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Factors Regulating the Growth of Algae in Continuous Culture in Diluted Secondary Sewage Treatment Plant Effluent and Subsequent Biodegradability

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Edward G. Foree
University of Kentucky

Caroline P. Wade
University of Kentucky

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FACTORS REGULATING THE GROWTH OF ALGAE IN CONTINUOUS CULTURE IN DILUTED SECONDARY SEWAGE TREATMENT PLANT EFFLUENT AND SUBSEQUENT BIODEGRADABILITY

Edward G. Foree
Principal Investigator

Caroline P. Wade
Research Assistant

University of Kentucky Water Resources Institute
Lexington, Kentucky

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PREFACE

This report on the factors regulating the growth of algae in continuous culture in diluted secondary sewage treatment plant effluent and subsequent biodegradability presents the results of the second part of a study entitled Algal Growth and Decomposition: Effects on Water Quality, Phase 2 (OWRR Project No. A-023-KY). The results of the first part have been published as University of Kentucky Water Resources Institute Research Report No. 31 (October 1970). Two additional studies on the kinetics of algal growth in continuous culture and on the decomposition and nutrient regeneration of plankton samples collected from central Kentucky surface waters have been completed and the results will be published in two subsequent research reports.

The cooperation afforded the authors during this study by Dr. Robert A. Lauderdale, Director of the University of Kentucky Water Resources Institute, is greatly acknowledged. The assistance of Mrs. Pat Hammond in preparation of the report is greatly appreciated.

ABSTRACT

Heterogeneous algal cultures were grown in laboratory continuous culture in continuous flow, completely mixed chemostats in secondary sewage treatment plant effluent diluted to give an ammonia nitrogen concentration of 10 mg/l. Variables were lighting, pH, carbon dioxide availability, and hydraulic residence time.

Optimum growth occurred under pH 7.0, excess CO_2, and continuous lighting conditions. The availability of artifically supplied excess CO_2 greatly increased the mass (standing crop) at steady-state over that produced under otherwise identical conditions for all residence times studied. For the case of excess CO_2 availability, the nitrogen concentration in the algal cells regulated growth rather than the concentration of nutrients in solution. A mathematical expression was hypothesized to describe this phenomenon and was confirmed by the experimental results.

Under dark-aerobic conditions, the algal cultures exerted a two-stage BOD, the second stage apparently beginning after the death of the algal cells. Longer chemostat residence times during growth produced cultures with lower percentage biodegradability. Carbon dioxide enriched growth conditions produced cultures with lower percentage biodegradability than cultures grown in a carbon dioxide deficient medium.

KEY WORDS: algae, carbon, nitrogen, hydrogen, ion concentration, biochemical oxygen demand, sewage effluents

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INTRODUCTION

The fertilization of surface waters with excess nutrients may cause massive growths of algae and other aquatic plants and thus serious associated water quality problems. Eutrophication, the aging of waters by fertilization, has become a major concern in many regions and will be one of the most significant water quality problems of the future. The nutrients which seem to limit algal growth in most cases are nitrogen, phosphorus, and carbon. When the nutrients enter a natural water through domestic and industrial discharges or agricultural drainage, under favorable environmental conditions large blooms of algae can occur causing water quality deterioration from an aesthetic standpoint.

Algal growth will continue until some factor becomes growth limiting. If environmental conditions become unfavorable the algae will begin to decompose, many times causing serious oxygen depletion in the water. Under conditions of severe oxygen depletion the water may become anaerobic and anaerobic decomposition will occur with its associated detrimental water quality effects.

Considerable research has been conducted on the entry of algal nutrients into receiving waters and ways of preventing algal growth in fertilized waters. However, it is generally expensive to remove small quantities of nutrients from wastewater streams and nearly impossible to regulate agricultural drainage; therefore, it is important to determine the factors which affect algal growth and decomposition and how this information can be used to minimize the associated water quality problems.

In the first phase of this two phase study a continuous flow system was used to evaluate algal growth under different growth conditions and to determine the effects of these conditions on the level of mass supported by the particular environment. In the second phase the algal cultures were then decomposed aerobically to determine the rate and extent of decomposition of the algae grown under different environmental conditions. The results from the growth and decomposition studies can be applied to similar natural situations to determine the effect of the algae on the natural water quality.

CHAPTER II

BACKGROUND

A. Continuous Flow Chemostat Theory

The use of continuous flow culture vessels allows the study of steady state conditions and enables a determination of differences in growth behavior and characteristics as a function of the growth rate. Data from chemostat studies can be applied to natural ecosystems and the laboratory environment can be adjusted to simulate natural conditions.

Continuous flow analysis has been used in the past to study many aspects of algal growth such as the effects of light intensity and temperature, and the relative roles of various nutrients in regulating algal growth. In a laboratory environment it is possible to determine the level of growth at any particular hydraulic residence time for any given set of growth conditions and concentration of growth regulating nutrients. When the system reaches steady state, growth will be regulated by the concentration of the rate limiting nutrient, so for any particular concentration of the limiting nutrient a maximum level of mass which the system will support can be determined.

1. Characteristics of Chemostat Systems. The chemostat is a reactor in which a constant flow rate and volume produce an outflow equal to the inflow. The system is completely mixed with a uniform distribution of algae and is maintained in a uniform environment. The desired hydraulic residence time (θ) is obtained by adjusting the flow rate through the constant volume chemostat.

Steady state conditions are characterized by constant cell mass concentration in the effluent equal to that in the chemostat and a constant specific growth rate ($\frac{dX}{dt}$ / X) and nutrient concentration in the reactor.

Due to the completely mixed system, the mass in the reactor will be washed out at a rate equal to the flow rate and in order to maintain steady state the rate of growth must be adequate to replace this washout rate. Thus a constant level of mass is maintained.

Growth may be limited by either an energy source (organic material, light) or by nutrients (CO_2, N, P, trace elements). When the energy sources are in excess the maximum mass level the system will support will be regulated by the concentration of the limiting nutrient.

2. Nutrient Limitation. Porcella (22) found in chemostat studies of phosphorus limitation that the phosphorus concentration decreased rapidly and then remained at a constant low level. He found that the nutrient concentration deminished faster than would be expected based on a constant yield of algal cells for each unit of phosphorus removed from the influent. This tends to indicate that the algal cells store excess nutrients during nutrient abundant growth and the growth rate is therefore not constant with respect to the nutrient concentration in solution. These stored nutrients could then be used to produce added growth after the nutrient concentration in solution had been exhausted. Foree and Tapp (7) reported cases in which excess nitrogen was taken up during nutrient abundant growth and stored rather than converted directly to protein. Once the nitrogen became depleted from solution the nitrogen stored in the algal cells was converted to protein causing additional growth.

This storage of excess nutrients would tend to indicate a growth rate proportional to the cellular nutrient concentration rather than the concentration of the limiting nutrient in solution. Jewell and McCarty (11) reported this effect as they found that although cell division ceases when the limiting nutrient is depleted from solution, growth continues by increased cell sizes until a minimum cellular nutrient concentration is reached. Gerloff and Skoog (8), on the other hand, proposed that growth continues until some

minimum critical level of the growth regulating nutrient is reached in the cells at which time both cell division and growth will cease. Both these explanations of nutrient defficient growth would indicate a growth rate based on cellular nutrient concentration as opposed to one based on the nutrient concentration in solution.

3. Growth Kinetics Theory. Based on the previous discussion and preliminary studies during this research, it was concluded that the kinetic theory outlined in Provisional Algal Assay Procedure (PAAP) (4) was not always applicable. The PAAP theory describes a growth rate proportional to the nutrient concentration in solution and based on a constant yield of cells per unit of nutrient removed.

A more reasonable kinetic equation to describe the observed data is hypothesized with the specific growth rate proportional to the cellular nutrient concentration. The specific growth rate may be defined as:

$$\text{specific growth rate} = \frac{dX}{dt} \; / \; X$$

where X is a measure of mass and t is time. Assuming that growth will cease when the cellular nutrient concentration reaches some minimum level and allowing for the respiration rate, the specific growth rate may be expressed as:

$$\frac{dX}{dt} \; / \; X = k(N/X - [N/X]_{min}) - b$$

where k is a constant of proportionality, N is the quantity of growth regulating nutrient incorporated in a total quantity of cellular mass X (thus N/X is the cellular nutrient concentration), and b is the respiration rate of the algae. Combining the respiration rate and the minimum cellular concentration in a term b' representing an "effective respiration rate" and using the continuous flow assumptions:

$$\frac{dX}{dt} \; / \; X = k\,(N/X) - b' = 1/\theta$$

where θ is the hydraulic residence time or the mean algal cell age in the system.

B. Carbon Dioxide Limitation

Much research has been done in the past concerning the influence of limiting nutrients on the growth of algae. Most of this research has been concerned with nitrogen and phosphorus. Carbon, although it has long been known to be an important growth limiting nutrient for all plant life, has until recently been all but disregarded.

Carbon dioxide was long considered as readily available through access to the atmospheric supply and therefore not limiting in natural situations. However, as pointed out by Hutchinson (10), both the movement of free CO_2 through the water interface and CO_2 replacement by carbonate salts are slow processes, with the quantity of CO_2 normally available lying between 0.4 and 1.0 mg/l. Kuentzel (15) described cases of growth which would have required as much as 110 mg/l of CO_2 to produce, far above the maximum available from most natural inorganic sources.

Kuentzel postulated that this tremendous amount of CO_2 came from bacterial action on organic carbon sources stimulated by the quantities of O_2 produced by the fast-growing algae. He described a bacteria-algae symbiosis which can supply as much as 20 mg/l of CO_2 in a supersaturated state causing explosive algal growth rates. He pointed out many examples in which massive algal blooms are associated with large bacterial populations and decomposable carbohydrates and cited much research in which CO_2 growth limitation was indicated.

Kuentzel (15) and Oswald (21) described a system such as that depicted in Figure 2.1 as that responsible for large mass blooms.

In this situation an organic carbon source, usually from a sewage effluent, stimulates the growth of large amounts of bacteria which produce CO_2 as the organic carbon source is degraded. The algae, in turn, use the CO_2

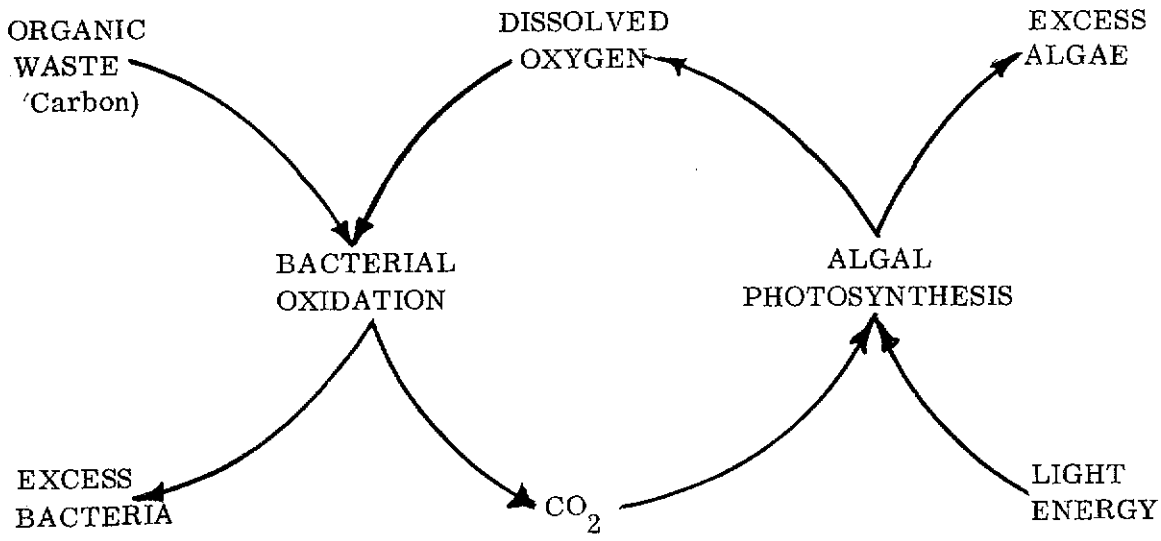

Figure 2.1 The Carbon Dioxide-Oxygen Cycle in a Mixed Algae-Bacteria Environment.

and produce more O_2 which, with that from the atmosphere, further stimulates the growth of bacteria.

Thus many times it may be the availability of CO_2 which limits algal growth and not one of the other major nutrients, nitrogen or phosphorus. Phosphorus, for example, is needed in very small quantities, about 0.01 mg/l, to stimulate and support massive blooms. Since most lakes and rivers contain at least this much, and the use and distribution of phosphorus is so widespread, it would be almost impossible to limit algal growth by restricting phosphorus. Kuentzel (15) suggested that more appropriate means might be to

provide more complete removal of biogradeable organic matter from effluents or use of a bactericide to eliminate the CO_2 producing bacteria. The former suggestion is a practical possibility, the latter is not.

C. Biochemical Oxygen Demand of Algae Under Unfavorable Environmental Conditions.

Waste stabilization lagoon studies by Meron, et al. (18) and others have shown that much of the organic material in the lagoon effluent is comprised of algal cell mass as opposed to the waste organics of the influent. There is considerable interest inthe effects of this algae on water quality in receiving streams. The results of previous studies, Jewell (11) and Foree (6), have shown that the rate and extent of aerobic decomposition, and thus oxygen demand, depend upon algal cell age and conditions of growth. Thus it would be desirable to evaluate the relationship between algal growth parameters in simulated lagoon situations and the oxygen demanding potential of the effluent in simulated receiving water situations.

The conventional 5 dya BOD test has some shortcomings when applied to the analysis of samples containing significant quantities of algae. Previous studies, Fitzgerald (5), have shown that living algal cells are relatively resistant to attack by bacteria, but become highly susceptible to bacterial attack and decomposition upon death. Thus when heterogeneous cultures of algae and bacteria are subjected to dark aerobic conditions, the oxygen demand is characterized by three phases: an initial phase in which an oxygen demand is exerted at a relatively slow rate as a result of algal respiration, an intermediate phase in which an oxygen demand is exerted at an accelerated rate as a result of bacterial respiration. This second stage BOD usually begins after a few days of incubation in the dark and may not be measured in the conventional 5 day test. An electrolysis BOD apparatus allows for a continuous determination of BOD as a function of time and provides a convenient technique

for determining the BOD characteristics of an algal culture for extended periods of time.

In this study algal cultures were grown in the continuous flow chemostats under various environmental conditions and detention times, and once steady-state conditions were established, aliquots were placed on the electrolysis BOD apparatus for observation. This allowed the desired correlation between algal growth parameters in simulated lagoon situations and the oxygen demanding potential of the effluent in simulated receiving water situations.

CHAPTER III

EXPERIMENTAL PROCEDURE

The purpose of this chapter is to describe the growth apparatus and procedure and the sampling methods used during this study. Analytical techniques are described in the next chapter.

A. Growth Procedure

1. Apparatus: The algae were grown in completely mixed continuous flow chemostats using methods similar to those outlined in Provisional Algal Assay Procedure (PAAP) (4). Details of the chemostat and the system arrangement can be seen in Figures 3.1 and 3.2. The chemostats were constructed of plexiglas tubing with ports for influent, sampling, and effluent, as well as provisions for diffused air and/or carbon dioxide supply. Two sets of chemostats were used during the study in order to compare the effect of different growth factors on the systems.

One set consisted of four chemostats, each of which were 61 centimeters high and contained 1.2 liters. These chemostats were maintained at hydraulic residence times of 2, 4, 8, and 16 days throughout the study. A constant influent flow rate, determined from the residence time and volume, was provided by using metering syringe pumps.

The second set consisted of six chemostats each of which was 61 cm high and contained 2.0 liters. The hydraulic residence times were 1, 2, 4, 8, 16, and 32 days in this set.

All chemostats were mixed continuously by magnetic stirrers using teflon stirring bars to ensure complete mixing. The stirring speed was adjusted to prevent formation of a vortex at the surface of the liquid.

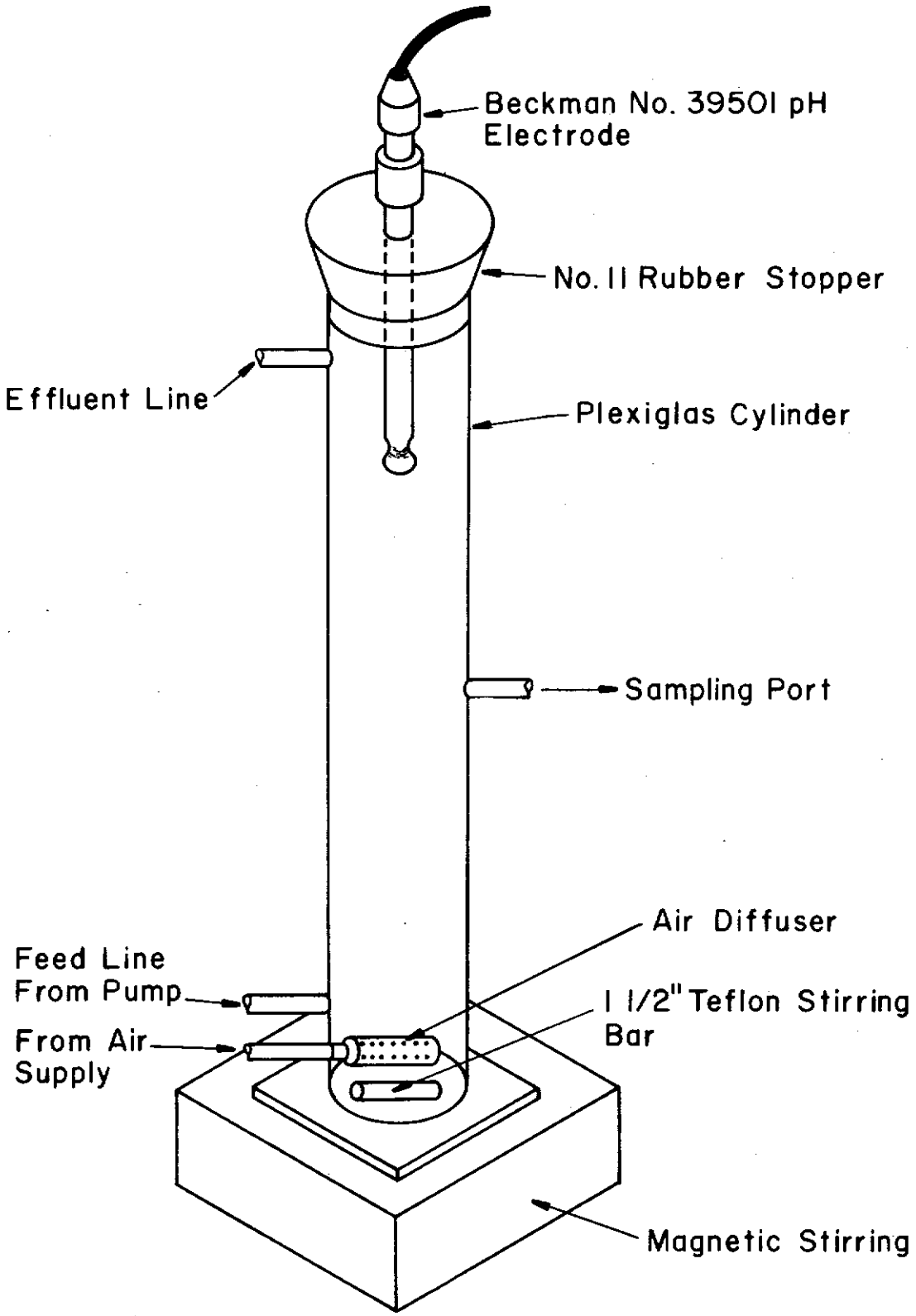

Figure 3.1. Detail of the Continuous Flow Culture Apparatus (Chemostat).

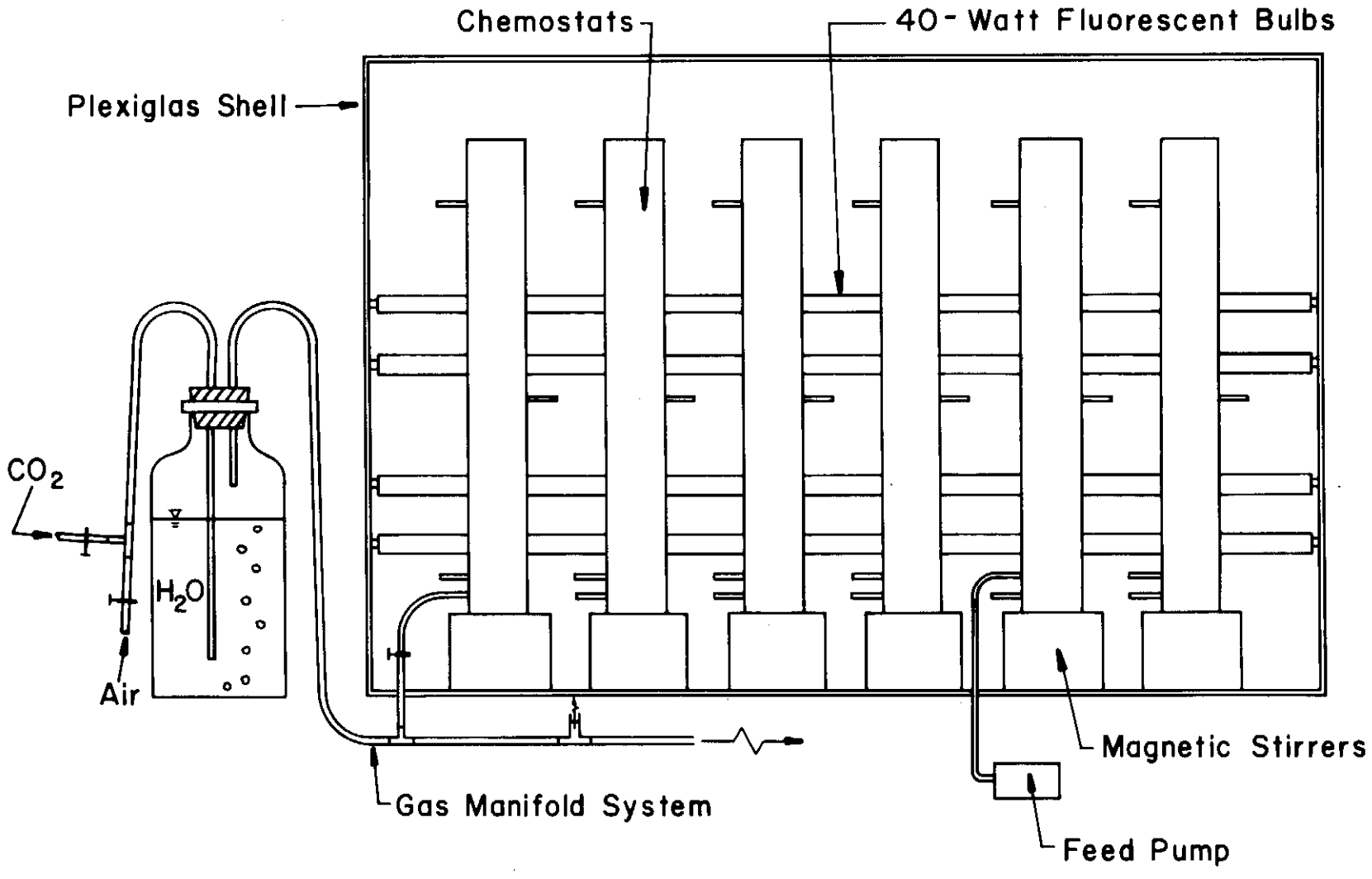

Figure 3.2. Arrangement of the Chemostat System.

2. Gas Supply System. Provisions for gas supply were furnished at the bottom of each chemostat by means of glass porous diffusors. By using a system of glass "T" connections and rubber tubing, it was possible to provide either a mixture of air and CO_2 or air alone to each set of chemostats. The air used was from a laboratory compressed air system and the CO_2 was supplied from pure bottled CO_2.

After the junction of the air and CO_2, the mixture was bubbled through a 9.0 liter pyrex bottle containing distilled water to ensure saturation of the gas and thereby minimize evaporation losses in the system. The flow of each chemostat air supply was regulated individually with the use of a castaloy clamp to compensate for pressure differences.

Since the pH of the culture was controlled by the free CO_2 in solution, pH was used as a monitor of adequate CO_2 concentration in the culture vessels. pH was maintained in the 7.0 to 8.0 range by increasing the CO_2 flow when the pH reached 8.0 and decreasing it when the pH fell below 7.0.

It was determined that when no CO_2 was supplied in the aeration mixture the pH would stabilize at about 10 due to the carbon dioxide depletion from the growth medium by the algae. Since in one phase of the study it was desired to evaluate the role of the absence of added CO_2 in regulating the algal growth while maintaining the pH in the same range as when CO_2 was supplied, it was necessary to chemically regulate the pH. This was accomplished by the addition of sufficient 1.0 N HCl to the feed to maintain the pH in the chemostats in the 7.0 to 8.0 range.

3. Light. The light source used for set one, containing the four chemostats, consisted of five, 20 watt, cool-white fluorescent lamps. The bulbs were mounted vertically between the chemostats and on each end of the set. Lighting for set two, which contained the six chemostats, was supplied by four horizontally supported 40 watt cool-white fluorescent bulbs. The average light intensity measured at the surface of the vessels was 250 and 320 foot-candles for sets one and two, respectively.

Each set of chemostats was enclosed in a white plexiglas shell with an open front to ensure a more uniform lighting intensity. To allow study of diurnal lighting conditions, each set of lights was controlled by an AMF electric 24 hour timer which automatically controlled the 12 hours-on: 12 hours-off cycle.

4. Temperature. The temperature of the laboratory housing the culture apparatus was controlled by a thermostat which was maintained at approximately 22°C during the growth period.

5. Feed Material. To simulate natural conditions, settled secondary activated sludge effluent obtained from the Town Branch Sewage Treatment Plant in Lexington, Kentucky, was used as a source of nutrients.

After each collection of the sewage effluent, it was returned to the laboratory and allowed to settle for several hours. In order to ensure a relatively constant influent composition the supernatant was then diluted to give an ammonia-nitrogen (NH_3-N) concentration of 10.0 mg/l (as N). The feed was then stored in a refrigerator at 4°C for a maximum time of three days to minimize biological activity, and enough withdrawn daily to supply the feed requirements of the cultures.

6. Growth Procedure. At the beginning of the growth period, each chemostat was filled from a large bottle containing a mixture of feed solution and mixed algal culture indigenous to Kentucky. The algae in the mixture had been cultured in the same sewage treatment plant effluent to ensure acclimation and was blended for approximately 30 seconds in a Waring Blender to break up any clumps before it was added.

During growth the cultures were sampled periodically to determine when steady state conditions existed. Once steady state was reached in all the vessels each was sampled and five chemostats selected for decomposition studies on an electrolysis Biological Oxygen Demand (BOD) apparatus.

B. Sampling Procedure

All samples were collected from the sampling ports at mid-height of the culture vessels. Before sampling, the inside of the chemostat was brushed down to remove any algae which adhered to the sides, and the port was allowed to run sufficiently to dislodge any algae in the port opening. Approximately 250 ml of sample was then collected directly into a plastic bottle and, after shaking, half was transfered to centrifuge tubes and the remainder marked "total sample." The tubes were centrifuged for 15 minutes at 17,000 rpm and 20°C on an International Equipment Company Model B-20 refrigerated centrifuge. The supernatant was then passed through Whatman Glas Fibre Paper, grade GF/C, using a millipore filter apparatus, and stored in another plastic bottle marked "soluble fraction."

All samples were stored in a -30°C freezer and thawed at +4°C for analysis. Samples taken after the BOD decompcsition were pipetted into plastic bottles and separated into total and soluble fractions in the same manner as the chemostat samples.

C. Analysis

Appropriate combinations of analyses from the following list were run in accordance with the objectives of the various phases of the studies:

a. Chemical Oxygen Demand
b. Biological Oxygen Demand
c. Total Carbon
d. Organic Carbon
e. Total and Volatile Suspended Solids
f. Ammonia Nitrogen
g. Kjeldahl-Nitrogen (Organic plus Ammonia Nitrogen)
h. Nitrate Nitrogen
i. Total Phosphorus
j. Inorganic Phosphorus

k. Orthophosphate

l. pH

m. Alkalinity

CHAPTER IV

ANALYTICAL PROCEDURE

All tests were run in accordance with Standard Methods for the Examination of Water and Wastewater, 12th ed. (25) unless otherwise indicated and are described briefly below.

A. Biological Oxygen Demand (BOD)

In order to obtain a direct and continuous measure of the dissolved oxygen used during decomposition of the algae, the electrolysis method of BOD determination was used. Using the design of Young and Baumann (1), a unit was fabricated consisting of six, one-liter Pyrex bottles to serve as reaction vessels, each of which had an electrolysis cell and a direct current power source.

The electrolysis cell consisted of a potassium hydroxide solution well for absorbing the carbon dioxide produced by the microorganisms, a weak acid solution to serve as an electrolyte and three electrodes. As oxygen in the reaction vessel is depleted, a pressure differential is created, causing the electrolyte level to fall and activate the switching electrode. This switches on the current and oxygen is produced by electrolysis at the positive electrode. The oxygen production reestablishes the gas pressure in atmosphere over the reaction vessel and shuts off the current until depletion of oxygen again activates the switching electrode. The negative electrode produces hydrogen which is allowed to escape to the atmosphere.

The apparatus is constructed so that the current is constant and a timer records the accumulated time of current flow. Thus, by using Faraday's Law, the accumulated oxygen production at any time is computed as a direct multiple of the timer meter reading. For this particular set-up the oxygen

production in mg is computed by multiplying the meter reading in minutes by 0.100. The oxygen consumption in mg/l is then computed knowing the size of the sample in the reaction vessel. By taking periodic meter readings a BOD vs. elapsed time relationship is established.

In running the algal samples, a one-liter sample was taken from each chemostat after growth had reached steady state and placed in the reaction vessels. The only seed material used was that already contained in the aglal cultures; therefore no seed correction was necessary. The sample was used undiluted and unbuffered and was stirred continuously by a magnetic stirrer built into the electrolysis BOD unit. The decomposition was allowed to continue for 30 days in a constant temperature room at 20°C and readings of BOD were taken daily.

B. Chemical Oxygen Demand (COD)

All COD tests were run using the dichromate reflux method in accordance with Standard Methods, pp. 510-514. Twenty ml sample sizes and the corresponding volumes of reagents for the 0.1 N standard ferrous ammonium sulfate titrant were used.

C. Nitrogen

1. Ammonia Nitrogen. A micro-kjeldahl steam distillation and nesslerization procedure was used to determine the ammonia nitrogen concentration of the samples. All reagents were made in accordance with Standard Methods, pp. 391-392.

2. Total Kjeldahl Nitrogen. To measure the total nitrogen (organic + NH_3-N) content of the samples, the organic nitrogen was converted to NH_3-N using a micro-kjeldahl digestion procedure described in Standard Methods, pp. 208-210 and measured using the previously mentioned ammonia nitrogen test.

D. Phosphorus

1. Total Phosphorus. In order to convert the organic phosphorus to orthophosphate (23), the sample with 1.0 ml of 70 gm/l magnesium chloride reagent added was dried in a vycor dish at 100°C, and then burned for 10 minutes in a muffle furnace at 600°C. The resulting pyrophosphate was then hydrolyzed to orthosphosphate by boiling in acidic solution as described in Standard Methods, p. 236. Total phosphorus was then determined as orthophosphate as indicated below.

2. Orthophosphate. The orphophosphate concentrations were determined by the stannous chloride method for orthophosphate described in Standard Methods, pp. 234-236 utilizing the Beckman spectrophotometer.

3. Inorganic phosphorus. To determine the inorganic portion of the phosphorus in the sample, all but the organic portion was converted to orthophosphate by acid hydrolysis and measured as previously described according to Standard Methods, pp. 234-236.

E. Suspended Solids

1. Total Suspended Solids. Total suspended solids concentration was obtained using the method described in Standard Methods 13th ed., pp. 537-538 (26). Whatman Glass Fibre Filter Papers grade GF/C and a Millipore Filter Apparatus were used for filtering the sample and a distilled water blank was carried through the test.

2. Volatile Suspended Solids. The filter pads from the total suspended solids determination were then analyzed according to Standard Methods 13th ed., pp. 538-539 (26) by burning for 10 minutes in a 580°C muffle furnace. The volatile suspended solids value was then determined as the portion of the total suspended solids which was lost during combustion. The furnace was maintained below 600°C since that is the melting point of the glass filter pads.

F. Carbon

1. Total Carbon determinations were made by passing 20 μl of sample through a Beckman infrared total carbon Analyzer Model 1R315. Dilutions were sometimes necessary to produce on-scale readings.

2. Soluble Carbon was measured using the same techniques as total carbon but on the "soluble fraction" of the sample.

3. Soluble Organic Carbon. A portion of the "soluble fraction" sample was acidified to pH 2.0 and then purged with nitrogen for 15 minutes to drive off the inorganic carbon as CO_2 leaving the organic form for measurement. To obtain soluble inorganic carbon values the soluble organic portion was subtacted from the total soluble carbon value.

G. Alkalinity and pH

Alkalinity and pH were run in accordance with Standard Methods, pp. 48-52, using a Corning Model 10 pH meter with a Beckman No. 39501 combination pH electrode.

CHAPTER V

RESULTS AND DISCUSSION

A. General Comments

In this two phase study, algal cultures were grown in parallel chemostats at different hydraulic residence times under four different sets of growth conditions. In each run several residence times (calculated as the volume of the reactor divided by the feed or effluent rate) were observed and comparisons made between residence times for the same growth condition and between growth conditions for the same residence times.

In the first phase, the growth regulating factor was determined for each set of growth conditions and the rate and extent of growth was evaluated as a function of the hydraulic residence time and the growth condition. For the second phase samples from the cultures were allowed to decompose on an electrolysis BOD apparatus to determine the relationship between growth conditions and the rate and extent of oxygen utilization during decomposition.

1. Algae Cultures Used. The original inoculation of algae was a mixture of several unialgal cultures (Chlorella, Scenedesmus, and Anabaena) and a mixed natural pond culture indigenous to Kentucky. After each run samples from each chemostat were mixed and used for inoculation of the next run to ensure the same algal types for future comparison.

An examination of the samples after a growth period revealed presence of the algal genera presented in Table 5.1.

During a growth period in which CO_2 was supplied to the cultures a population shift was observed after a steady state level had been reached. The samples of shorter detention times were predominantly Chlorella, a green

TABLE 5.1

Algal Genera Present in Cultures

CO_2 ENRICHED CULTURES

1. 16-day residence time

   Anabaena - blue green - dominant

   Chlorella - green

   Scenedesmus - green

   Chlamydomonas - green

2. 8-day residence time

   Chlorella - green - dominant

   Oscillatoria - blue green

   Cylindrospermum - blue green

   Chlamydomonas - green

   Chlorococcum - green

3. 4-day residence time

   Chlorella - green - dominant

   Closterium - green - few

   Unknown blue green filamentous form-few

4. 2-day residence time

   Chlorella - green - dominant

   Anabaena - blue green - few

CO_2 DEFICIENT CULTURES

5. 32-day and 8-day residence time

   Chlorella - green - dominant

   Chlorococcum - green

   Oscillatoria - blue green

alga, while the 16-day detention sample contained more Anabaena, a blue-green alga. Samples with no CO_2 added during growth generally all showed the same algal types present and all were dominated by Chlorella.

2. Characteristics of Feed Solution. The feed solution was secondary sewage treatment plant effluent diluted so as to contain approximately 10 mg/l ammonia nitrogen. Other parameters measured are listed in Table 5.2 and represent approximate values as each batch collected varied in strength. This feed solution was used in order to simulate a natural treatment plant effluent condition.

TABLE 5.2

Diluted Sewage Feed Characteristics

	Approx. value (mg/l)	Range
Total Kjeldahl Nitrogen	13	
Soluble Kjeldahl Nitrogen	11	
Ammonia Nitrogen	10	8.0 - 10.9
Total COD	40	36 - 72
Total Phosphorus	3.0	2.7 - 4.0
Total Suspended Solids	less than 5	
pH	7.5	
Alkalinity	130	
Total Carbon	50	
Soluble Organic Carbon	15	

3. Symbols Used. The symbols used for the measured parameters presented in the results and the method by which they were determined are presented in Table 5.3.

TABLE 5.3

Abbreviated Symbols

Abbreviation		Means of Determination
M_T	Total Chemical Oxygen Demand	Direct Measurement
M_S	Soluble Chemical Oxygen Demand	Direct Measurement
M	Particulate Chemical Oxygen Demand	Calculated as $(M_T - M_S)$
S	Volatile Suspended Solids	Direct Measurement
C_T	Total Carbon	Direct Measurement
C_{TS}	Total Soluble Carbon	Direct Measurement
C	Particulate Organic Carbon	Calculated as $(C_T - C_{TS})$
C_{SO}	Soluble Organic Carbon	Direct Measurement
C_{SI}	Soluble Inorganic Carbon	Calculated as $(C_{TS} - C_{SO})$
N_{TK}	Total Kjedahl Nitrogen	Direct Measurement
N_{SK}	Soluble Kjedahl Nitrogen	Direct Measurement
N_A	Ammonia Nitrogen	Direct Measurement
N	Particulate Organic Nitrogen	Calculated as $(N_{TK} - N_{SK})$
%N	Nitrogen Content of Particulate Material expressed as % of S	Calculated as 100 (N/S)
P_T	Total Phosphorus	Direct Measurement
P_S	Soluble Phosphorus	Direct Measurement
P	Particulate Phosphorus	Calculated as $(P_T - P_S)$
%P	Phosphorus Content of Particulate Material Expressed as % of S	Calculated as 100 (P/S)
BOD	Biological Oxygen Demand	Direct Measurement
θ	Hydraulic Residence Time	Volume of Reactor/ Flow Rate

Note: All symbols except %N, %P and θ represent concentrations in (mg/l).

B. Growth Phase Results

The objective of this phase of the study was to evaluate the effects of various environmental conditions upon the rate and extent of algal growth. By varying factors such as pH, CO_2 availability, and lighting, while holding all other conditions constant, these factors were evaluated as to their effect on growth potential. By using the same algal cultures throughout the study it was also possible to study growth regulating factors under each set of conditions.

1. Diurnal pH Variation. An example of an observed variation in pH through the light-dark cycle is shown in Figure 5.1. During the light cycle algal photosynthesis requires carbon dioxide and light energy to produce algal protoplasm according to the following equation:

$$CO_2 + H_2O + NH_3 + PO_4^{\equiv} + \text{light energy} \rightarrow \text{protoplasm} + O_2$$

If a direct source of CO_2 is not available then the algae must obtain carbon from the bicarbonate alkalinity as noted by Meron and Rebhum (18). The bicarbonate disassociates to give CO_2 as a usable carbon source:

$$HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O$$

As the algae use this available CO_2, the equilibrium shifts to the right, decreasing the hydrogen ion concentration and causing the pH to rise as observed during the light cycle as the algae use CO_2. This pH variation was noted even though the total alkalinity remained fairly constant. This is possible according to King (14) in waters where there is largely carbonate alkalinity unless there is formation and precipitation of calcite as the pH rises. During the dark cycle the process is reversed as the algae respire, producing CO_2 according to:

$$\text{Algal protoplasm} + O_2 \rightarrow CO_2 + H_2O$$

This shifts the bicarbonate equilibrium to the left decreasing pH by increasing the hydrogen ion concentration. The pH of the samples begins to decrease at

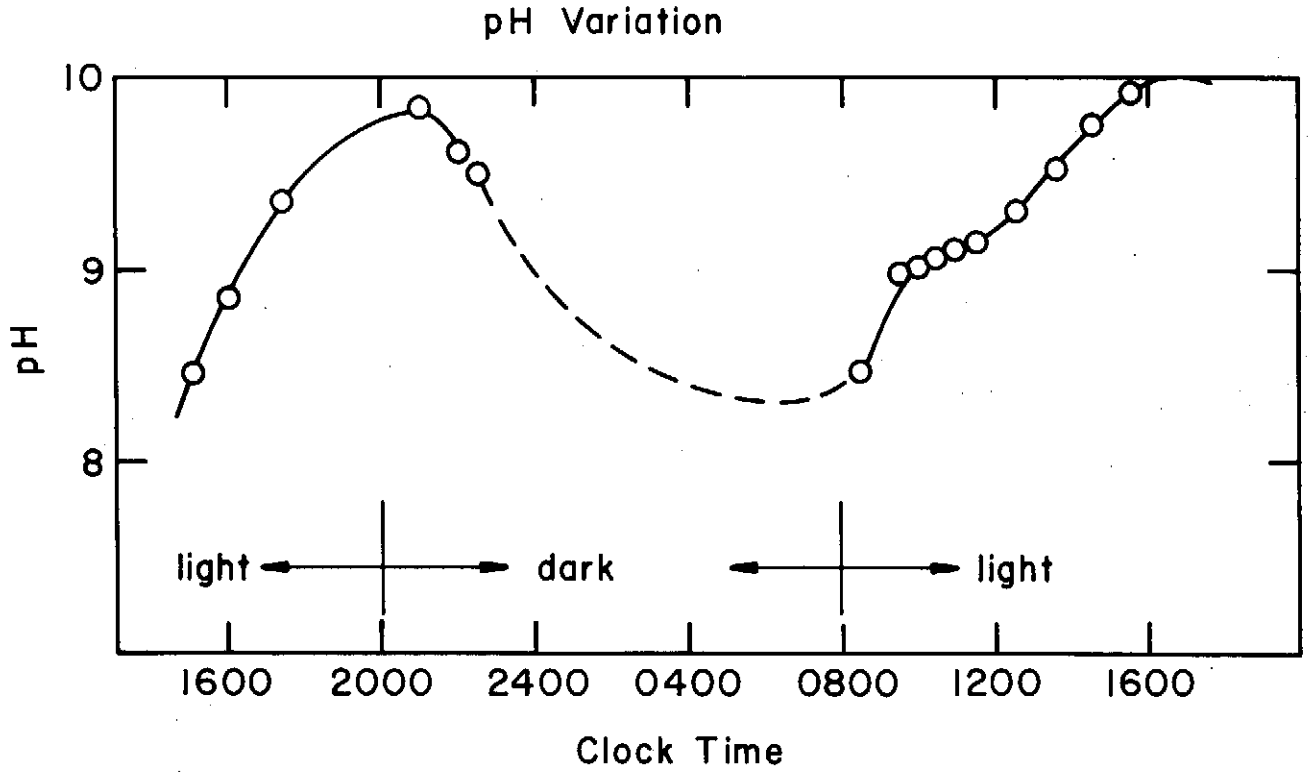

Figure 5.1. Typical pH Variation with Time in an Algal Culture Observed During Diurnal Light Cycling.

the end of the light cycle and continues until it reaches its previous level at the end of the dark period.

2. Mass Variation with Hydraulic Residence Time. Figure 5.2 shows the variation of Total COD (M_T) and Particulate COD (M) with the hydraulic residence time during several growth conditions. For the carbon dioxide enriched samples on a diurnal lighting cycle it can be noted that the pH maintained during the growth period had a decided effect on the total COD at all residence times. In the observed pH range from 5.0 to 9.0 it was found that the apparent optimum pH level for growth was around 7 and that cultures maintained either above or below this value produced less total growth. Of all the samples grown under the same conditions it can be seen that a pH level of about 5 produced the least growth, and in order of increasing growth were pH 6 and pH 8 to 9 with pH 7 showing the maximum of all values tested.

Similar results were reported by Soltero and Lee (24) in the testing of an automatic pH controller for algal cultures. In their studies on unialgal cultures they found pH 7.0 to be optimum followed by pH 8.0 and 9.0, and 6.0, respectively. Although the data cannot be compared directly, as batch cultures were used by Soltero and Lee, the results show the same trend in growth at different pH levels.

Also from Figure 5.2 differences in growth for the same conditions with and without CO_2 can be observed. At pH 7.0 and diurnal lighting conditions the algae grown with excess CO_2 showed as much as seven times the growth based on total COD as that grown in the absence of CO_2. In order to maintain the same pH conditions the CO_2 deficient samples were adjusted to pH 7.0 using HCl daily. Both the CO_2 enriched and the CO_2 deficient cultures produced the same curve, although the slope of the CO_2 supplied cultures was far greater than that of the CO_2 deficient case.

Lighting effects were also studied using the CO_2 deficient case. Samples grown without CO_2 under continuous lighting showed a significantly

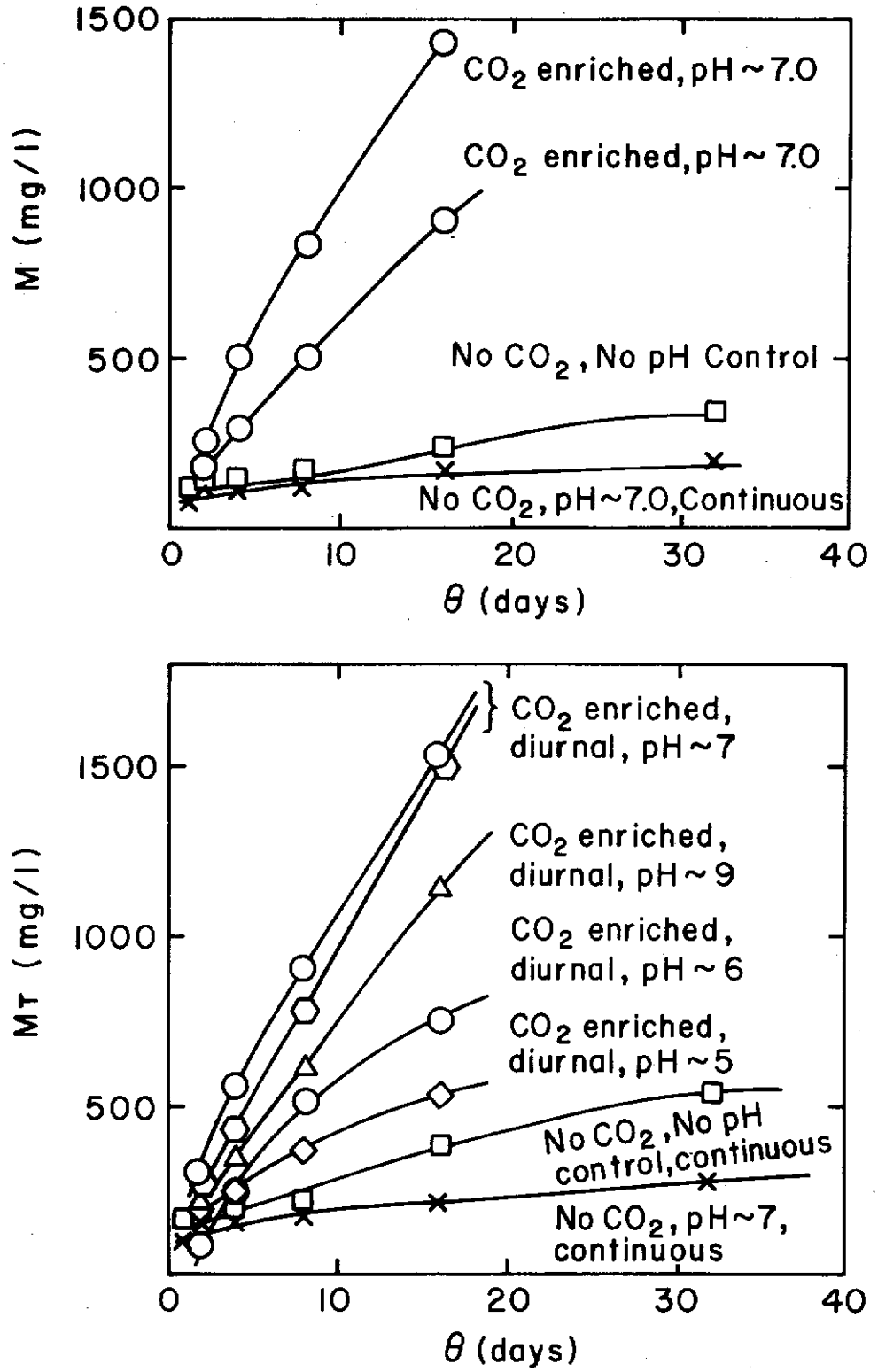

Figure 5.2. Total and Particulate COD as a Function of Hydraulic Residence Time Under Different Growth Conditions.

higher total COD than samples grown under diurnal cycles of light. For the shorter detention times only a small effect was noted, while the longest detention time showed almost twice the growth for the continuously lighted sample.

In the run made using continuous lighting no attempt was made to control pH and it ranged to 10.6 to 11.0 as compared to the manually adjusted pH of near 7 for the diurnal lighted samples. From the previous results, this would tend to cause a lower total COD than would have been observed at pH 7 and would point to a greater difference in growth due to lighting. Considering these data in light of a stabilization lagoon situation, the longer days during the summer months, along with higher ambient temperatures, contribute to the greater masses of algae usually observed.

3. Mass Variation with Time of Growth. Figure 5.3 shows the variation in the total COD (M_T) during the growth period for the different residence times of both the CO_2 enriched and the CO_2 deficient runs. It can be noted that all samples showed a pattern of reaching a maximum value and then decreasing slightly to a fairly constant steady state level. This same effect was reported by Porcella (22) in his studies using chemostat analysis and sewage effluents to grow algae. He found this pattern to hold when using direct cell counts, COD, and suspended solids measurements. For similar conditions of growth Porcella observed the maximum mass between 15 and 20 days after inoculation, the same range of time observed in Figure 5.3. Figure 5.3 also depicts the significance of carbon dioxide availability on the growth rate of the cultures as can be seen from the slope of the lines immediately after inoculation. The CO_2 enriched samples showed very steep curves indicating a fast growth rate, with greater variation between residence times than the CO_2 deficient cases. For all the residence times of the CO_2 deficient cultures, the curves were similar and all showed wash out rates greater than growth rates for about 15 days before peaking and reaching steady state. The large difference

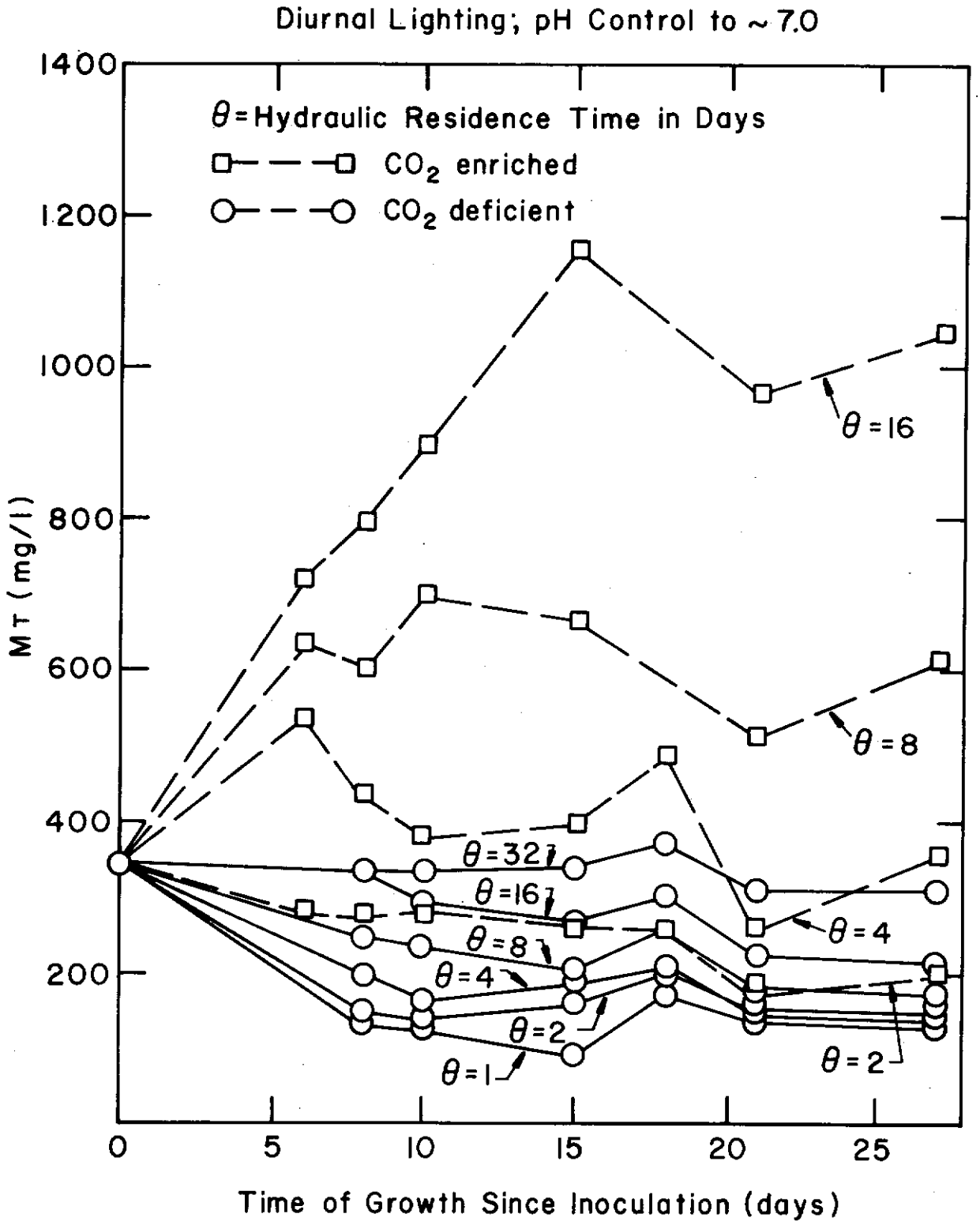

Figure 5.3. Total COD as a Function of Growth Time at Various Residence Times for Two Conditions of Carbon Dioxide Supply.

in growth rate and total COD shows the significance of CO_2 availability. In comparing the two conditions at the same residence time it can be seen that there is a much greater effect at the long detention times while at 2 days there is very little significant difference in the steady state Total COD values.

4. Growth Limiting Factors. Table 5.4 shows a comparison of values of pertinant parameters at steady state conditions for four separate chemostat runs. Two runs contained sets of CO_2 enriched chemostats grown under the same conditions and the other two runs contained CO_2 deficient chemostats. In run 3, a CO_2 deficient run, there was no effort made to control the pH of the cultures. Due to photosynthesis and the corresponding depletion of CO_2 as discussed previously, the average pH remained high, normally about 10.8. In trying to determine the growth regulating factor under these conditions, it was difficult to determine what in fact limited growth. As reported by King (14) at pH's of 10.0 and above, phosphorus in solution will precipitate as $CaPO_4$ and growth may then become phosphorus limited. The values observed for soluble phosphorus were fairly low and could have possibly been limiting in this situation. Ranging from 0.09 mg/l to 0.21 mg/l phosphorus, they were in fact the lowest observed during this research.

Since CO_2 was purposely withheld, the values reported for soluble inorganic carbon were low and randomly distributed, indicating possible carbon limitation. There was, however, a certain amount of CO_2 supplied by the bacteria in the system as they degraded the organic matter in the sewage feed, as discussed by Kuentzel (15) and Kerr et al. (12). The third possibility for growth limitation during this run was the high pH per se, which has a decided effect on growth as previously discussed and shown in Figure 5.2.

In an effort to isolate the growth regulating factor in this CO_2 deficient situation, a second run (run 4) was made in which the pH was adjusted to 7.0 manually each morning as described in Chapter III. Under these conditions the maximum pH recorded was 9.3 in the afternoon which may

TABLE 5.4

Comparison of Steady State Parameters for Different Growth Conditions

θ	pH	M	C	C_{SI}	N_A	P_S
days		mg/l	mg/l	mg/l	mg/l	mg/l
Run 1 with CO_2 enriched, diurnal lighting						
2	7.4	255	140	8.0	0.0	0.90
4	7.0	506	155	14.0	0.2	0.86
8	7.0	834	250	30.0	0.0	0.89
16	6.7	1427	430	40.0	0.0	0.81
Run 2 with CO_2 enriched, diurnal lighting						
2	7.9	176	49	7.0	0.0	1.17
4	7.3	298	97	9.0	0.0	0.14
8	7.6	505	162	16.0	0.0	0.34
16	7.0	905	276	13.0	0.0	0.23
Run 3 without CO_2 supplied, continuous lighting, no pH control						
1	11.1	114	33	4.0	3.0	0.09
2	10.9	126	38	1.0	4.0	0.11
4	10.9	126	42	3.0	4.2	0.10
8	10.7	129	32	7.0	8.6	0.21
16	10.8	235	56	11.0	2.0	0.15
32	10.7	341	74	1.0	0.2	0.21
Run 4 without CO_2 supplied, diurnal lighting, pH controlled						
1	9.3	66	12	16.0	3.0	0.99
2	9.1	110	36	12.0	2.0	0.76
4	8.4	102	19	8.0	1.9	0.49
8	8.0	150	43	5.0	1.9	1.66
16	7.1	162	44	3.0	1.9	1.47
32	6.0	196	67	4.0	1.4	1.31

account for the greater soluble phosphorus values recorded in Table 5.4. These values, which ranged from 0.49 mg/l to 1.66 mg/l phosphorus, were well above the previous run and similar to other runs where CO_2 was supplied. The soluble inorganic carbon (C_{SI}) values were again low and there was no increase in growth as measured by particulate COD even though phosphorus was in solution. The COD's were actually slightly lower in run 4 than in run 3. This was probably due to continuous lighting in run 3 and diurnal lighting in run 4. Since no greater growth was experienced when more favorable pH and phosphorus conditions were used it can be concluded that carbon was the regulating nutrient under the conditions of run 3 as well as run 4.

In both run 1 and run 2 for the CO_2 enriched samples there were soluble phosphorus and soluble inorganic carbon in solution at steady state conditions. However, in neither of these two identical runs was there any ammonia nitrogen in solution at steady state. Therefore, nitrogen appeared to be the growth limiting nutrient under the CO_2 enriched, diurnal lighting conditions. There was some difference in growth between run 1 and run 2 in part due to run 2 having a longer total lapse time of growth and an apparent population shift as discussed previously (Section A).

From Table 5.4 a trend in the soluble inorganic carbon results can be noted: that of increasing soluble carbon with increasing residence time. Each of the CO_2 enriched chemostats received the same amount of CO_2, and due to the higher growth rate at the shorter detention times, more CO_2 was used leaving less carbon in the solution. The inconsistancy of the inorganic carbon values for the CO_2 deficient runs was probably due to the fact that the analytical technique employed is rather insensitive at the low concentration range measured for these cultures.

When considering these results in light of natural situations there are several points to consider. Run 3 with no CO_2 added and uncontrolled pH conditions most nearly simulates a stabilization lagoon environment. Although

in the laboratory continuous lighting was used which increased the growth and restricted the normal pH variation observed with diurnal cycling, the same general trend was noted in the field for waste treatment lagoons. During the summer months of observation in 1971 when the days were long, the pH of the final lagoon of the West Hickman Creek Plant in Lexington, Kentucky, reached 10.0 during the afternoons. The results of run 3 suggest that while the longer lagoon detention times may increase the quality of sewage effluent there is considerably more algae grown, which actually adds to the organic load in the receiving stream. It is also evident that both nitrogen and phosphorus are removed from solution, the levels being much smaller than that of the feed, either by algal uptake or due to precipitation because of the algal effect on pH. This nutrient removal, however, is of little benefit unless the algae is prevented from entering the receiving stream by some means such as algae harvesting since the nutrients removed from solution are incorporated in the algal cells.

There is another implication in these results which has been the topic of much controversy in recent literature (2): the role of phosphorus as a regulating nutrient. In the runs simulating natural conditions, CO_2 was found to be the limiting factor even though phosphorus was probably being precipitated as a result of high pH. In subsequent runs when CO_2 was added in excess amounts, a four fold increase in growth was observed and nitrogen became growth regulating while phosphorus was still observable in solution. This seems to support the position of Kuentzel (15) and Kerr (12) that carbon is in fact many times the regulating nutrient rather than nitrogen or phosphorus. In this case efforts to remove nitrogen and phosphorus from the sewage effluent or restrict phosphorus contents in detergents would do little to limit algal growth unless highly efficient methods are employed. A more beneficial and practical approach might be to provide more complete removal of organic matter from sewage as bacterial action on such matter produces

quantities of CO_2 which are readily useable by the algae. The algae, in turn, produce oxygen and more organic matter and thereby a mutually supportitive system develops.

It might also be pointed out that the sewage effluent used was diluted to the weakest concentration of ammonia nitrogen observed during preliminary studies to produce a feed of approximately constant concentration. This implies that at most times greater nitrogen and phosphorus concentrations would be available than those actually used.

5. Measure of Growth. Figure 5.4 is a graphical representation of growth parameters for the data in Table 4. As can be seen from the figure the runs made with excess CO_2 added to the system produced greater COD (M), solids (S), and Carbon (C) concentrations, than the runs made without CO_2. Subscripts 1 and 2 denote the runs made under identical conditions with the exception of the observed population shift in run 2 discussed in Section A. Although the mass concentration measured as COD and solids seemed to vary somewhat the carbon concentrations for runs 1 and 2 were almost identical. The same effect is noted for runs 3 and 4 made without CO_2 although the conditions of growth varied as mentioned before for both lighting and pH control.

6. Variation of Cellular Nitrogen and Phosphorus Content. According to the theory outlined in PAAP (4) the growth rate is a function of the limiting substrate nutrient concentration and the mass yield is directly proportional to the quantity of the growth limiting nutrient depleted from solution. During preliminary studies and this research it was found that growth did not cease when the regulating nutrient was no longer available in solution. Figure 5.5 shows the total COD data from Figure 5.3 for the CO_2 enriched samples plotted along with the corresponding ammonia-nitrogen (N_A) values in solution. As can be seen the nitrogen in solution deminished quickly and remained there for the duration of the sampling period. Even though the ammonia-nitrogen in most cases was completely removed from solution

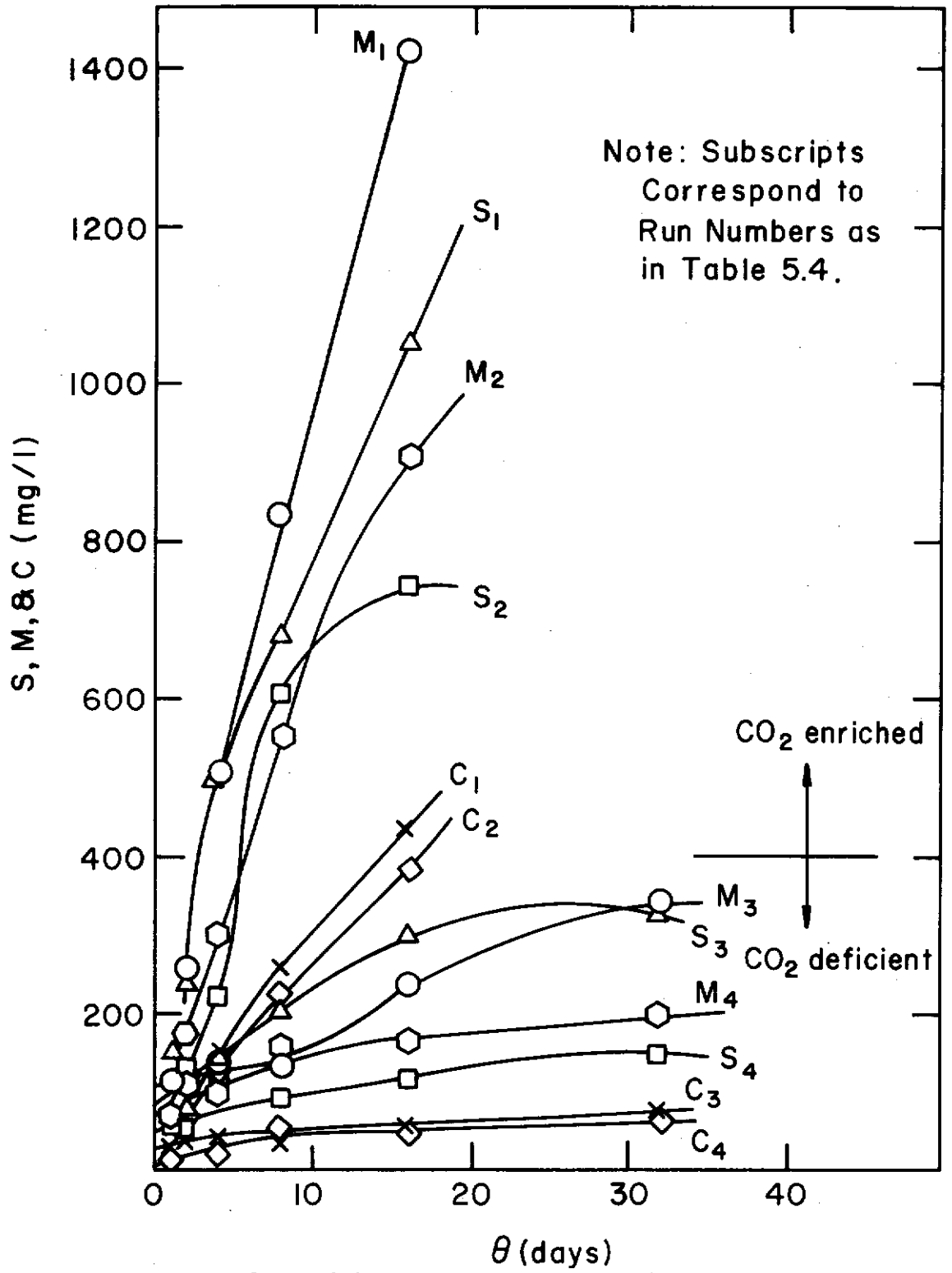

Figure 5.4. Particulate Solids (S), COD (M), and Carbon (C) Concentrations as a Function of Residence Time for Four Sets of Growth Conditions.

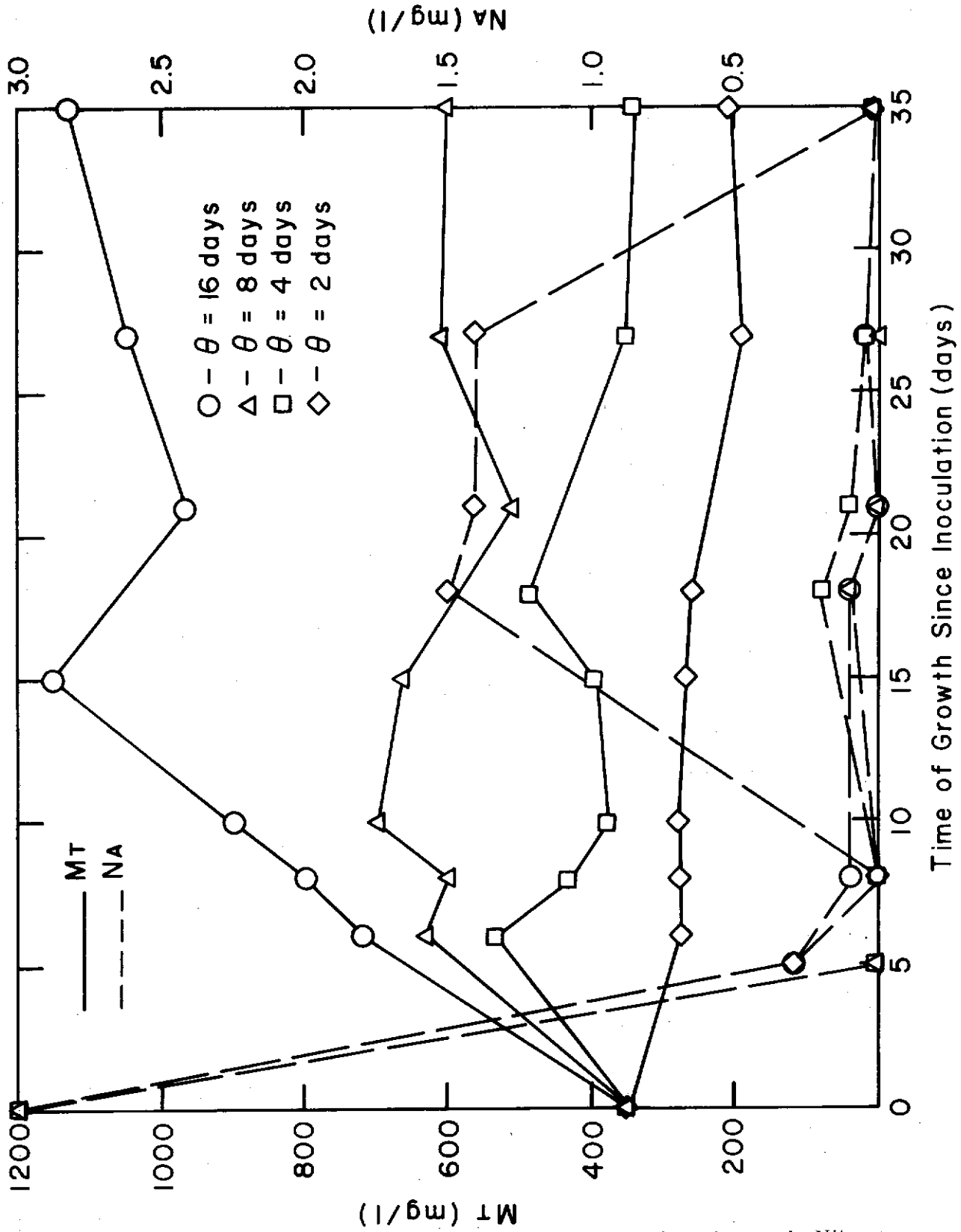

Figure 5.5. Total COD Concentrations and the Corresponding Ammonia Nitrogen in Solution as a Function of the Time of Growth.

rapidly, growth continued for sometime before a steady state level was obtained.

This trend seems to follow those observed by either Gerloof and Skoog (8) or Jewell and McCarty(11) as discussed in Chapter II. Although they did not agree on the point at which cell division actually ceases, both their theories state the cellular nutrient content, not the nutrient concentration in solution, actually limits growth. As discussed by Foree and Tapp (7), algae store excess nutrients in their cells during nutrient abundent growth and can then use these stored nutrients for continued growth during nutrient deficient conditions.

This phenomenon has an effect on the percent nutrients in the cells as seen from Figure 5.6. For the longer detention times the total mass is higher due to synthesis of cellular material (primarily lipids and carbohydrates) which contains no nitrogen or phosphorus while the limiting nutrient in solution is low or absent. The result is if essentially all the nutrients are depleted from solution and stored by the algae, the longer residence times with higher cell masses will result in smaller percentages of the nutrients. This was observed for both nitrogen and phosphorus in all the cases studied with the exception of run 2 for the 16 day residence time where there was apparent nitrogen fixation due to the observed population shift. For this particular case the cellular nitrogen concentration was very much greater than the ammonia-nitrogen concentration in the feed solution.

From Figure 5.6 the cellular phosphorus content was below 2% for the longer residence times and below 0.4% for the heavy growth, CO_2 enriched cases even though phosphorus was not found to be growth regulating. Most observed nitrogen concentrations were below 3% and in the nitrogen limited case they were about 1%. These values are in agreement with those found by Foree and Tapp (7) for a Kentucky Mixed sample of algae under batch culture conditions.

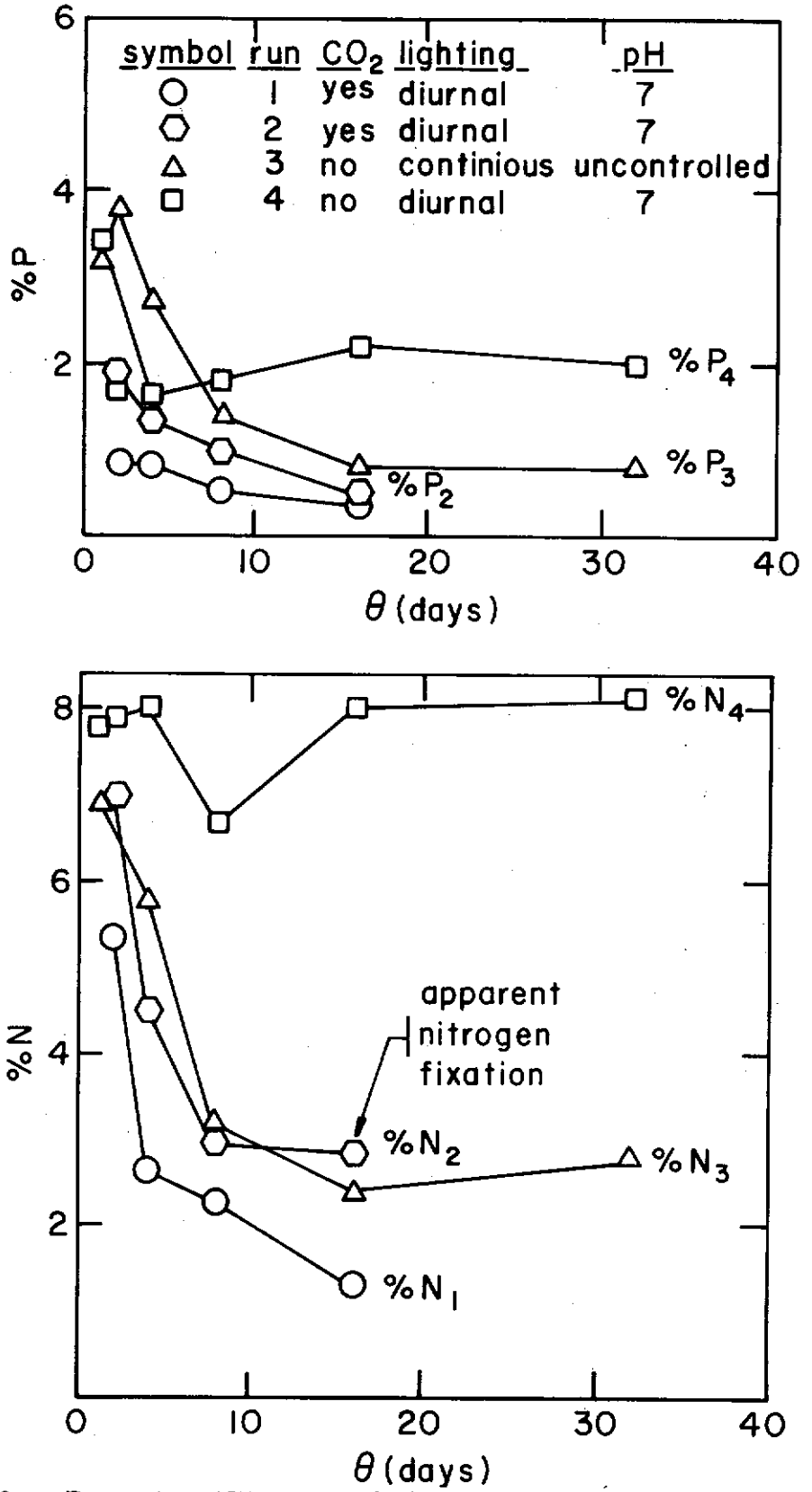

Figure 5.6. Percentage Nitrogen and Phosphorus in the Algal Cells as a Function of Hydraulic Residence Time for Four Conditions of Growth.

These low percentages would tend to support Kuentzel's (15) and Kerr's (12) position that limitation of nitrogen and phosphorus might be of little value as many lakes and rivers already contain sufficient quantities of these nutrients to produce large algal blooms if all other conditions were optimum. The population shift would tend to indicate that the algal culture which was limited by nitrogen shifted to a predominant species that could fix atmospheric nitrogen and continue to grow.

7. Nutrient Limitation Theory. As stated previously, the kinetic theory outlined in PAAP (4) for nutrient limited growth does not seem to apply in the case when nitrogen or phosphorus is growth limiting. Since preliminary studies pointed out this fact, a kinetic theory was hypothesized and developed in Chapter II which describes a growth rate proportional to the cellular nutrient concentration on a mass basis. This theory applies only to nutrients such as nitrogen and phosphorus and not to the observed case of carbon limitation, as lipid and carbohydrate synthesis, and thus mass increase, requires an available source of extracellular carbon in the substrate. The growth is therefore regulated, in the carbon deficient case, by the available inorganic carbon in the substrate.

For completely mixed, continuous flow systems the specific growth rate ($\frac{dX/dt}{X}$) is equal to the reciprocal of the hydraulic residence time ($1/\theta$) and by the hypothesized equation is proportional to the cellular nutrient concentration minus an effective respiration rate.

$$\frac{1}{\theta} = \frac{dX}{dt} /X = k\,(N/X) - b'$$

To test this equation, $1/\theta$ can be plotted against N/X to check for proportionality. If the expression is applicable, then a straight line should result with a slope equal to k and a negative intercept equal to b'. The data for the nitrogen limited cases of runs 1 and 2 were plotted in Figure 5.7. The cellular nitrogen concentration on three different mass bases was used

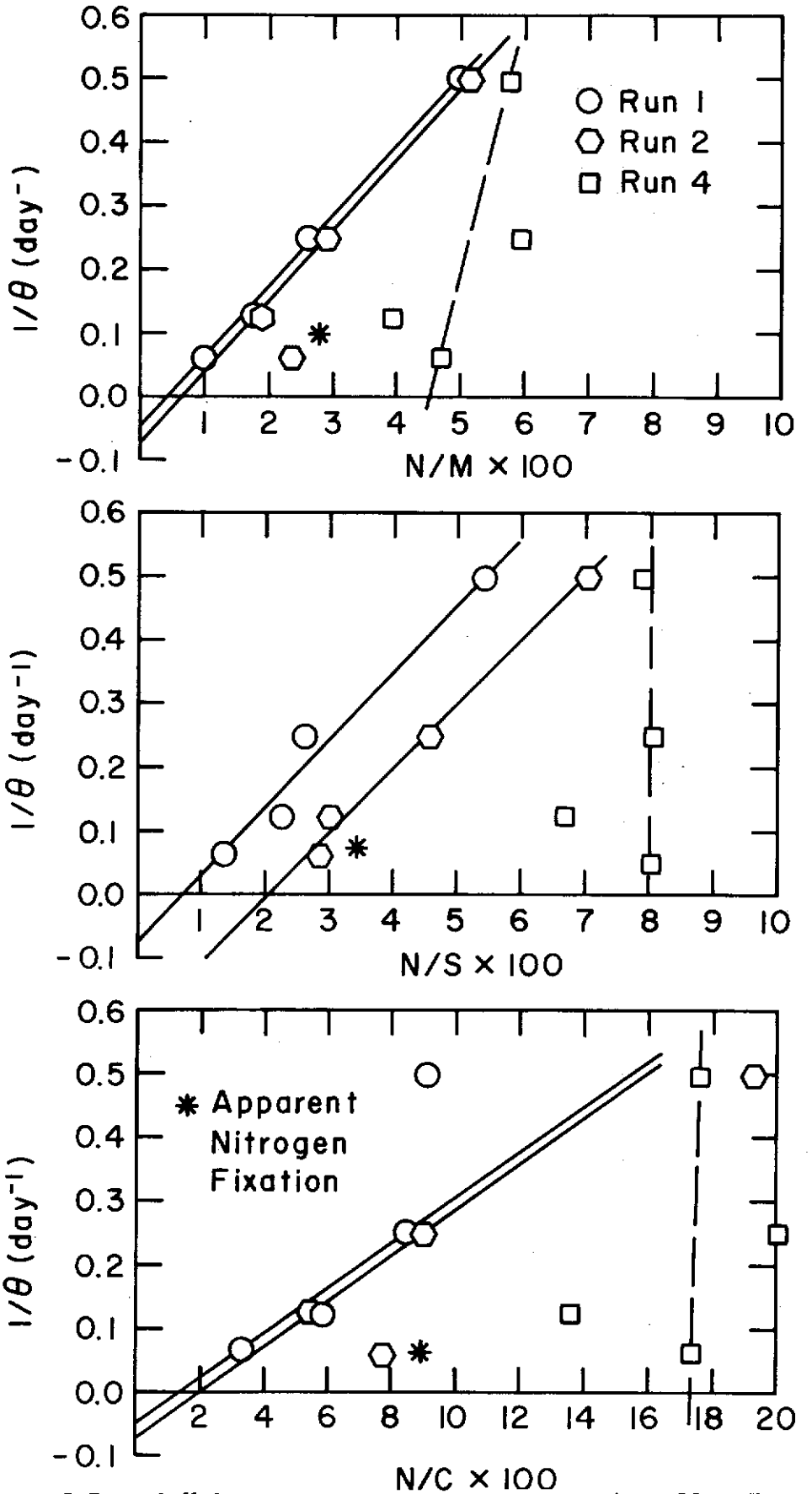

Figure 5.7. Cellular Nitrogen Concentrations on Three Mass Bases as a Function of the Reciprocal of the Mean Algal Cell Age ($1/\theta$).

as nitrogen was the limiting nutrient. It can be seen that the observed data were reasonably well fitted to straight lines for both runs for all three mass parameters. Values for k and b' were determined from Figure 5.7 and are summarized in Table 5.5.

TABLE 5.5

Summary of Kinetic Growth Parameters for Nitrogen Regulated Growth from Figure 5.7

Basis for Cellular Nitrogen Concentration	Run	$k\left(\frac{\text{mg X}}{\text{mg N} \cdot \text{day}}\right)$	$b'\ (\text{day}^{-1})$
N/M	1	10.9	0.05
	2	11.4	0.07
N/S	1	10.7	0.08
	2	10.1	0.22
N/C	1	3.6	0.05
	2	3.8	0.07

The values for k and b' were consistent except for run 2 on a solids basis. This inconsistency was probably due to a systematic error in this set of suspended solids analyses which tended to make all the determined solids values somewhat smaller than the actual values. The ratio of k for the COD basis to k for the carbon basis was approximately 3 to 1 and the ratio for the COD basis to solids basis was 1.13 to 1 which were within the normal range of COD: carbon and COD: solids values previously reported for algae (11).

Also plotted in Figure 5.7 were the data from Run 3 in which carbon was the limiting nutrient. As contrasted to the nitrogen limited runs, it can be seen that there was no such linear relationship for cellular nitrogen concentration as it appeared to be almost constant with respect to the specific growth rate.

C. Decomposition Phase

In the decomposition phase the algae grown under different conditions were allowed to decompose in the dark on an electrolysis BOD apparatus in order to determine the rate and extent of oxygen utilization. From these data it was determined what effects, if any, the different growth conditions made on the biodegradability of the algal cultures.

Since there is much concern over the added organic load of dying algae in eutrofied lakes, these data might have application in determining the amount of oxygen depletion associated with different algal growth factors.

1. Shape of the Algae BOD Curve. In all the BOD studies a characteristic curve was noted (Figures 5.8 and 5.9) showing a two stage decomposition effect. There was usually a first stage BOD exerted followed by a leveling off, then between 5 and 10 days there was an increased rate of BOD exertion. This effect was due (27, 16, 28) to the fact that algae incubated in the dark will continue to live for a period and respire which exerts a low oxygen demand. After 5 to 10 days in the dark, death and autolysis of the algal cells begin. This results in additional biodegradable organic matter becoming available to the decomposer organisms and thus the second stage increase in the BOD. Fitzgerald (5) found that living algal cells are highly resistant to attack and degradation by bacteria, but become susceptible to bacterial decomposition upon death.

This phenomenon may have an important bearing when evaluating treatment plant and lagoon effluents containing algae as the conventional 5 day BOD will not show the added effect of the algal mass load downstream. As long as the algae continue to live it will not produce an increased organic load and may actually be beneficial if the receiving stream environment is favorable for photosynthesis and thus O_2 production. However, if downstream conditions become unfavorable for photosynthesis, the algae will produce the same effect on the stream dissolved oxygen resources as unstabilized organics from a sewage effluent.

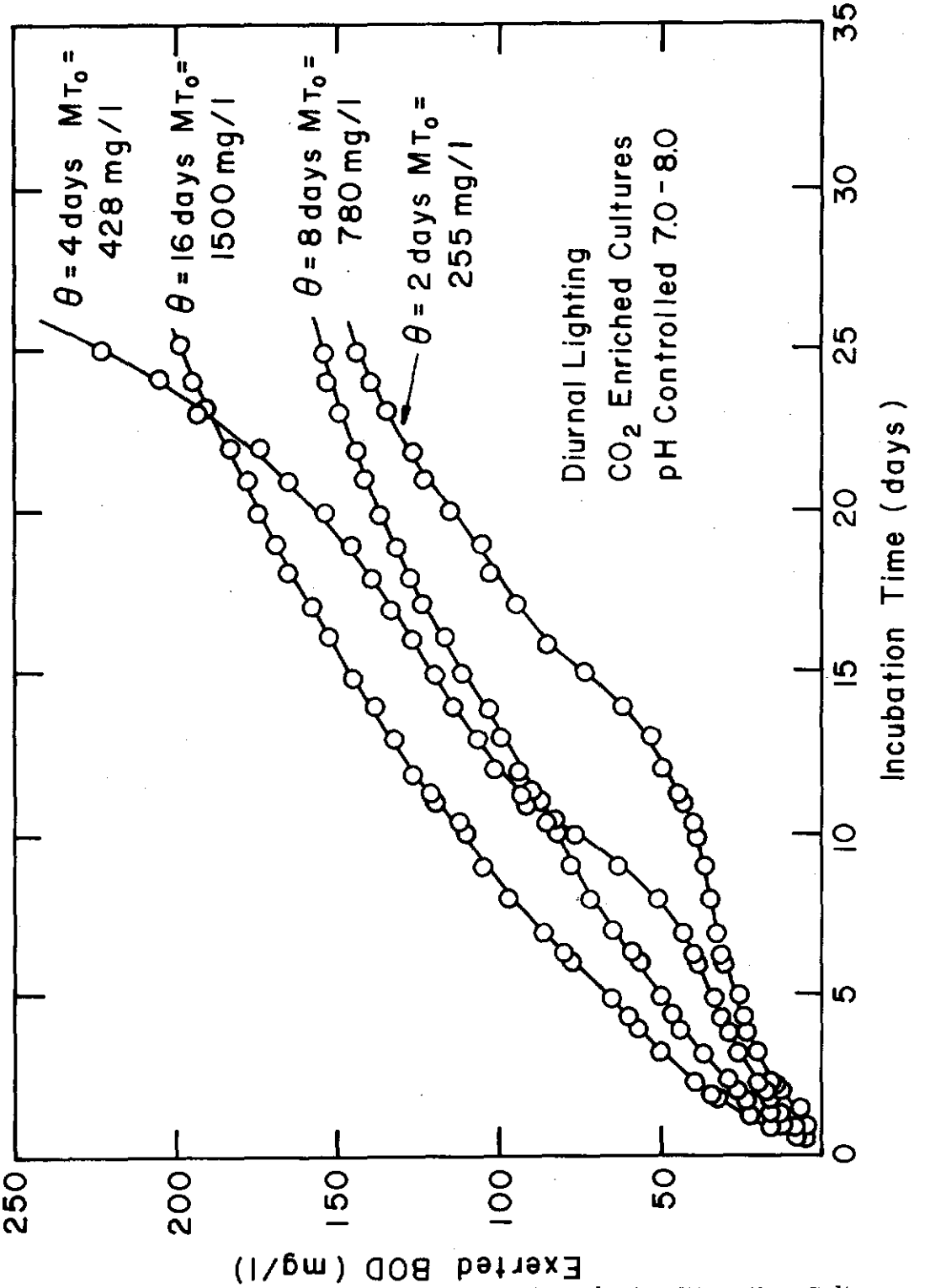

Figure 5.8. Continuous BOD as a Function of Incubation Time For Cultures Grown Under the Same Conditions at Different Residence Times.

Symbol	Curve	θ (days)	CO_2	M_T initial (mg/l)	initial pH	final pH
□	A	8	yes	600	8.1	8.1
⬡	B	8	no	184	9.0	5.4
△	C	4	yes	340	8.2	7.5
○	D	4	no	152	8.3	5.3
◇	E	2	no	156	7.8	5.3

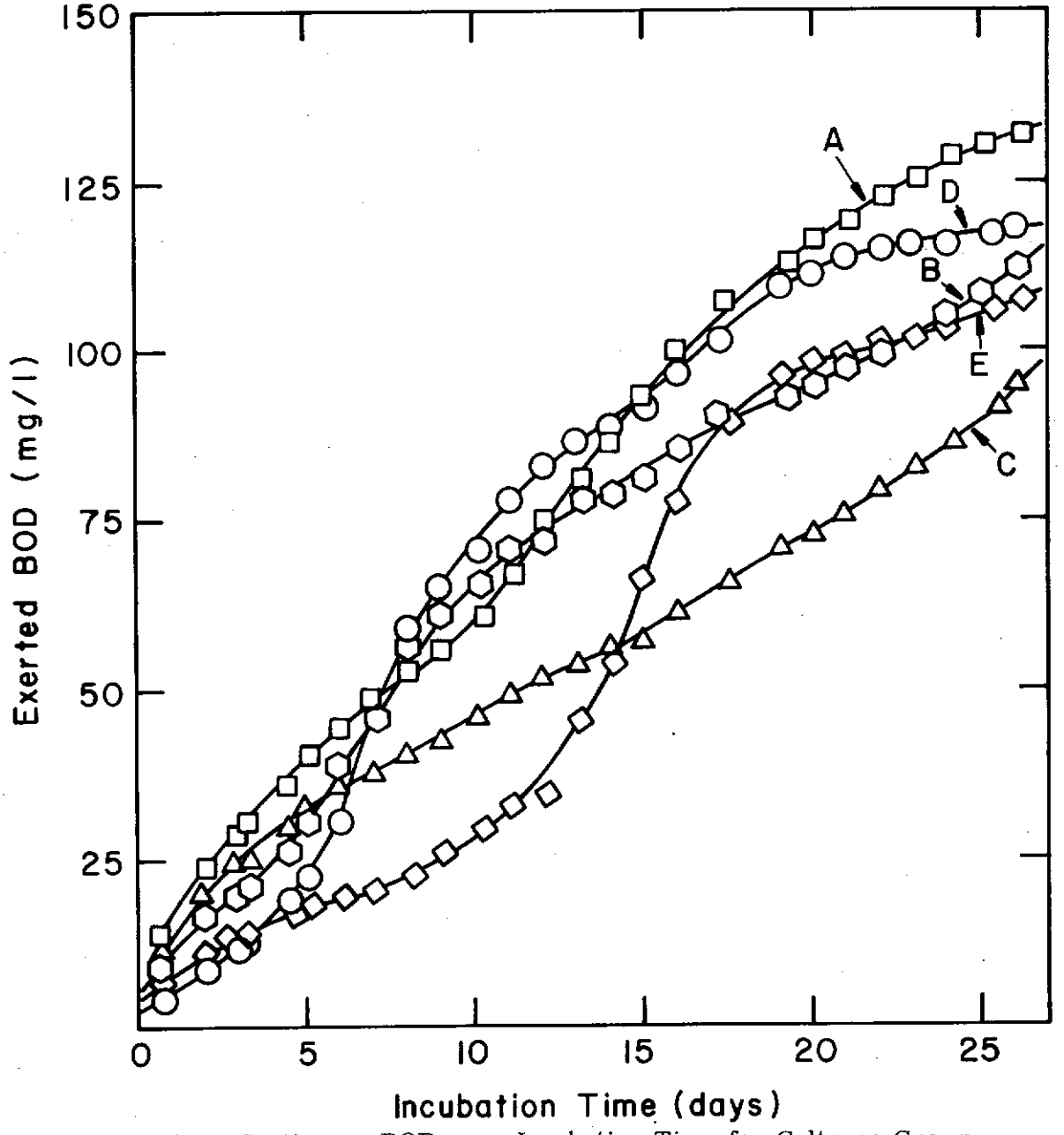

Figure 5.9. Continuous BOD vs. Incubation Time for Cultures Grown with and without Carbon Dioxide Under Diurnal Lightning.

2. BOD Variation with Growth Residence Time. To determine the effect of the hydraulic residence time or the mean algal cell age on the BOD, an electrolysis BOD test was run on a sample of each residence time grown under identical conditions. Figure 5.8 shows the continuous BOD plotted against the incubation time of four samples grown under diurnal lighting and sufficient carbon dioxide supplied to maintain the pH of the sample during growth at near 7.0. As expected the sample with the lowest initial COD and the shortest residence time had the lowest BOD. With the exception of the 4 day detention time, this was true for all samples although judging from the shape of the 4 day curve there was probably an error introduced due to machine malfunction. Nitrification did not seem to occur during any of the 25 day BOD tests. This was confirmed by analysis which showed no significant decrease in Kjeldahl nitrogen during the BOD tests.

As discussed in Section B, under these conditions of growth the hydraulic residence time had a large effect on the total mass supported by the system. Although the total initial COD was six times greater for the 16 day sample as for the 2 day sample as seen in Figure 5.8, the same difference was not observed for the 25 day BOD, the 16 day being only 1.4 times greater than the 2 day residence time. This would suggest that the age of the algal culture has a considerable effect on its degradability. Jewell and McCarty (11) have noted this same effect, that biodegradability generally decreases with culture age.

From Figure 5.8 it is also noticeable that there is more variation or a more pronounced second stage increase in the BOD curve for the shorter residence times. This again would indicate that the younger algal cells were more easily degraded once death occurred.

If this result is applied to a waste treatment lagoon situation it appears that although a long detention time would produce a larger mass of algae it would be more resistant to biological breakdown and would probably

cause little more oxygen depletion than would the smaller mass of algae from a lagoon of shorter detention time. However, although there is no great increase in the oxygen used for decomposition, there is an aesthetic problem of large quantities of fairly non-biodegradable algae flowing into a receiving stream.

3. BOD Variation with Growth Conditions. Figure 5.9 shows the BOD data for algae grown at several residence times under identical conditions except for the carbon dioxide supply. Again the continuous BOD was plotted against the incubation time in days and typical BOD curves were obtained. As noted previously in Figure 5.8 for the effect of detention time, very little variation was observed between the BOD of the CO_2 enriched and the CO_2 deficient sample for the same detention times. The 8 day sample with CO_2 supplied showed a higher BOD than did the 8 day without CO_2 supplied as would be expected from the initial COD; however, this difference is insignificant compared with the large difference in their total masses.

At the 25 day BOD all cultures showed similar BOD's with little variation for either detention time or CO_2 growth condition. It can also be noted that the pH in the CO_2 deficient samples dropped during the decomposition period from a level of around 8 down to 5.3. This was due to destruction of the buffering capacity of the waste feed by the HCl addition to maintain the pH near 7 during the growth period. These low pH levels could have caused unfavorable conditions for decomposition and lower BOD's of these samples than might have otherwise been obtained.

In order to depict the biodegradability of the different growth conditions, Figure 5.10 shows the ratio of the BOD to the initial COD plotted against the incubation time. It can be seen that the cultures which were not fed CO_2 were 60 to 70% degraded while the CO_2 enriched samples remained fairly low, between 20 and 30% degraded after 25 days. As in the case of the age of the algal cultures, this seems to suggest that the CO_2 enriched growth conditions produce a more refractory algae which is more resistant to

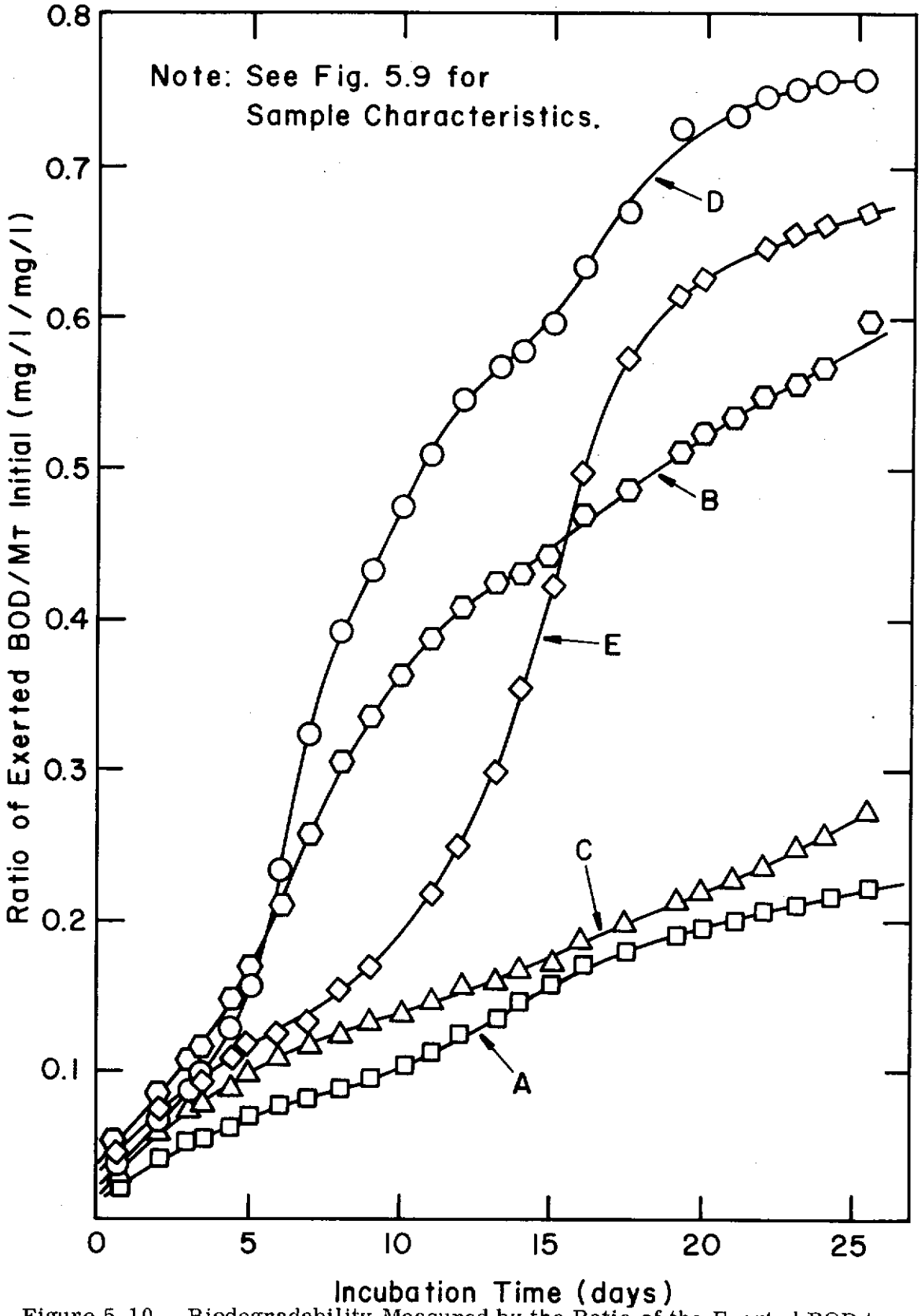

Figure 5.10. Biodegradability Measured by the Ratio of the Exerted BOD to the Initial Total COD as a Function of Incubation Time.

decomposition over the 25 day period studied. It is also noted that the residence time made less difference in biodegradability of the samples enriched with CO_2 than those without CO_2.

Since these results suggest that it would make little difference on the short term BOD load in a receiving stream, it might be argued that bacterial action on organic matter in sewage which produces excess CO_2, should cause little concern. However, from the COD data it is clear that there are actually far greater masses of algae produced in the CO_2 enriched environment and, although it is resistant to short term decomposition and the associated dissolved oxygen depletion, it retards a water's self cleansing process and causes aesthetic problems. Other resulting problems are taste and odor in water, filter clogging, dead algae washing up on beaches, and reduced recreational benefits.

CHAPTER VI

SUMMARY

Using the continuous flow algal cultures it was found that optimum growth occurred when the culture was maintained at pH 7.0. Decreased levels of growth were noted at pH 8 - 9 and pH 6.0, and pH 5.0 showed the least observed growth during this research.

Samples grown under CO_2 enriched conditions showed faster growth rates and seven times the total steady-state standing crop mass on a COD basis as identical cultures grown under CO_2 deficient conditions. The growth conditions were constant otherwise with pH = 7.0 and diurnal lighting. Greater mass was noted for all three mass bases: COD, solids (dry weight), and organic carbon concentration.

Cultures maintained at similar conditions with the exception of the lighting cycle were used to study the effects of diurnal lighting and continuous lighting. The continuously lighted samples produced twice the total COD for the longer residence times, but there was little difference for the shorter residence times.

Carbon dioxide was found to be the growth regulating nutrient in runs made at pH 7.0, diurnal lighting, and the diluted sewage treatment plant effluent feed. When pH was uncontrolled phosphorus was precipitated from solution, but was determined not to cause lower growth levels. In the cultures which were CO_2 enriched, but grown under the above conditions, growth was determined to be regulated by the nitrogen concentration in the algal cells. For these conditions, cultures with long residence times exhibited a population shift to blue-green forms of algae which had the capability for atmospheric nitrogen fixation and associated mass increase.

It was found that the growth regulating nutrient (nitrogen) was depleted from solution shortly after inoculation and remained low during the entire run, but growth (mass increase) continued for some time after this depletion from solution. Percentages of nitrogen and phosphorus in the cells showed trends of decreasing with increasing residence times due to the difference in growth rates. On a dry weight basis, the cellular phosphorus concentration ranged from 0.4 - 2.0% and the cellular nitrogen concentration ranged from 1.0 - 3.0%.

The data from this research for nitrogen limitation confirmed the hypothesized kinetic theory developed for cases of nitrogen and phosphorus limitation. Due to algal inability to synthesize mass without a carbon source this theory did not apply for the observed case of CO_2 limitation.

During the decomposition studies typical BOD curves were obtained showing a first stage BOD due to algal respiration followed by a leveling off and then a second stage increased exertion after the death of the algae. For samples grown under identical conditions the longer residence times showed the greatest 25 day BOD. However, there was less difference in BOD between the 2 day residence and the 16 day residence than the difference in the respective total initial COD.

Samples grown with and without CO_2 showed differences in BOD similar to the differences observed in the COD. The CO_2 enriched samples produced greater 25 day BOD than samples of the same residence times grown without CO_2. As in the case of residence times (algal cell age) this difference was less pronounced for BOD than for total initial COD. This points to a difference in biodegradability due to cell age and conditions of growth. The algae grown under CO_2 deficient conditions were found to be 60 - 70% degraded, determined as the ratio of the BOD to the initial total COD, while those grown under CO_2 enriched conditions were only 20 - 30% degraded during the same 25 day decomposition period.

CHAPTER VII

CONCLUSIONS

The following conclusions are drawn based on this laboratory study of heterogeneous algal populations grown in continuous culture in diluted secondary sewage treatment plant effluent:

1. Optimum algal growth occurred under pH 7.0, excess CO_2, and continuous lighting conditions. Lower and higher pH, CO_2 deficiency, and diurnal lighting all resulted in decreased growth rate and mass (standing crop) at steady-state conditions.
2. For the case when excess CO_2 was provided, nitrogen was quickly depleted from solution after inoculation, but growth (mass increase) continued due to the storage of nitrogen in the algal cells during the previous period when excess nitrogen was available from solution.
3. The availability of artifically supplied excess CO_2 greatly increased the mass (standing crop) at steady-state over that produced under otherwise identical conditions for all residence times studied. Thus CO_2 availability would regulate growth in similar natural situations.
4. For the case of excess CO_2 availability, the nitrogen concentration in the algal cells regulated growth rather than the concentration of nutrients in solution. A mathematical expression was hypothesized to describe this phenomenon and was confirmed by the experimental results.
5. Under dark-aerobic conditions, the algal cultures exerted a two-stage BOD, the second stage apparently beginning after the death of the algal cells.

6. Algal cell age (as measured by the residence time) and environmental growth conditions influenced the biodegradability of the cultures. Increased cell age produced a lower percentage biodegradability. Cultures grown in CO_2 enriched medium (optimum growth conditions) were significantly less biodegradable on a percentage basis than cultures grown in a CO_2 deficient medium.

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