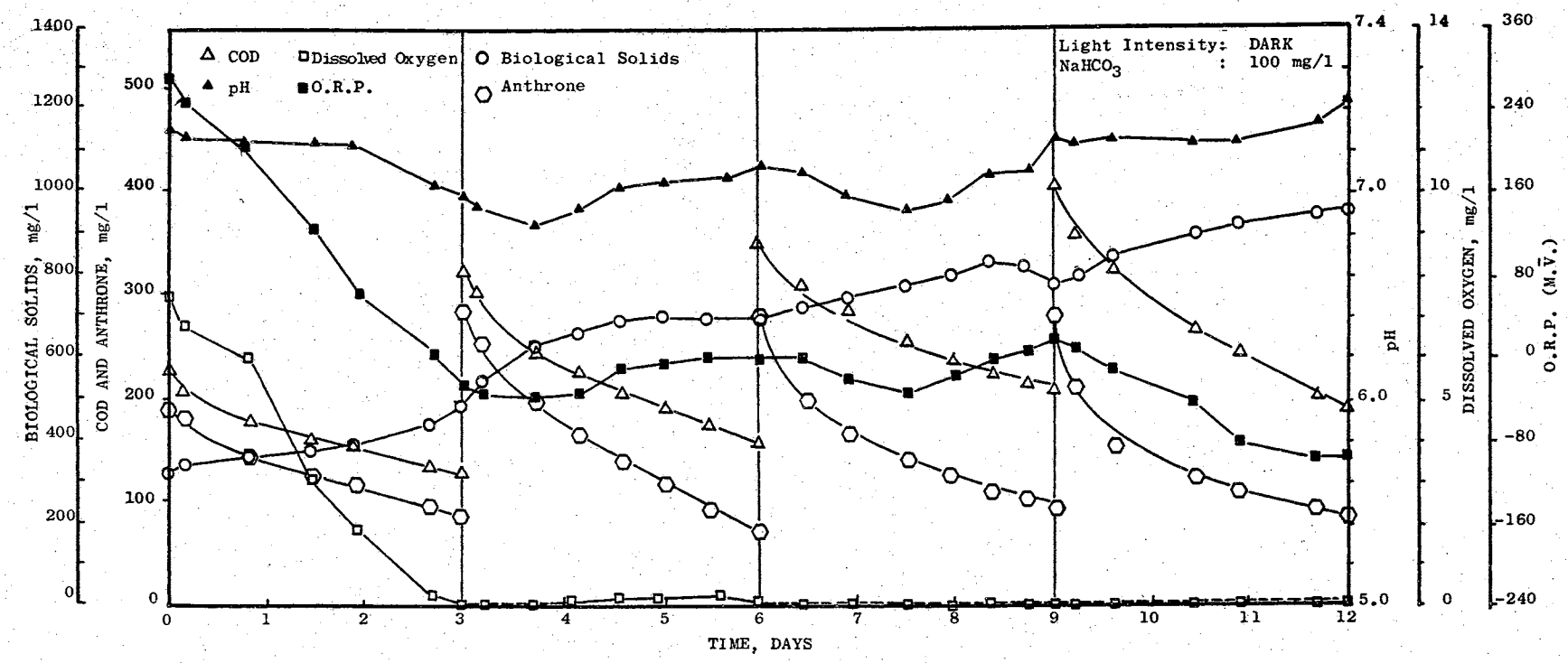

Figure 35. Purification of 200 mg/l Glucose Applied Every Third Day in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

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 Efficienty (%) 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

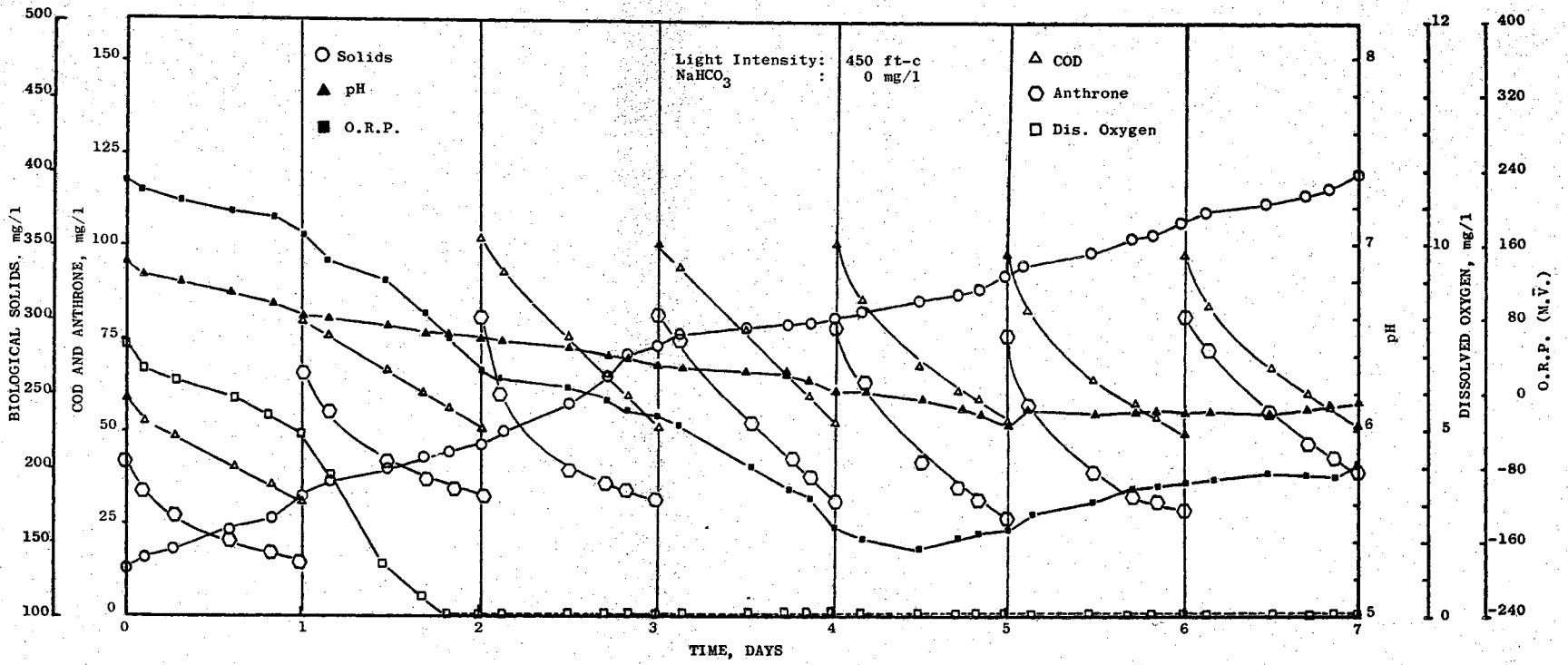

Figure 36. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with No Sodium Bicarbonate Added (Light Period - 12 Hrs/Day).

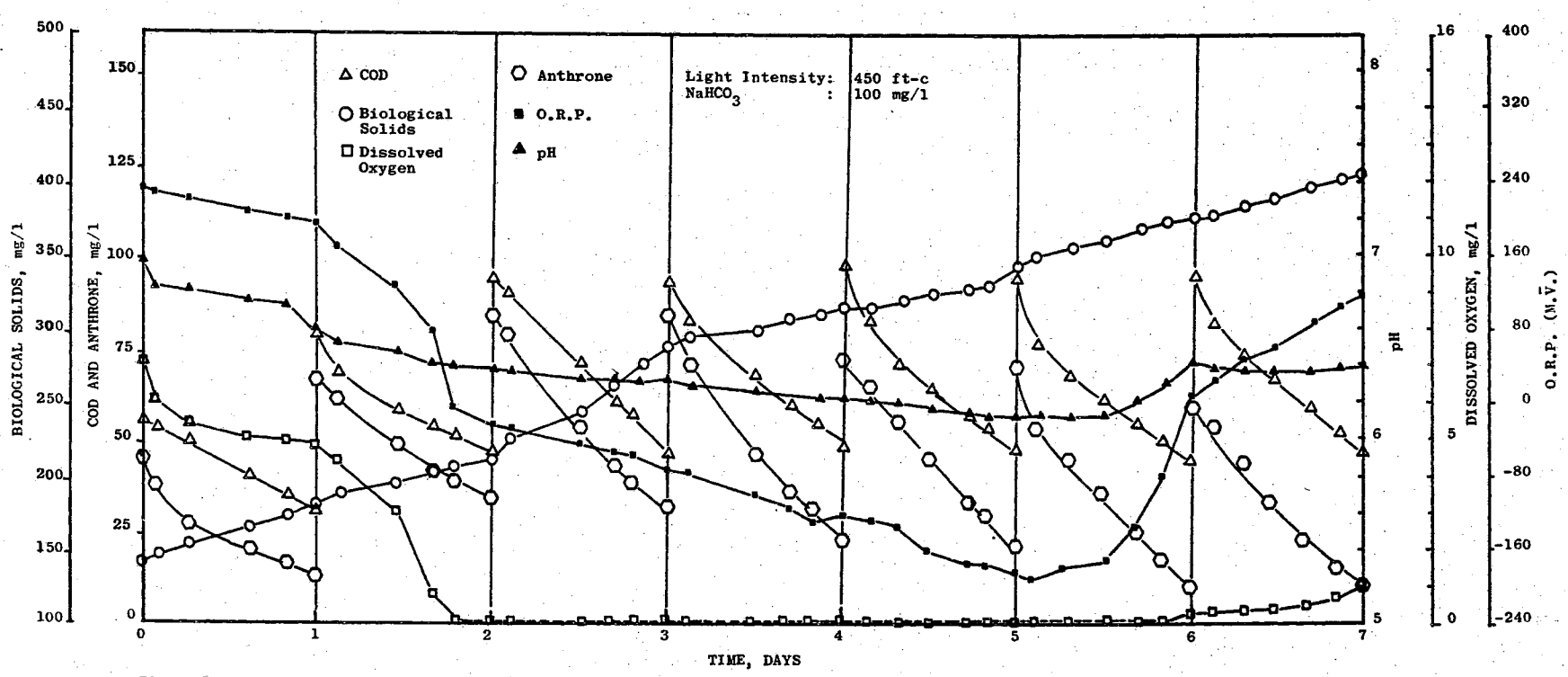

Figure 37. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

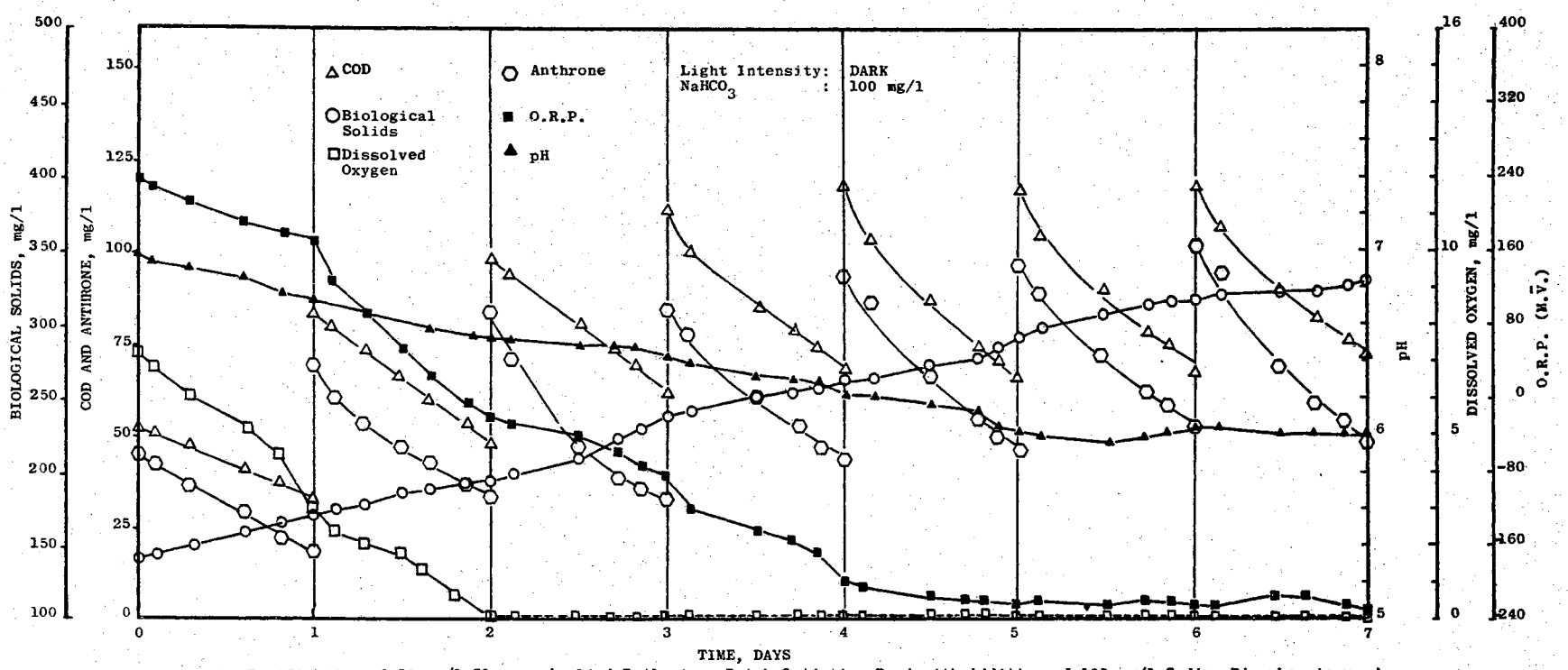

Figure 38. Purification of 50 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate used under Condition of No Lighting.

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.

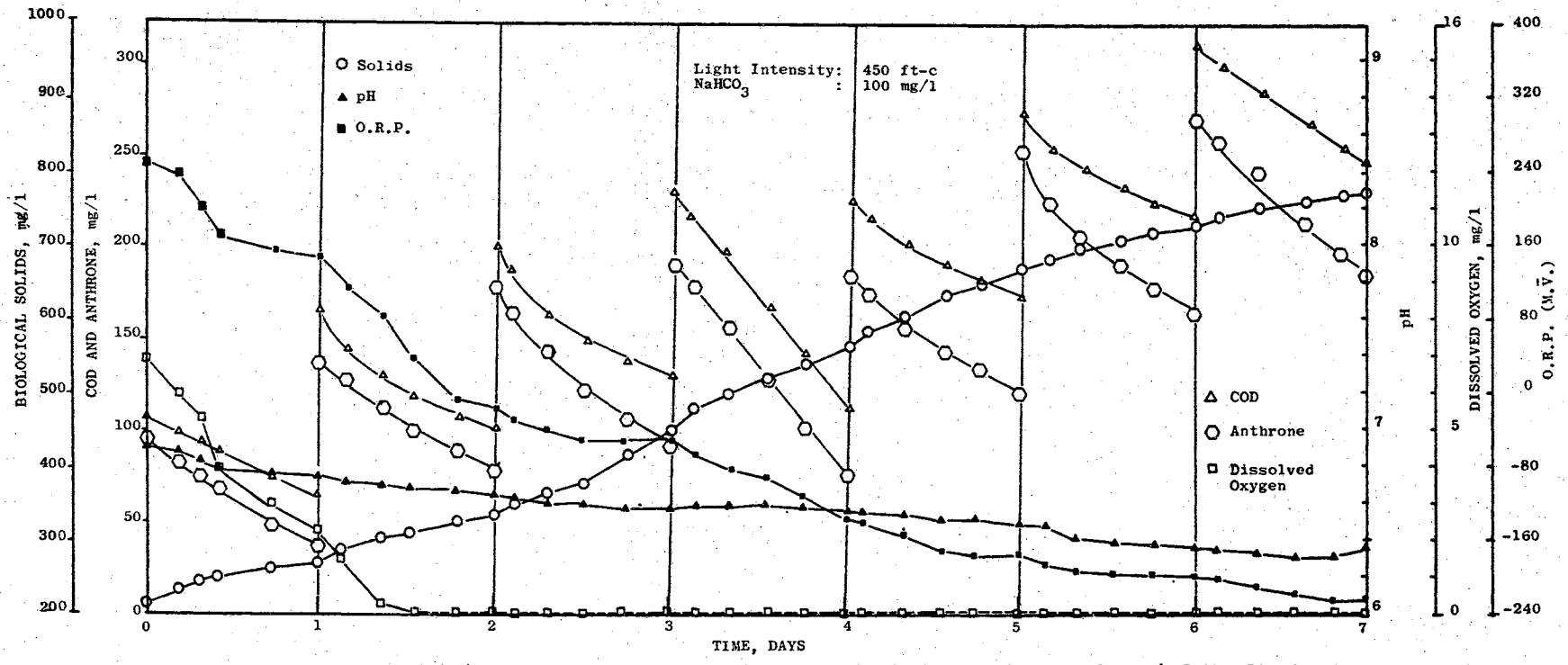

Figure 39. Purification of 100 mg/l Glucose Applied Daily in a Batch Oxidation Pond with Addition of 100 mg/l Sodium Bicarbonate (Light Period - 12 Hrs/Day).

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

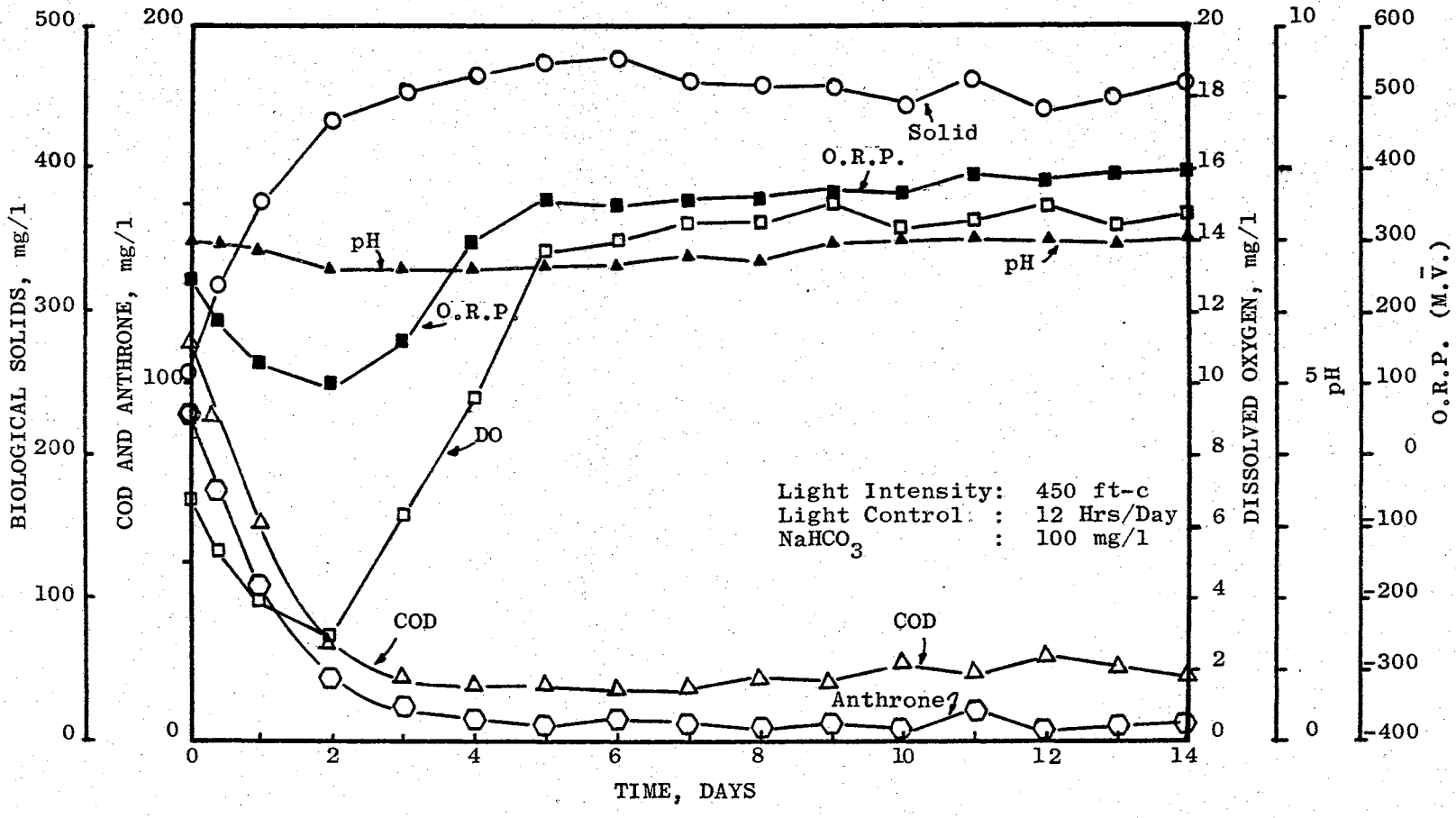

Figure 40. Purification of 100 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.

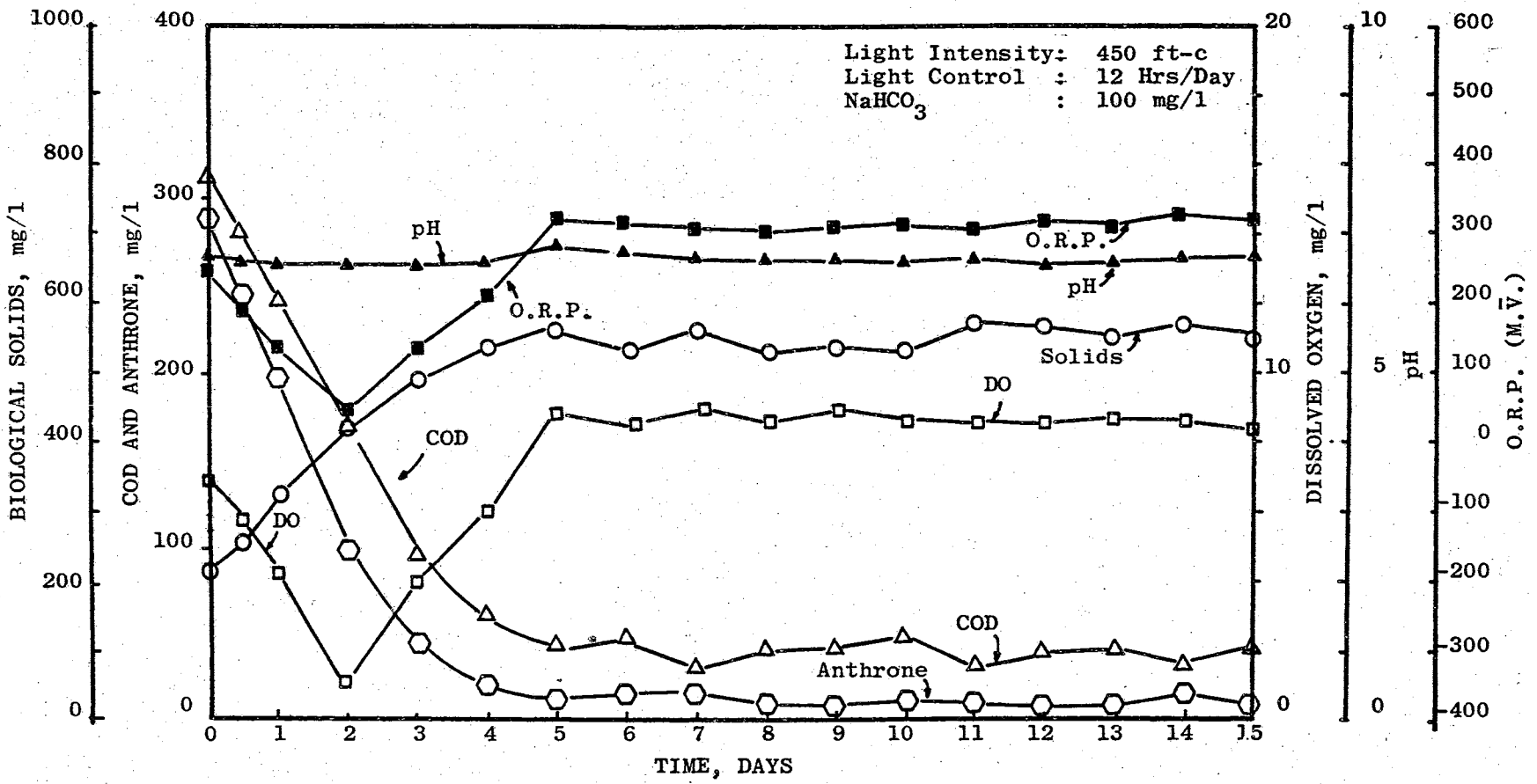

Figure 41. Purification of 300 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.

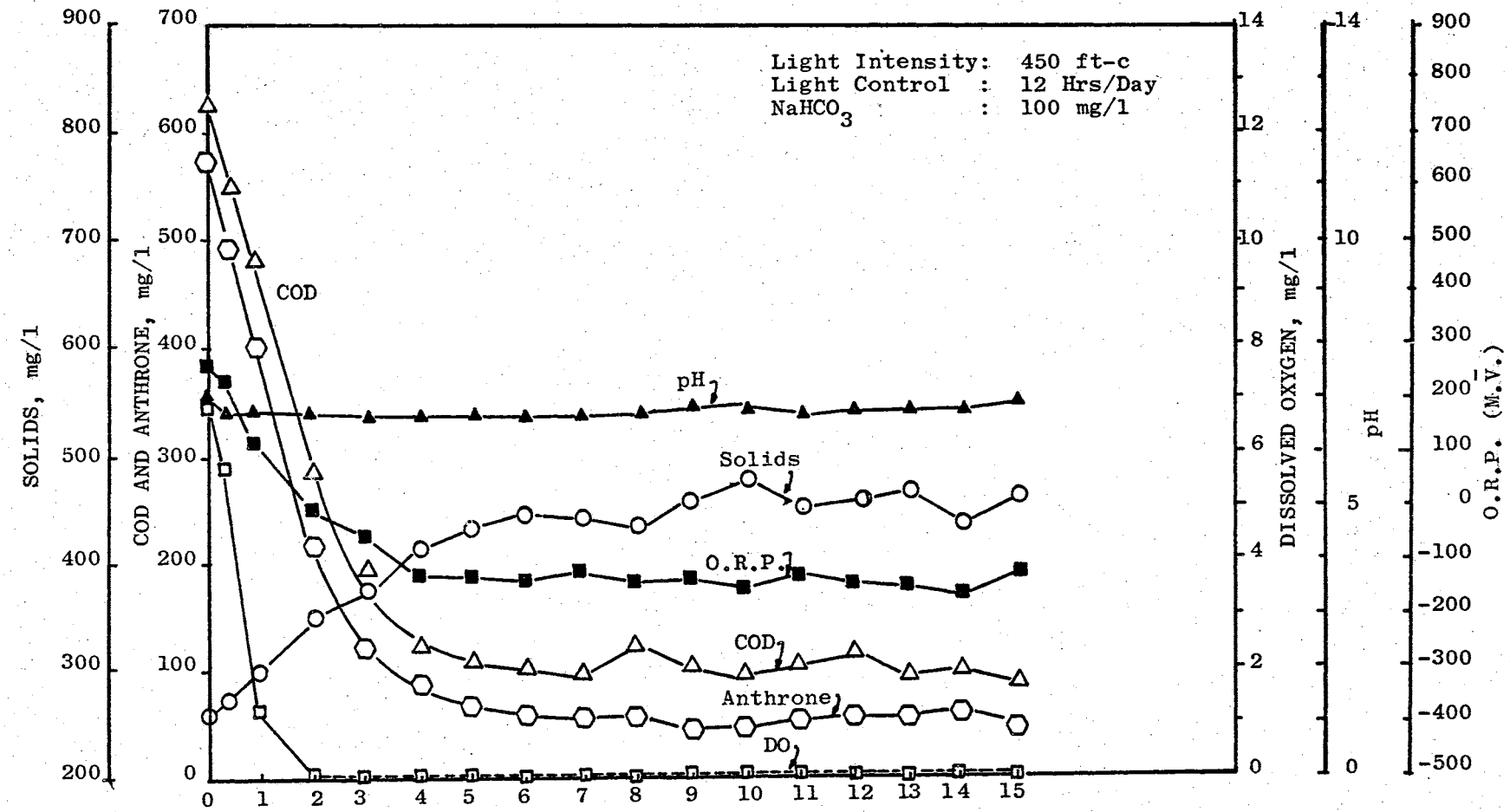

Figure 42. Purification of 600 mg/1 Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 10 Days.

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 42 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-

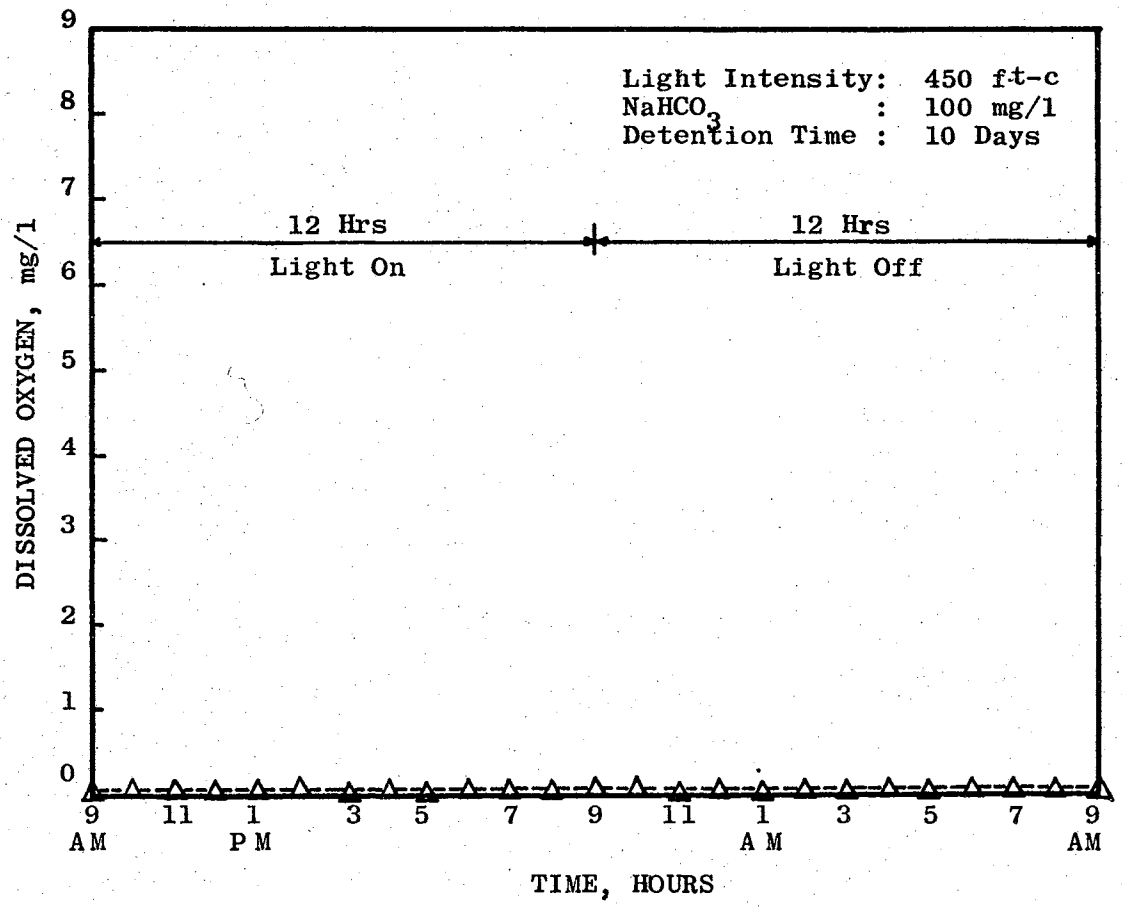

Figure 43. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose (Light Period - 12 Hrs/Day).

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 45 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.

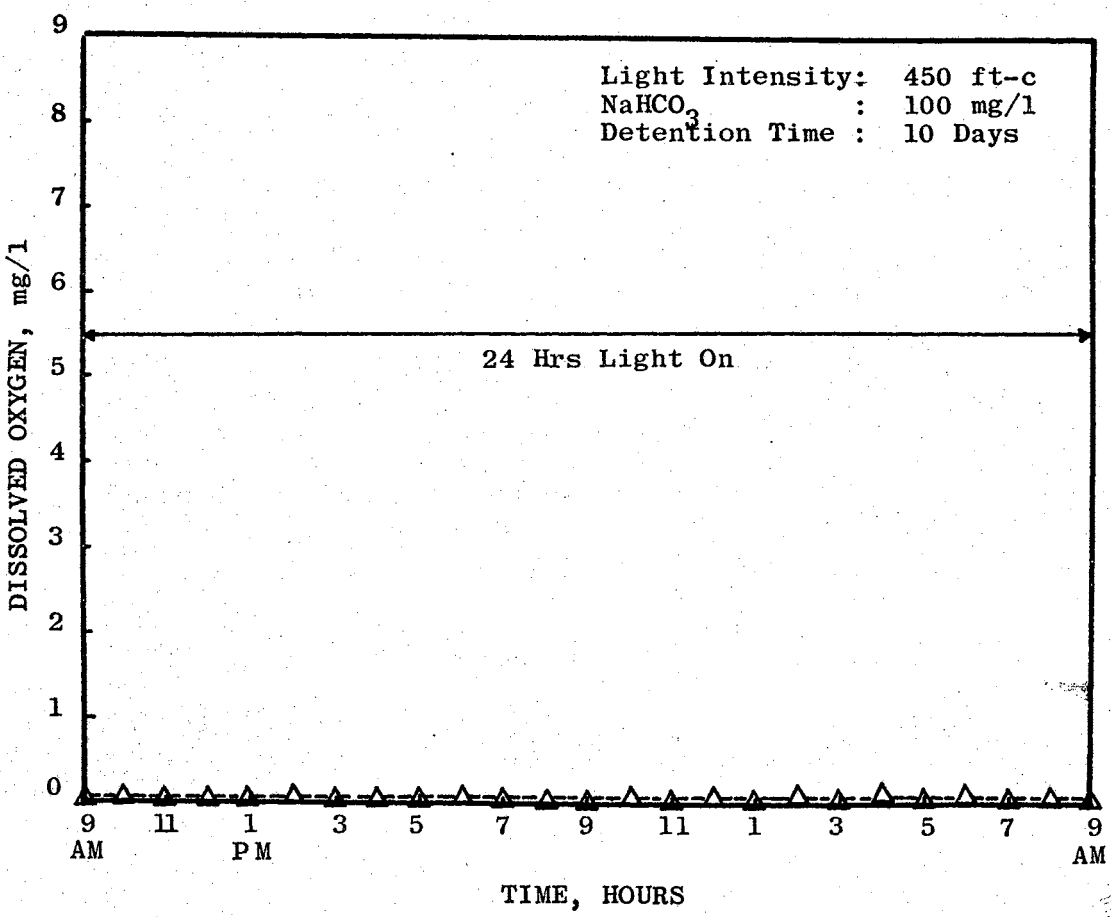

Figure 44. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose (Light Period - 24 Hrs/Day).

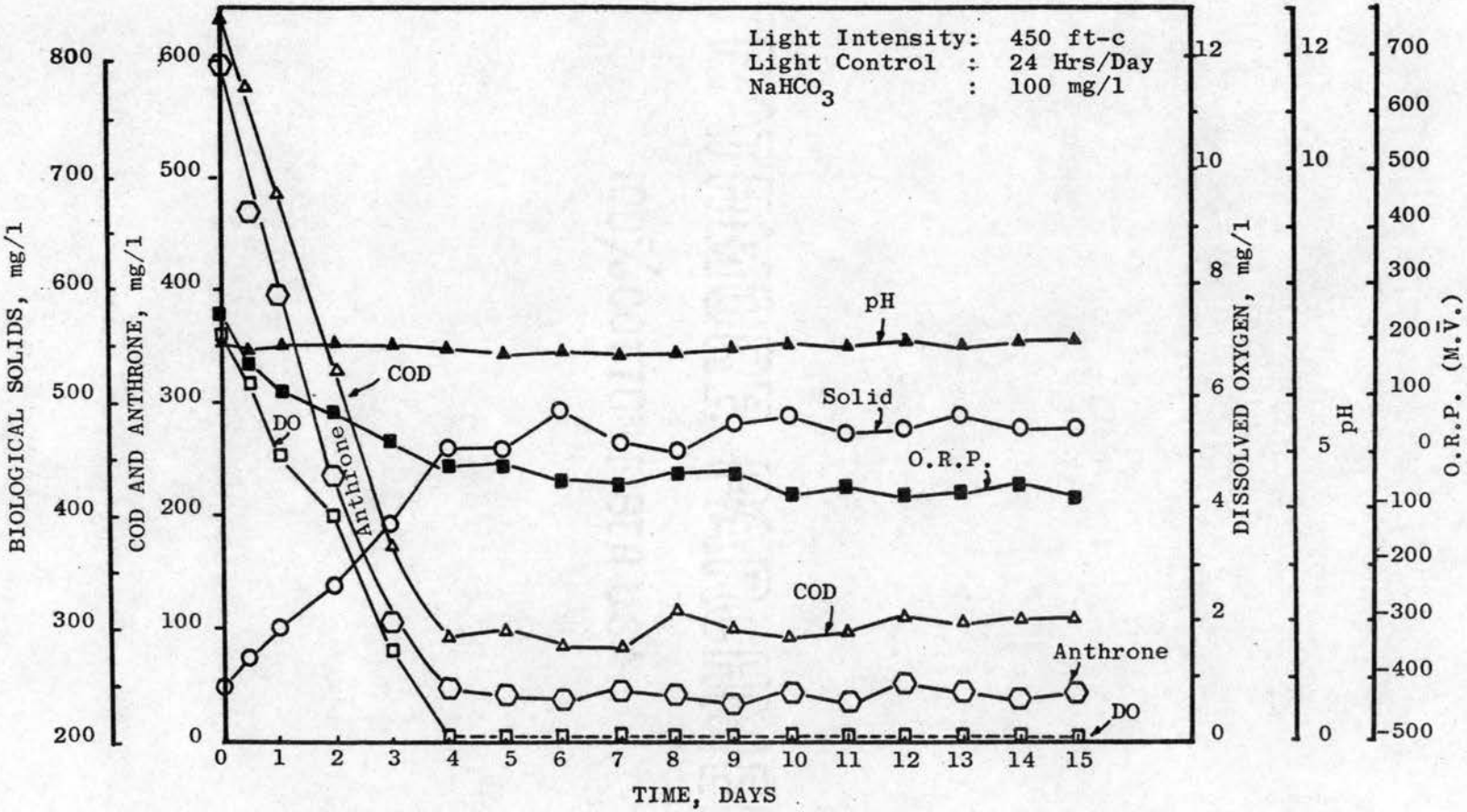

Figure 45. Purification of 600 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at Detention Time of 10 Days.

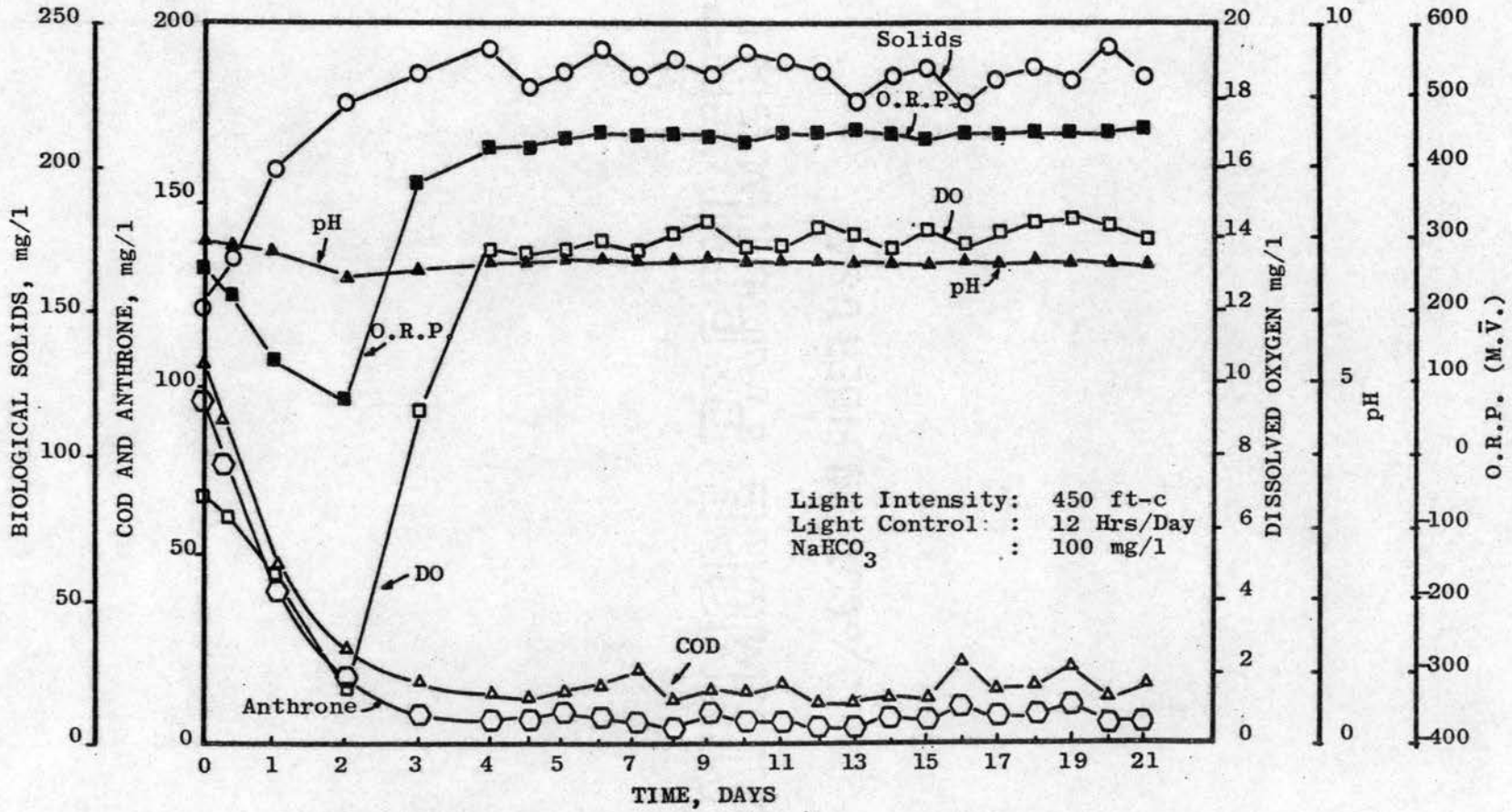

Figure 46. Purification of 100 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

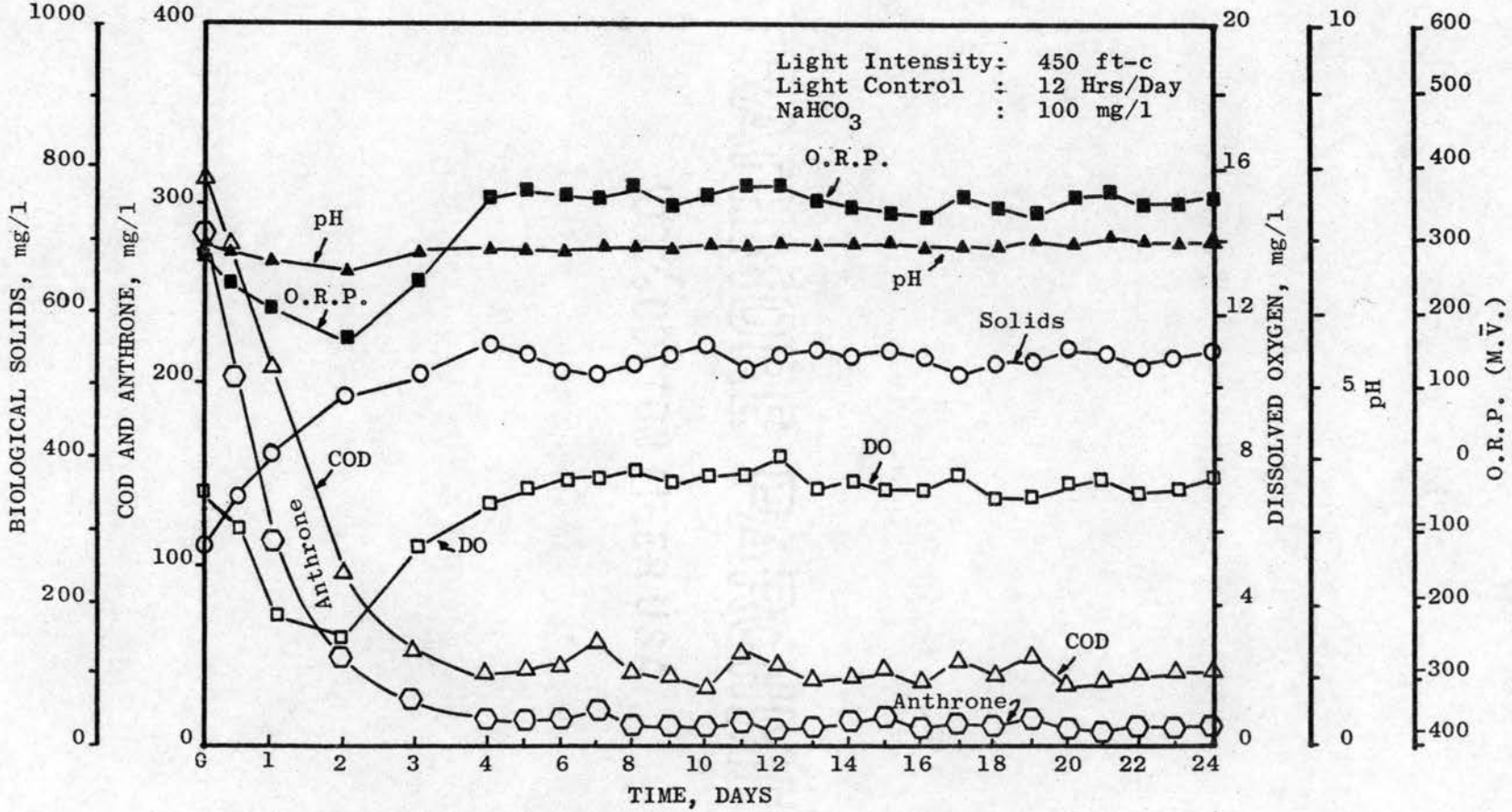

Figure 47. Purification of 300 mg/1 Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

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-

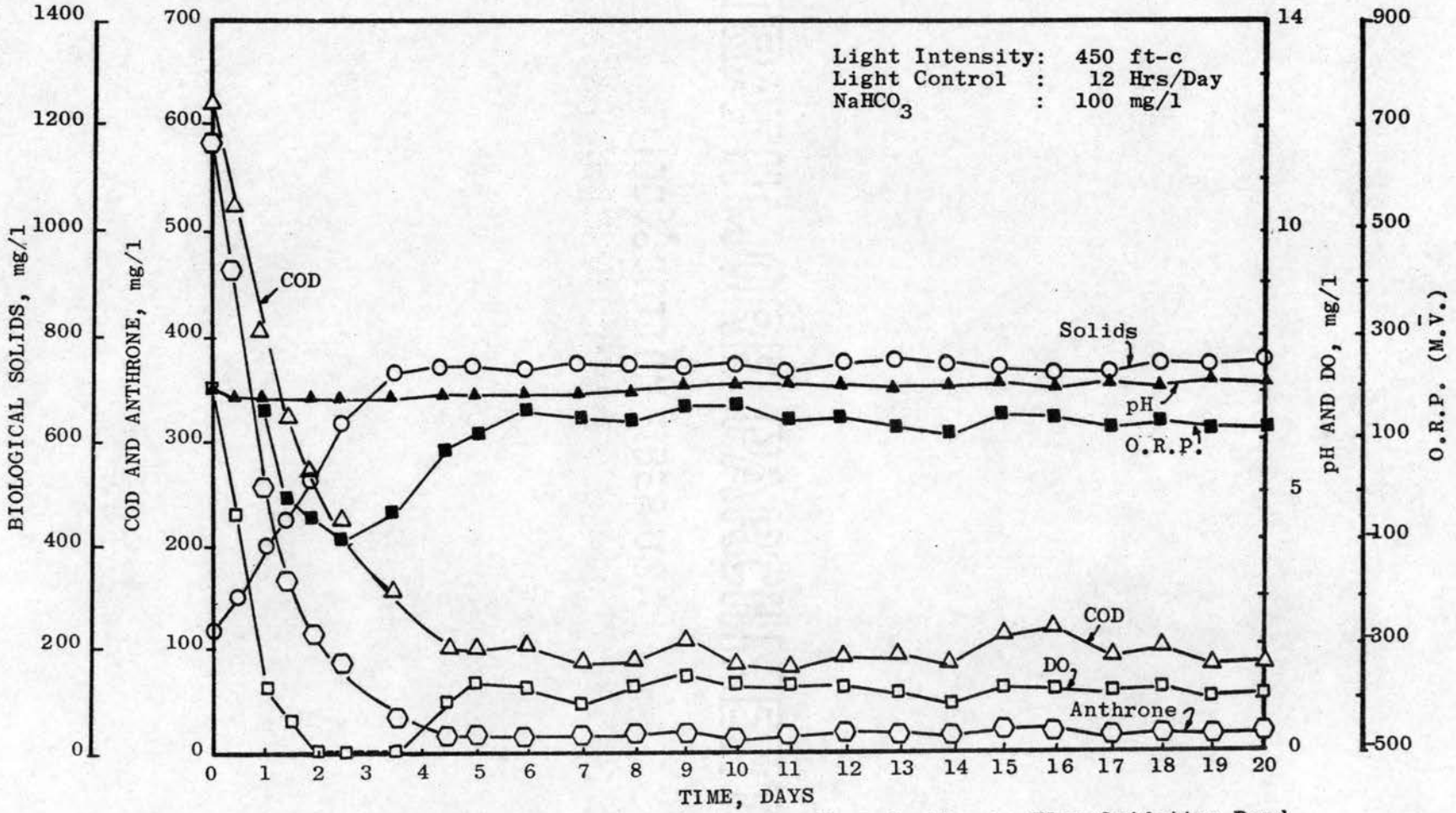

Figure 48. Purification of 600 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

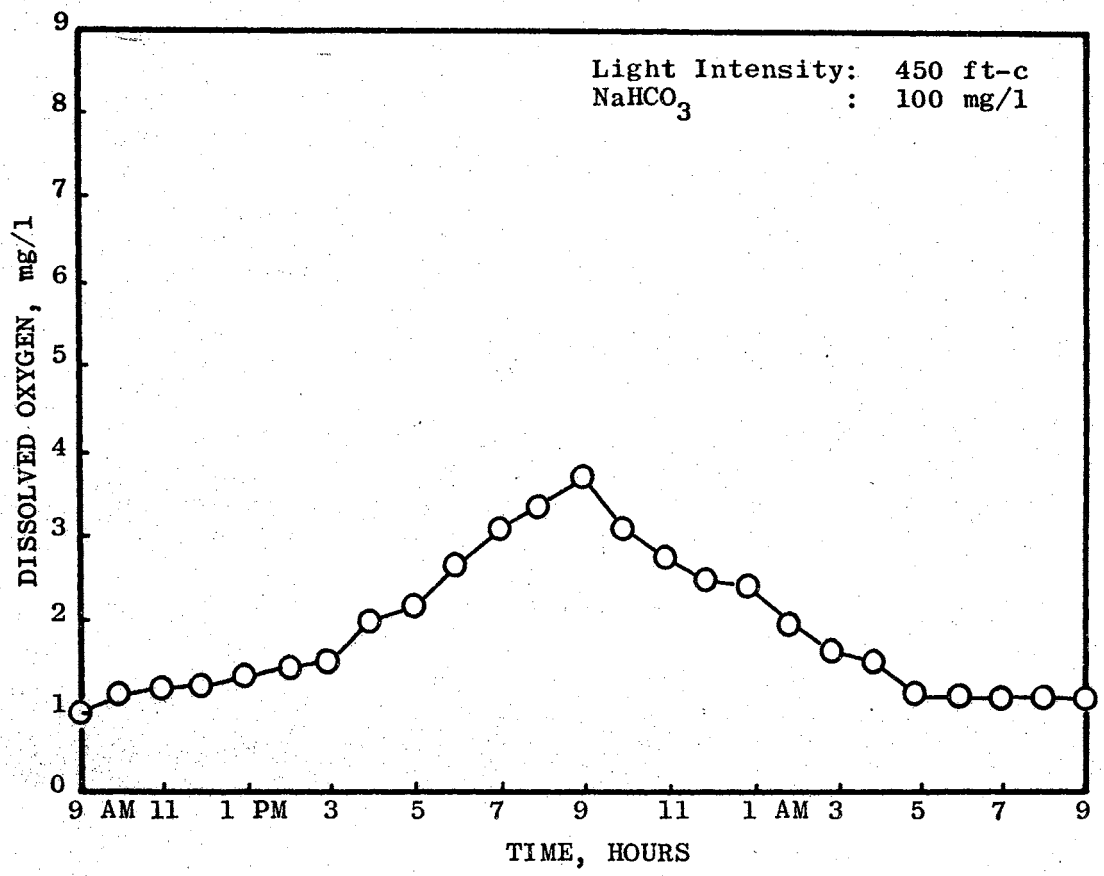

Figure 49. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose Operated at a Detention Time of 20 Days (Light Period - 12 Hrs/Day).

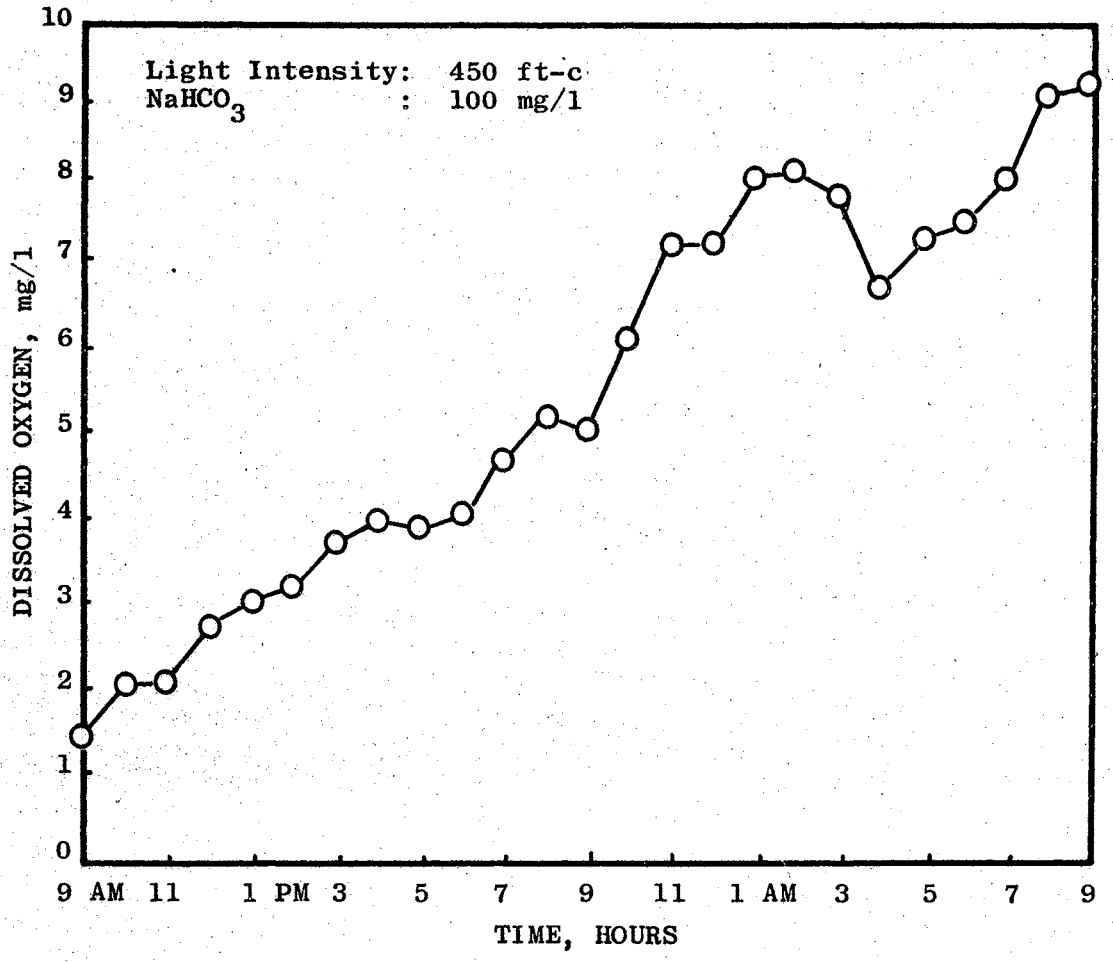

Figure 50. Variation of Dissolved Oxygen Concentration in the Steady State Continuous Flow Oxidation Pond with Influent Feed 600 mg/l Glucose Operated at a Detention Time of 20 Days (Light Period - 24 Hrs/Day).

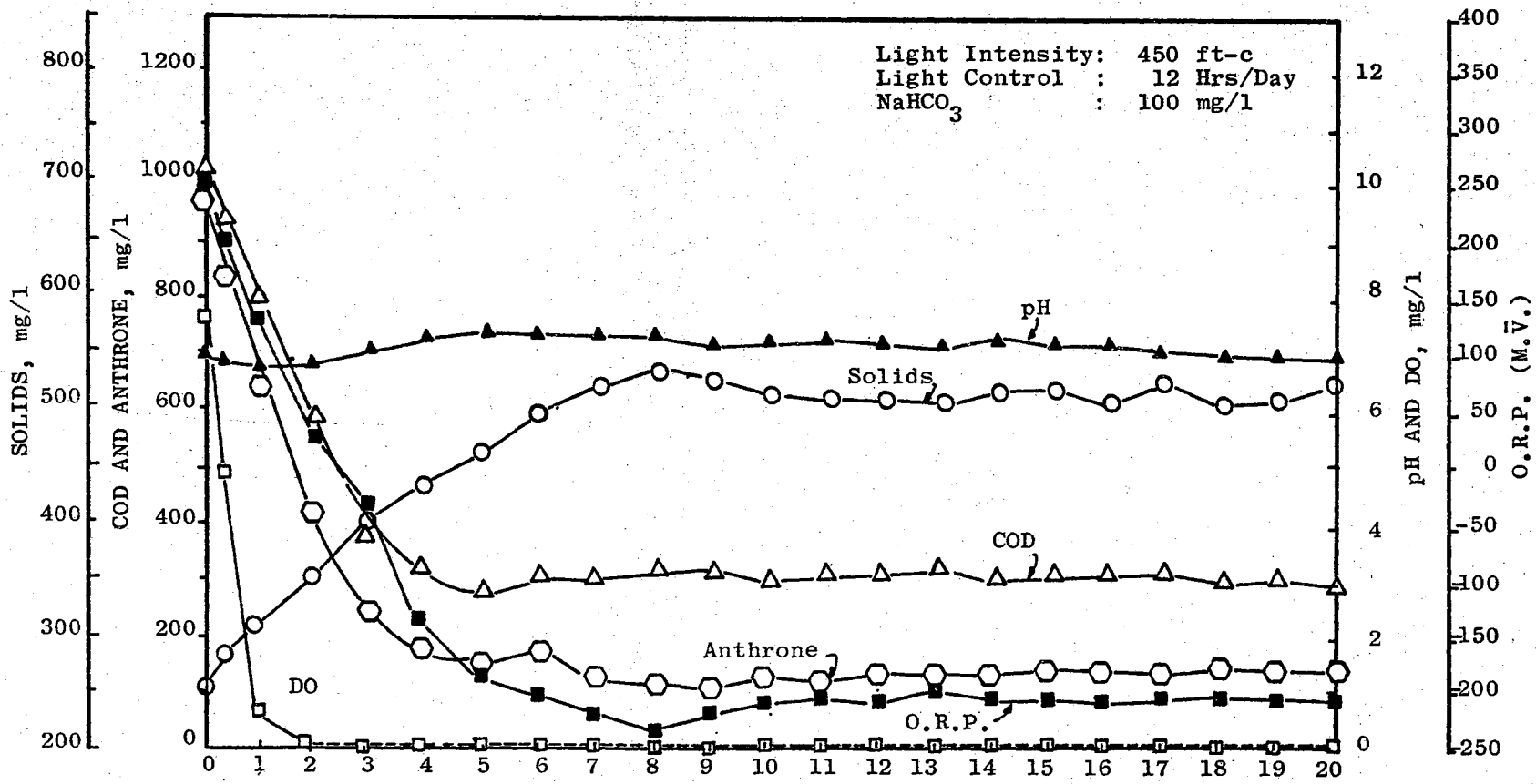

Figure 51. Purification of 1000 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

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.

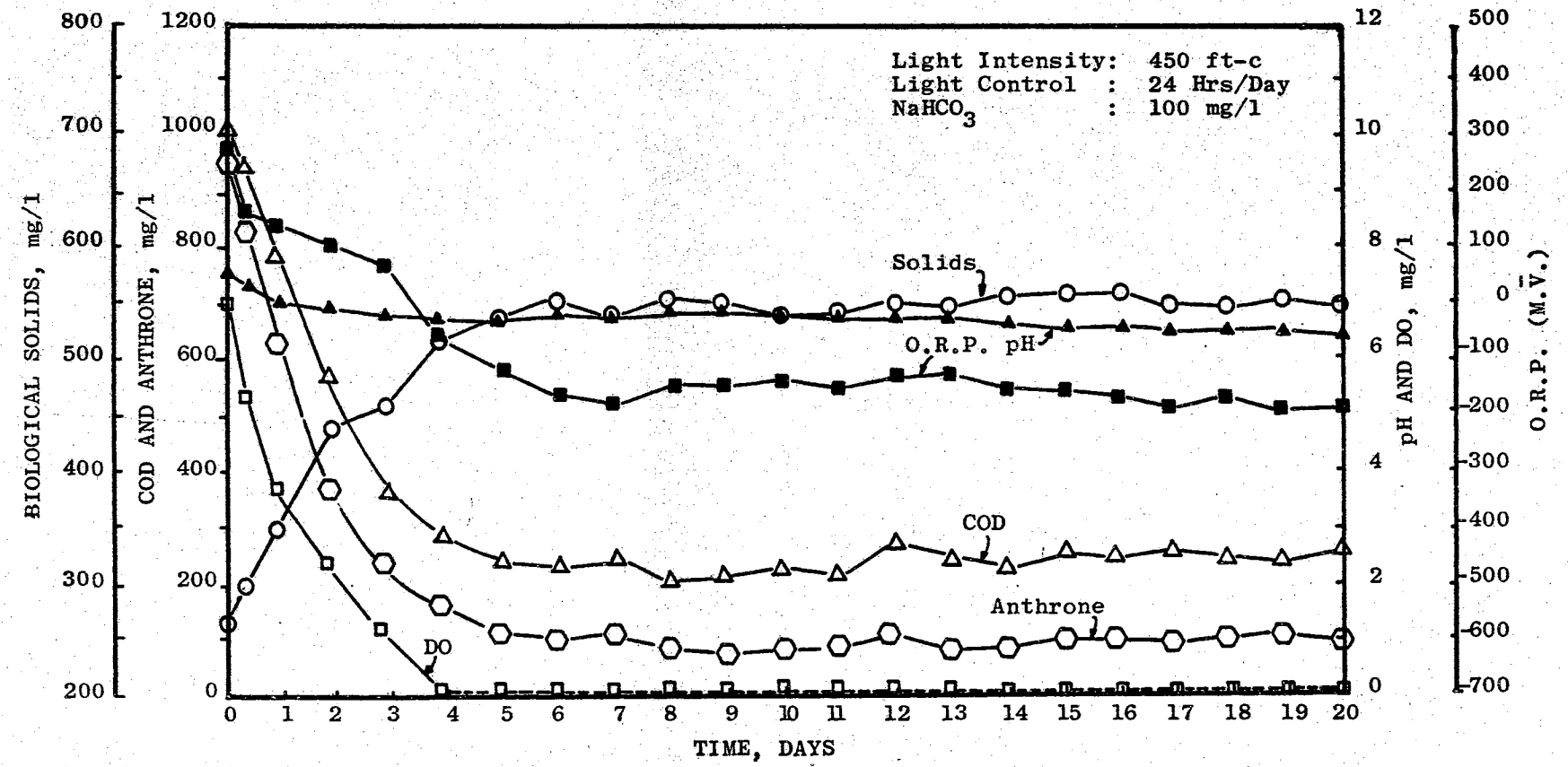

Figure 52. Purification of 1000 mg/l Glucose in a Continuous Flow Oxidation Pond Operated at a Detention Time of 20 Days.

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 soldium 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_2. 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

Glucose, mg/l	Glucose, lb/acre/day	COD, lb/acre/day
Batch Studies, Single Loading, 7-Day Detention		
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
Batch Studies, Loading at 3-Day Intervals		
100	64.4	68.7
200	128.8	137.4
Batch Studies, Loading Applied Daily		
50	96.6	103.1
100	193.2	206.2
Continuous Flow Studies, 10-Day Detention		
100	19.3	20.6
300	57.9	61.7
600	115.8	123.3
Continuous Flow Studies, 20-Day Detention		
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 reoxygenation 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.

SELECTED BIBLIOGRAPHY

1. Edminston, F. E., "Fish Ponds for the Farm." Chas. Scribner's Sons, 1947.

2. Schillinger, Vona, Vom Wasser, II. 200 (1928) translated by G. P. Edwards, "The Construction of Sewage Fish Pond as Relief Work." Sewage Works Journal, 6, 829 (1934).

3. Oswald, William J., "Fundamental Factors in Oxidation Pond Design." Conference on Biological Waste Treatment, Manhattan College, April 20-22, 1960.

4. Gillespie, C. G., "Emergency Land Disposal of Sewage." Sewage Works Journal, 16, 740 (1936).

5. Giesecks, F. E., and Zeller, P. J. A., "Secondary Treatment of Sewage in Artificial Lakes." Eng. News Rec., 117, 674 (1936).

6. Parker, C. D., Jones, H. L., Taylor, W. S., and Greene, N. C., "Purification of Sewage in Lagoons," "Performance of Large Sewage Lagoons at Melbourne, Australia." Sewage and Ind. Wastes, 22, 760 (1950), and 31, 133 (1959).

7. Keel, J. K., and Hopkins, Glen J., "Experimental Lagooning of Raw Sewage." Sewage and Ind. Wastes, 28, 1351-1355 (1956).

8. Towne, W. W., Bortsch, A. F., and Davis, W. H., "Raw Sewage Stabilization Ponds in the Dakotas." Sewage and Ind. Wastes, 29, 377 (1957).

9. Caldwell, D. H., "Sewage Oxidation Pond Performance, Operation and Design." Sewage and Ind. Wastes, 18, 433-458 (1946).

10. Van Heuvelen, W., and Svorve, J. H., "Sewage Lagoons in North Dakota." Sewage and Ind. Wastes, 26, 771 (1954).

11. Hermann, E. R., and Gloyna, E. F., "Waste Stabilization Ponds - III. Formulation of Design Equations." Sewage and Ind. Wastes, 30, 973-974 (1958).

12. Hermann, E. R., and Gloyna, E. F., "Waste Stabilization Pond. I. Experimental Investigation." Sewage and Ind. Wastes, 30, 511-693 (1958).

13. Fitzgerald, G. P., and Rohlich, G. A., "An Evaluation of Stabilization Pond Literature." Sewage and Ind. Wastes, 30, 1213 (1958).

14. Amer. Soc. Civ. Eng., "Manual of Engineering Practice," 36, 185 (1959).

15. Burden, J. P., "Oxidation Pond." Southwest Water Works Journal, 40, 23-24 (1961).

16. Oswald, William J., Gotaas, H. B., Ludwig, Harvey F., and Lynch, Victoria, "Algae Symbiosis in Oxidation Ponds." Sewage Works Journal, 23 (1340-1341 (1953).

17. McGoodwin, L. M., "Design and Construction of Oxidation Ponds." Southwest Water Works Journal, 44 (1962).

18. O'Connell, W. J., Jr., and Gray, H. F., "Emergency Land Disposal of Sewage." Sewage and Ind. Wastes, 16, 740-744 (1944).

19. Myers, J., "Studies of Sewage Lagoons." Public Works, 79, 25-28 (1948).

20. Williams, Louis G., "Can Sewage be Converted to Human Food?" Furman University Bull. 2, 16-24 (1955).

21. Allen, M. B., "General Features of Algal Growth in Sewage Oxidation Ponds." State Water Pollution Control Board, Sacramento, California, Publication No. 13 (1955).

22. Silva, P. C., and Papenfuss, G. F., "Report on a Systematic Study of the Algae of Sewage Oxidation Ponds." S.W.P.C.B. Publ. No. 7, Sacramento (1953).

23. Rich, Linvil G., "Unit Processes of Sanitary Engineering." John Wiley and Sons, Inc., New York (1963).

24. Rosenberg, Jerome L., "Photosynthesis - The Basic Process of Food-making in Green Plants." Holt, Rinehart and Winston, Inc., New York (1965).

25. Bassham, J. A., and Calvin, M., "The Path of Carbon in Photosynthesis." Prentice-Hall, Inc., Englewood Cliffs, New Jersey (1957).

26. Fogg, G. W., "The Metabolism of Algae." John Wiley and Sons, Inc., New York (1952).

27. Bassham, J. A., and Calvin, M., "New Aspects of Photosynthesis." Fifth Int. Cong. of Biochemistry Preprint No. 48 (1961).

28. Hill, R., "Oxygen Evolved by Isolated Chloroplasts." Nature, 139, 881-882 (1937).

29. Arnon, D. I., "The Role of Light in Photosynthesis." Scientific American, 203 (1959).

30. Davis, P. D., Giovanelli, J., Rees, T. A., "Plant Biochemistry." Chapter 6. F. A. Davis Company, Philadelphia, Pa. (1964).

31. Blackman, F. F., and Smith, A. M., "Experimental Researches on Vegetable Assimilation and Respiration. IX. On Assimilation in Submerged Water Plants and Its Relation to the Concentration of Carbon Dioxide and Other Factors." Proc. Roy. Soc., 83, 389-412 (1911).

32. Warburg, O., "Über die Geschwindigkeit der Photochemischen Kohlen." Biochem. 100, 230-270 (1919).

33. Brown, W. H., "The Relation between Light Intensity and Carbon Dioxide Assimilation." Phil. Journ. Sci., C. Bot. 12, 85-95 (1917).

34. Daubeny, C., "On the Action of Light Upon the Plants and of Plants upon the Atmosphere." Trans. Roy. Soc. London, 62, 149-176 (1886).

35. Oswald, William J., Gotaas, H. B., Ludwig, Harvey F., and Lynch, Victoria, "Algae Symbiosis in Oxidation Ponds. I. Growth Characteristics of E. gracilis." Sewage and Ind. Wastes, 23, 1337 (1951).

36. Matthali, Gabrielle L. C., "Experimental Researches on Vegetable Assimilation and Respiration. III. On the Effect of Temperature on Carbon Dioxide Assimilation." Trans. Roy. Soc. London, 197, 47-105 (1904).

37. Eyster, H. Clyde, Brown, Thomas E., and Tanner, Howard A., "Mineral Requirement for Chlorella pyrenoidosa under Autotrophic and Heterotrophic Conditions," in Trace Elements, 157-173, Academic Press, Inc., New York and London (1958).

38. Standard Methods for the Examination of Water and Waste Water, 11th Ed., A.P.H.A., New York, 1960.

39. Gaudy, A. F. Jr., "Colorimetric Determination of Protein and Carbohydrate." Industrial Water and Wastes, 7, 17, 1962.

40. Gaudy, A. F. Jr., Komolrit, K., and Bhatla, M. N., "Sequential Substrate Removal in Heterogeneous Populations." Jour. Water Poll. Cont. Fed., 35, 903-963 (1963).

41. Beckman Operating and Maintenance Instructions Zeromatic pH Meter, Beckman Instruments, Inc., Scientific and Process Instruments Division, Fullerton, California, October, 1961.

42. Harden, A., and Norris, R. V., "The Enzymes of Washed Zymin and Dried Yeast (Lebedeu) II. Reductase Biochem. Journal, 8, 100, 1914.

43. Gaudy, A. F. Jr., Bhatla, M. N., Follett, R. H., and Abu-Niaaj, F., "Factors Affecting the Existence of the Plateau during the Exertion of BOD," J. Water Pollution Control Fed., 37, 444 (1965).

44. Bhatla, M. N., and Gaudy, A. F. Jr., "The Role of Protozoa in the Diphasic Exertion of BOD," J. San. Eng. Div., Proc. Am. Soc. Civ. Eng., SA3 (4371), 63 (1965).

45. Bhatla, M. N., and Gaudy, A. F. Jr., "Studies on the Plateau in Oxygen Uptake during Exertion of Biochemical Oxygen Demand by Pure Cultures of Bacteria," Biotech. and Bioeng. VII, 387 (1965).

46. Kowallik, W., "Die Proteinproduktion von Chlorella im Licht Verscheidener Wellenlangen," Planta Arch. Wiss. Bot., 64, 191 (1965).

47. Harris, R. V., Harris, P., and James, A. T., "The Fatty Acid Metabolism of Chlorella vulgaris," Biochem. Biophys. Acta, 106, 465 (1965).

48. Gaffron, H., and Rubin, J., "Fermentation and Photochemical Production in Algae," J. Gen. Physiol., 26, 219 (1942).

49. Danforth, W., "Oxidative Metabolism of Euglena," Arch. Biochem. Biophys., 46, 164 (1953).

50. Franck, J., and Gaffron, H., "Photosynthesis, Facts and Interpretations," Adv. Enzymol., 1, 199 (1962).

51. Ludwig, H. F., and Oswald, W. J., "Role of Algae in Sewage Oxidation Ponds," Scient. Monthly, 74, 3 (1952).

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: Sanitary and Public Health Engineering

Biographical:

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

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

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