INTERACTING CARBON AND LIGHT LIMITATION OF THE KINETIC SINKING RATE OF CHLORELLA VULGARIS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

MARK THORNE HILL

1977

INTERACTING CARBON AND LIGHT LIMITATION OF THE KINETIC SINKING RATE OF *CHLORELLA VULGARIS*

by

Mark Thorne Hill

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1977

ABSTRACT

INTERACTING CARBON AND LIGHT LIMITATION
OF THE KINETIC SINKING RATE OF
CHLORELLA VULGARIS

by

Mark Thorne Hill

Light and carbon limits interact in a multiplicative fashion to control the specific growth rate (μ_g), the specific sinking rate (μ_s) and the specific plankton biomass accumulation rate (μ_{ab}) of Chlorella vulgaris. μ_g decreases, μ_s increases and μ_{ab} decreases as functions of increased stress on the alga induced by interactions between carbon and light. The effect of the decrease in μ_{ab} as a function of the interacting limits is to limit the ability of Chlorella vulgaris to compete in natural systems at free carbon dioxide concentrations three orders of magnitude greater than those required to sustain photosynthetic carbon fixation. This suggests that application of results from chemostat studies of plankton algal kinetic response to environmental limits incorporates a significant error associated with the sinking of algae in natural systems.

ACKNOWLEDGMENTS

I would like to acknowledge the love and inspiration of Susan, whose patience with a part-time husband and father made this study possible.

A very special thanks is given to Dr. Darrell King for his generous devotion of time, energy and knowledge throughout my degree program.

This research was supported by grant USDI A-090-MICH from the Office of Water Research and Technology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES--continued

LIST OF FIGURES--continued

INTRODUCTION

Increasing interest in municipal sewage lagoon disposal systems and eutrophication of freshwater lakes and estuaries has generated numerous algal growth studies. These investigations have progressed from macroscopic effects of nutrient enrichment to the complex microscopic interactions within the aquatic system. The focus of most of these algal studies has been the evaluation of limiting nutrients within controlled laboratory microcosms.

Microcosm studies have provided more experimental control and less environmental variation of the parameters in question. Consequently, investigators have been able to concentrate on specific conditions which limit algal growth.

King (1970, 1972) described the association between algal succession and carbonate alkalinity and the levels of carbon required for blue-greens to dominate natural systems or sewage lagoons. Young and King (1973), Gavis and Ferguson (1975), and King and King (1974) provided mathematical models to define these carbon limitations on algal growth kinetics. Each algal species has unique environmental requirements for growth. If these requirements are not met for a particular alga, it cannot compete with another species in the same environment.

When an alga can no longer compete in a particular set of environmental conditions, it simply sinks out of the photic zone (Zeisemer, 1974). The phenomenon of algal sinking has been investigated from several viewpoints. Bella (1970) mathematically described the rate and effect of sinking of algae. Kalantyrenko (1972) reported on the concentration of algae biomass upon settling and Smayda (1974) related temperature, light and silicon uptake to the sinking of diatoms. Titman and Kilham (1976) studied algal sinking in relation to phosphorus limitations and Langmuir circulation. Zeisemer (1974) concentrated on the effects of carbon and light limitations on growth kinetics but recognized and explored the significance of sinking rate on algal growth. His results indicated that growth of algal populations is directly influenced by carbon availability illumination and sink rate.

The object of this study was to examine the effect of algal sinking rate vs. growth rate under varying conditions of carbon and light limitations. This study focused primarily on multiple stress factors in relation to the sinking rate of algae. Zeisemer (1974) described a reciprocal relationship between algal growth rate and sinking rate under a constant environmental light condition with carbon as the limiting parameter. In this investigation varying light conditions were used to test the general applicability of the reciprocity of sinking rate to growth rate and to quantify the effects of carbon and light interaction on algal growth kinetics.

A unicellular green alga, Chlorella vulgaris, was selected for the study and grown under a variety of light conditions. The microcosms contained inorganic growth medium and controlled carbonate alkalinity. The first phase of the study was performed to define workable experimental conditions and to eliminate procedural errors. The results of this study were used in the second part to pursue the study objective in a more definitive manner.

MATERIALS AND METHODS

Apparatus

Light microcosms and light-dark microcosms similar to those employed by Zeisemer (1974) and Haase (1973) were used in this investigation. The light microcosms were one liter Erlenmeyer flasks fitted with a No. 11 stopper as seen in Figure 1. One hole was drilled in the stopper to fit on air lock which minimized recarbonation from the atmosphere and maintained atmospheric pressure. The air lock was described by Zeisemer (1974) and is basicly a water filled manometer. A second hole was drilled in the stopper to fit a rubber serum cap through which samples were removed using a hypodermic syringe. This method prevented exposure of the culture to the atmosphere.

The light-dark microcosms were constructed from three 500 ml wide mouth Erlenmeyer flasks fused together in the manner shown in Figure 2. The bottom flasks were painted black to prevent light penetration, thus creating a non-photic zone; an area of the microcosm in which sinking algae are removed from the active photosynthetic portion of the culture biomass. These flasks were capped with No. 10 stoppers fitted with serum caps and air locks.

Illumination of the microcosms was accomplished with two 40 watt "Gro Lux" lights fitted into 48 inch fluorescent light

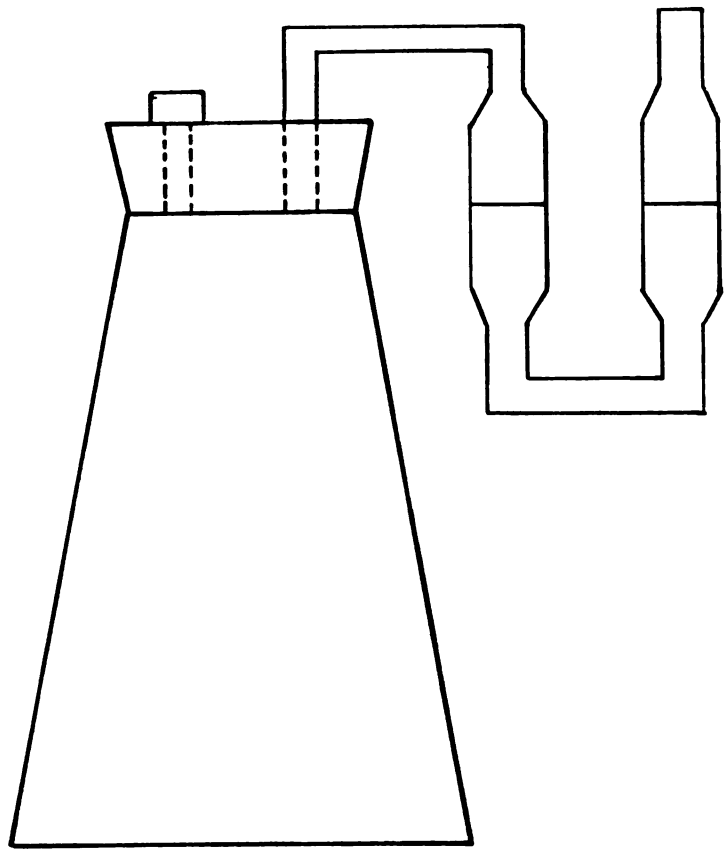


Figure 1. Light microcosm with air lock used in Chlorella vulgaris study.

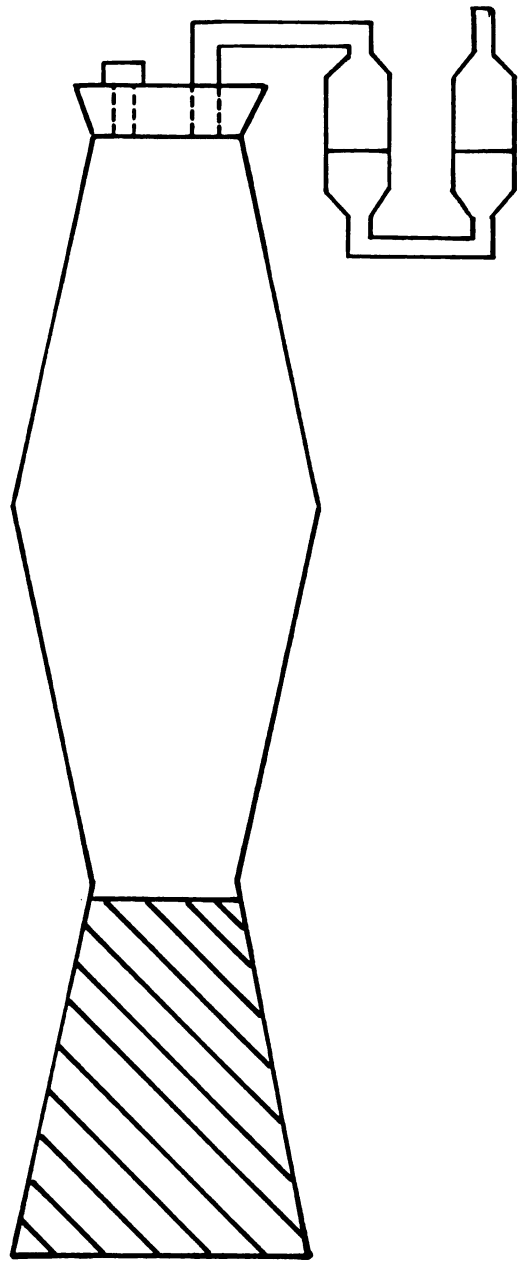


Figure 2. Light-dark microcosm with air lock used in Chlorella vulgaris study.

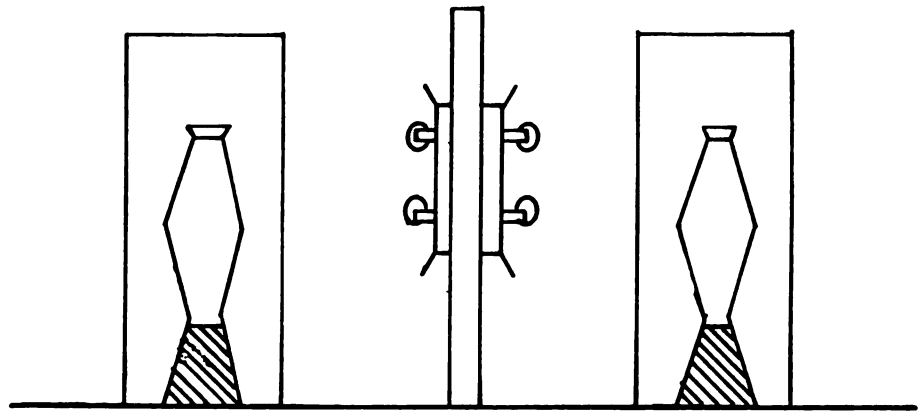
fixtures and mounted on wooden frames as shown in Figure 3. These light frames were constructed so that two light and two light-dark microcosms were illuminated under each light intensity. A platform was used for the light microcosms to compensate for the height difference between the two types of microcosms. Each microcosm was side illuminated to minimize illumination of the dark portion of the light-dark microcosm as shown in Figure 3.

A range of light intensities was achieved by covering each of the four light frames with various combinations of black nylon cloth and fine mesh wire screen painted black to prevent alteration of the light spectrum (Luebbers and Parikh, 1966). Table 1 lists the four light intensities used in this investigation and the coverings necessary to obtain the intensities. Illumination was measured with a Weston Model 756 footcandle meter.

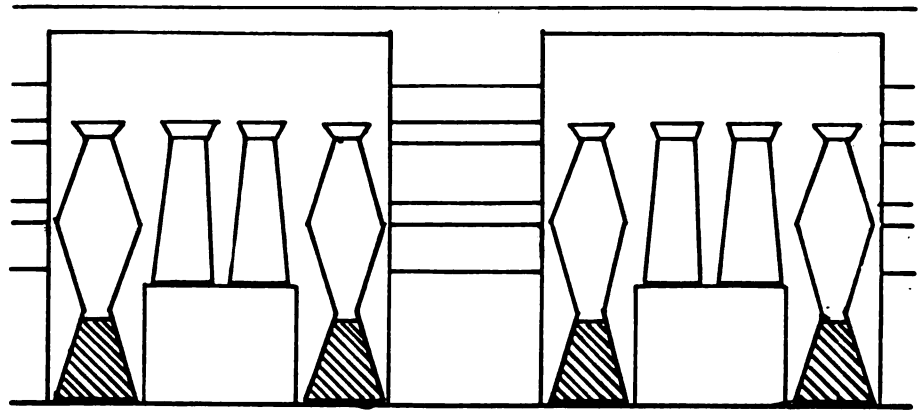
Growth Medium and Culture Methods

A growth medium similar to that used by King and King (1974) and Zeismer (1974) was used in this study for all microcosms. All nutrients were in excess and the medium was growth limiting with respect to carbon. The medium composition shown in Appendix A was dominated by monovalent cations which prevented the reduction of alkalinity by carbonate precipitation (Young and King, 1973).

The medium was added to the flasks and autoclaved at 250 degrees F, 15 psi for 20 minutes and aerated for 24 hours prior



Side View



Front View

Figure 3. Experimental apparatus layout used in Chlorella vulgaris study.

Table 1. Materials used to cover the lights to vary incident light transmission with the resulting light intensities delivered to the microcosms.

Coverings	Light Intensity Transmitted (foot candles)
No light cover	360
1 layer window screen	240
1 layer window screen + layer black nylon	50
2 layers of black nylon	16

to seeding to allow the free carbon dioxide concentration to return to atmospheric saturation. Following Zeisemer's (1974) suggestion EDTA was added after autoclaving and aeration to prevent precipitation of iron. After aeration and stabilization of carbon dioxide, a sample of the medium was titrated for alkalinity using the potentiometric method (Standard Methods, 1973).

The algal seed used in this study was Chlorella vulgaris (Culture No. 260) obtained from the Indiana University Type Culture Collection. The seed was aseptically subcultured in light microcosms under the same medium, alkalinity and light conditions as the test microcosms. After the algae raised the pH one to one and a half units they were centrifuged to concentrate the biomass and were then reintroduced into fresh medium. This concentration method was followed twice for each culture. As Zeisemer (1974) reported, this concentration and reintroduction procedure provided a sufficient biomass while maintaining the pH in the range of that of the fresh medium.

The total inorganic carbon content of the seed culture was determined with a Beckman Model IR-315 infrared carbonaceous analyzer. This allowed a uniform addition of seed to each microcosm in terms of a uniform organic carbon loading.

pH Determinations

All pH values were obtained with a Corning Model 12 research pH meter with a general purpose glass semi-microelectrode. The

pH meter was standardized frequently against prepared standard buffer solutions.

A 20 ml sample was withdrawn through the serum cap of the microcosms and injected into a 50 ml beaker in which the air was replaced with nitrogen gas. This method minimized recarbonation of the sample with atmospheric carbon dioxide. A number 9 1/2 stopper sealed the beaker and contained one hole to inject the nitrogen gas and fit the pH electrode and another hole fitted with a serum cap through which the sample was injected. Measurements of pH were taken once a day for those microcosms subjected to the two high light intensities and every other day for the two microcosm under low light intensity.

Carbon Calculations

The pH measurements collected from each microcosm were used with temperature and alkalinity data to determine carbon flux. These carbon calculations are fundamental to all data presented and are based on three principle equations, two of which were derived by Harvey (1957) and Park (1969) and expanded by King and Novak (1974).

The first equation deals with the total inorganic carbon dioxide available in the sealed microcosms.

$$\Sigma\ CO_2 = a \left[\frac{\frac{H^2}{K_1} + H + K_2}{H + 2\ K_2} \right] \quad (1)$$

Where: ΣCO_2 = Total inorganic carbon dioxide, moles C/l

a = Carbonate-bicarbonate alkalinity, corrected for hydroxyl ion concentration, eq/l

H = Hydrogen ion concentration, moles/l

K_1 = First dissociation constant of carbonic acid

K_2 = Second dissociation constant of carbonic acid

If equation 1 is time incremented using daily pH measurements, the amount of carbon fixed by algal photosynthesis may be calculated using equation 2.

$$C_{fixed} = \Delta\Sigma CO_2 = \Sigma CO_{2_{initial}} - \Sigma CO_{2_{final}} \qquad (2)$$

A third equation was used to calculate the free carbon dioxide concentration which is the form of carbon that algal cells use (King and Novak, 1974).

$$CO_{2f} = a \left[\frac{H^2}{K_1 (H + 2 K_2)} \right] \qquad (3)$$

Where: CO_{2f} = Free carbon dioxide concentration, moles C/l

a = Carbonate-bicarbonate alkalinity, corrected for hydroxyl ion concentration, eq/l

H = Hydrogen ion concentration, eq/l

K_1 = First dissociation constant for carbonic acid

K_2 = Second dissociation constant for carbonic acid

Growth Rate Calculation

Generally, algal biomass increase follows the first order growth equation.

$$M_t = M_o \, e^{\mu g t} \qquad (4)$$

Where: M_t = Mass at time (t)

M_o = Initial mass

μg = Specific growth rate

The specific growth rate (μg) is a growth rate normalized for the average biomass present during a given time interval.

$$\mu g = \frac{\frac{\Delta C_f}{\Delta t}}{\overline{\chi} \, C_f} \qquad (5)$$

Where: μg = Specific growth rate, hours $^{-1}$

ΔC_f = Change in carbon fixed (biomass increment), mMC/l

Δt = Time increment, hours

$\overline{\chi} C_f$ = Average carbon fixed (average biomass) over the time increment, mMC/l

The specific growth rate of algae generally follows the Michaelis-Menten or Monod equation (Goldman et al., 1974).

$$\mu g = \mu max \frac{S}{K_s + S} \quad (6)$$

Where: μg = Specific growth rate

μmax = Maximum specific growth rate

S = Concentration of the limiting substrate

K_s = Half saturation substrate concentration, where $\mu g = 1/2\ \mu max$

The μmax and K_s are constants for a particular environmental condition and are obtained from double reciprocal plots (Dowd and Riggs, 1965) of free carbon dioxide concentrations and specific growth rate.

King and Novak (1974) demonstrated the application of Monod kinetics of algal growth to levels of free CO_2 (CO_{2f}). Thus, in the present study where carbon is the limiting substrate, K_s and S in equation 6 carry units of mM CO_{2f}/l.

Active Biomass Accrual Rate Calculation

The relationship discussed in equation 6 applies only to algae maintained in a zone of abundant light. Algae in light microcosms sank to the bottom but continued photosynthesis and equation 6 can be used to calculate the specific growth rate (μg) of these algae as a function of CO_{2f}. However,

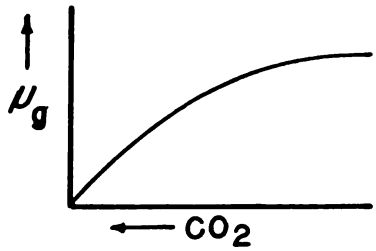
photosynthesis ceased in the light-dark microcosms after the algae sunk to the dark portion of the flasks. Since algal biomass was constantly sinking from the photic zone of these flasks, the specific growth rate (μg) could not be calculated directly for the light-dark bottles using equation 6.

Growth conditions were identical for each light, light-dark bottle pair and the specific growth rates were assumed to be identical at the same free CO_2 concentrations within the photic zone of each pair. Free CO_2 concentrations for the light-dark bottles were calculated for several short time increments over the growth period using equation 3. These values were then used to pick specific growth rates (μg) from the curve relating specific growth rate (μg) to CO_{2f} obtained from the companion light bottle. Figure 4 is a schematic representation of this calculation process.

When the algae sunk in the light-dark microcosms, the rate of biomass accrual is slower in these flasks than in the light microcosms. Therefore, indirect calculation of the specific growth rates for the light-dark bottles allow the calculation of average active biomass over a time increment in these flasks. As shown in Figure 4 this calculation is made by dividing the carbon fixed in the light-dark microcosms over an interval ($\Delta C_{LD}/\Delta t$) by the μg obtained from the calculated CO_{2f} concentration over that same interval in light-dark microcosms and the curve relating μg and CO_{2f} in the companion light bottle as shown in equation 7.

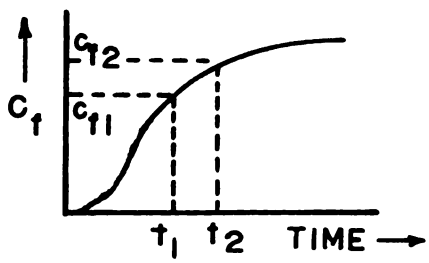
Equation 3: $CO_{2_f} = a\left[\frac{H^2}{K_1 (H + 2K_2)}\right]$

Equation 5: $\mu g = \frac{\frac{\Delta C_f}{\Delta t}}{\bar{x}\ C_f} = \frac{\frac{\Delta \text{ Biomass}}{\Delta t}}{\bar{x}\ \text{Biomass}}$



Light microcosm plot

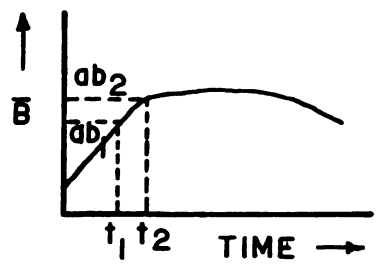
Equation 3 is used to calculate the free CO_2 for a particular light-dark microcosm. Return to the corresponding light microcosm plot to obtain μg values, for the light-dark microcosm.



Carbon fixed curve light-dark bottle

Equation 7:

$$\frac{\frac{\Delta C_{LD}}{\Delta t}}{\mu g} = \frac{\frac{C_{f_2} - C_{f_1}}{t_2 - t_1}}{\mu g} = ab$$



Equation 8 results when the active biomass curve is incremented.

$$\frac{\frac{\Delta ab}{\Delta t}}{\bar{x}\ ab} = \frac{\frac{ab_2 - ab_1}{t_2 - t_1}}{\bar{x}\ ab} = \mu ab$$

Equation 10: $\mu_s = \mu_g - \mu_{ab}$

Figure 4: Graphical explanation of the methods of data calculation.

$$\frac{\frac{\Delta C_{LD}}{\Delta t}}{\mu g} = \frac{\frac{\Delta C_{LD}}{\Delta t}}{\frac{\frac{\Delta C_L}{\Delta t}}{\bar{\chi}\, C_L}} = ab \qquad (7)$$

Where: ΔC_{LD} = Change in carbon fixed in the light-dark microcosm over the time interval Δt, mMC/l

ΔC_L = Change in carbon fixed in the light microcosm at equal CO_{2f} concentrations, mMC/l

$\bar{\chi}C_L$ = Average biomass in the light microcosm, mMC/l

μg = Specific growth rate at equal CO_{2f} concentrations, hr^{-1}

Δt = Time interval, hrs.

ab = Average active planktonic biomass

By incrementing the active biomass curve it is possible to calculate a specific active biomass accrual rate (μab) as shown in Figure 4 and equation 8. This rate is positive or negative depending on whether there is a net gain or loss of biomass over the period.

$$\mu ab = \frac{\frac{\Delta ab}{\Delta t}}{\bar{\chi} ab} \qquad (8)$$

Where: μab = Specific active biomass accrual rate, hr^{-1}

Δab = Change in active biomass over the time interval Δt, mMC/l

$\bar{\chi} ab$ = Average active biomass over the interval, Δt, mMC/l

Sink Rate Calculation

Equations 6 and 8 define methods to calculate specific growth rate and specific accrual rate over each time interval within the light-dark microcosms. The specific growth rate (μg) represents the overall rate for the system or the sum of biomass accrual rate (μab) and specific sink rate (μs) (Zeisemer, 1974). Such that:

$$\mu g = \mu ab + \mu s \qquad (9)$$

Therefore, the difference between specific growth rate (μg) and specific biomass accrual rate (μab) can be defined as the specific sink rate (μs).

$$\mu s = \mu g - \mu ab \qquad (10)$$

RESULTS AND DISCUSSION

Experiment 1

Algal Response to Culturing Method

The first phase of this investigation was designed to gather initial data on the growth response of Chlorella vulgaris in carbon and light limited environments. These data provided a means to examine and correct difficulties in the methods and to evaluate ranges of experimental conditions for the second phase of the study. Table 2 shows the experimental lattice, coding procedure, and biomass seed concentration used in this initial phase.

Chlorella responded well to the growth medium, culturing method and experimental light intensities. As a result of the biomass concentration method prior to seeding no initial growth lags were observed after seeding the microcosms. Fogg (1966) and Pritchard et al., (1962) reported that planktonic algae like Chlorella require a certain concentration of glycolic acid in the medium before growth begins. The absence of lag in this study suggested that the repeated biomass concentration and seeding method employed in this investigation allowed for a sufficient accumulation of such precursors to growth.

However, the large quantity of biomass seed added to each microcosm (0.03 m MC/l) produced such fast growth rates in the

Table 2. Experimental lattice, coding procedure and initial data for the first phase of the investigation.

Microcosm*	Light Intensity (Ft. Cd.)	Alkalinity (meq/l)	Seed mM C/l Concentration
362 L	360	1.88	0.03
362 LD	360	1.88	0.03
36.5 L	360	0.44	0.03
36.5 LD	360	0.44	0.03
242 L	240	1.88	0.03
242 LD	240	1.88	0.03
24.5 L	240	0.44	0.03
24.5 LD	240	0.44	0.03
52 L	50	1.88	0.03
52 LD	50	1.88	0.03
5.5 L	50	0.44	0.03
5.5 LD	50	0.44	0.03
12 L	16	1.88	0.03
12 LD	16	1.88	0.03
1.5 L	16	0.44	0.03
1.5 LD	16	0.44	0.03

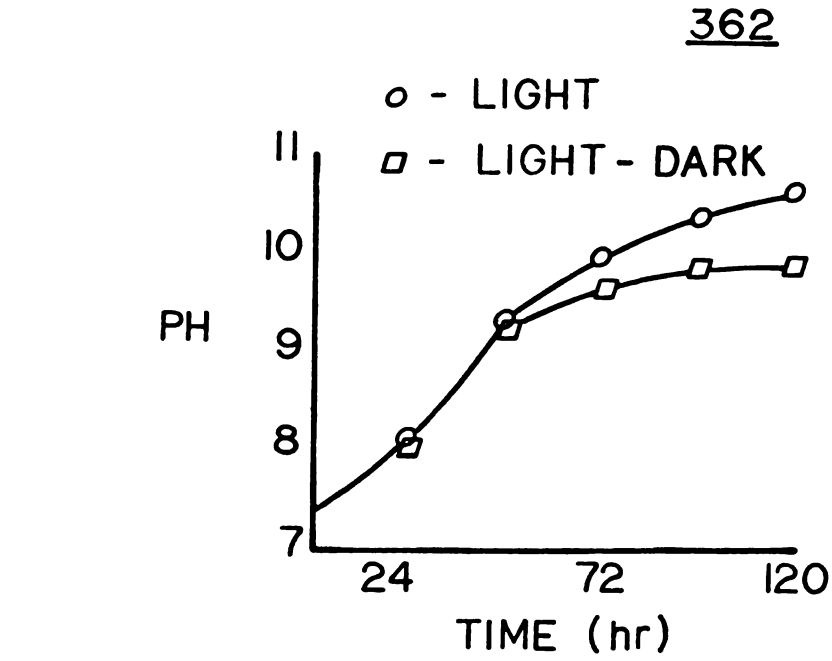
*The first one or two digits indicate the light intensity. The last digit indicates approximately the alkalinity. The L or LD indicate whether the microcosm is a light or light-dark bottle respectively.

microcosms under high light intensities that too few data points were obtained prior to carbon limitations. Periphyton growth occurred in several of the light-dark microcosms and no data were derived from these bottles. The effect of periphytic growth was that some algal cells which sunk became fixed to the walls of the photic portion of the light-dark microcosms and yielded a system similar to the light microcosms.

pH and Carbon Fixation

King (1970, 1972) reported that increased phosphorus and nitrogen loading of aquatic systems leads to increased carbon dioxide extraction by algae. This, in turn, results in an increased pH and a decrease in CO_{2f} concentration. However, light is the primary determinate of the minimum algal CO_{2f} requirement and maximum attainable pH if all other required nutrients are in excess (King and King, 1974). This relationship was evident in the present study as shown in Figures 5 and 6. These figures illustrate that with increased illumination, the rate of change of pH to maximum pH increased. Since increased pH reflects lower free CO_2 levels, the higher the algae raise pH the lower the CO_{2f} concentration.

Algal extraction of CO_2 from the alkalinity system results in equilibrium changes that (1) supply CO_2 to the algae, (2) increase pH and (3) decrease equilibrium CO_{2f} levels. Evidence of the role alkalinity plays can be seen in Figure 6. Both microcosms were subjected to the same light intensity (16 foot candles) but with different alkalinities. The microcosm pair



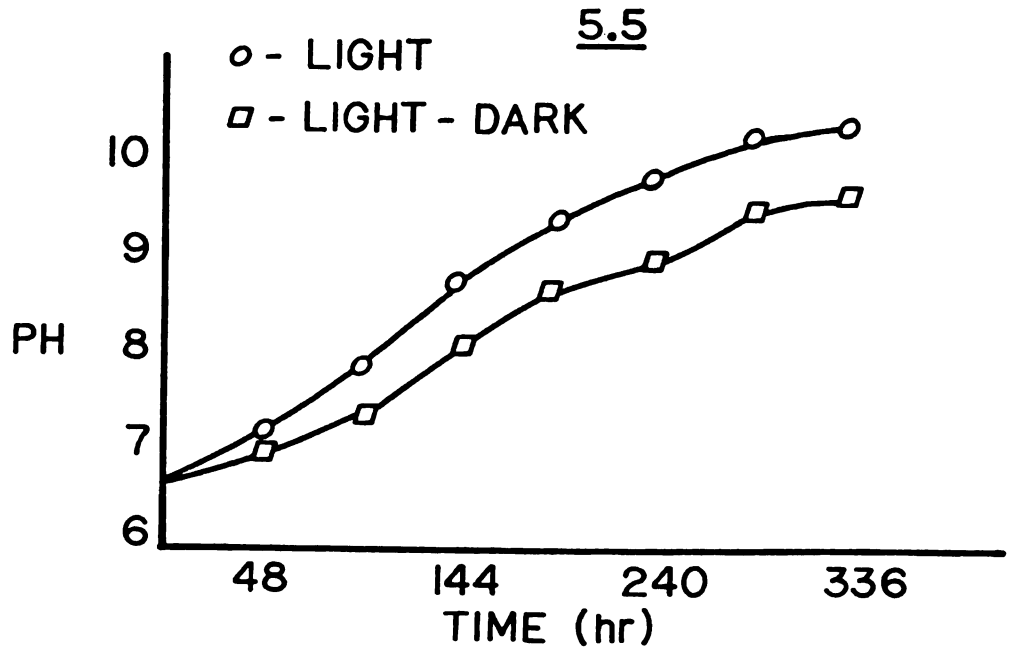


Figure 5. Time related pH response of Chlorella vulgaris at various light intensities and alkalinities for both light and light-dark microcosms.

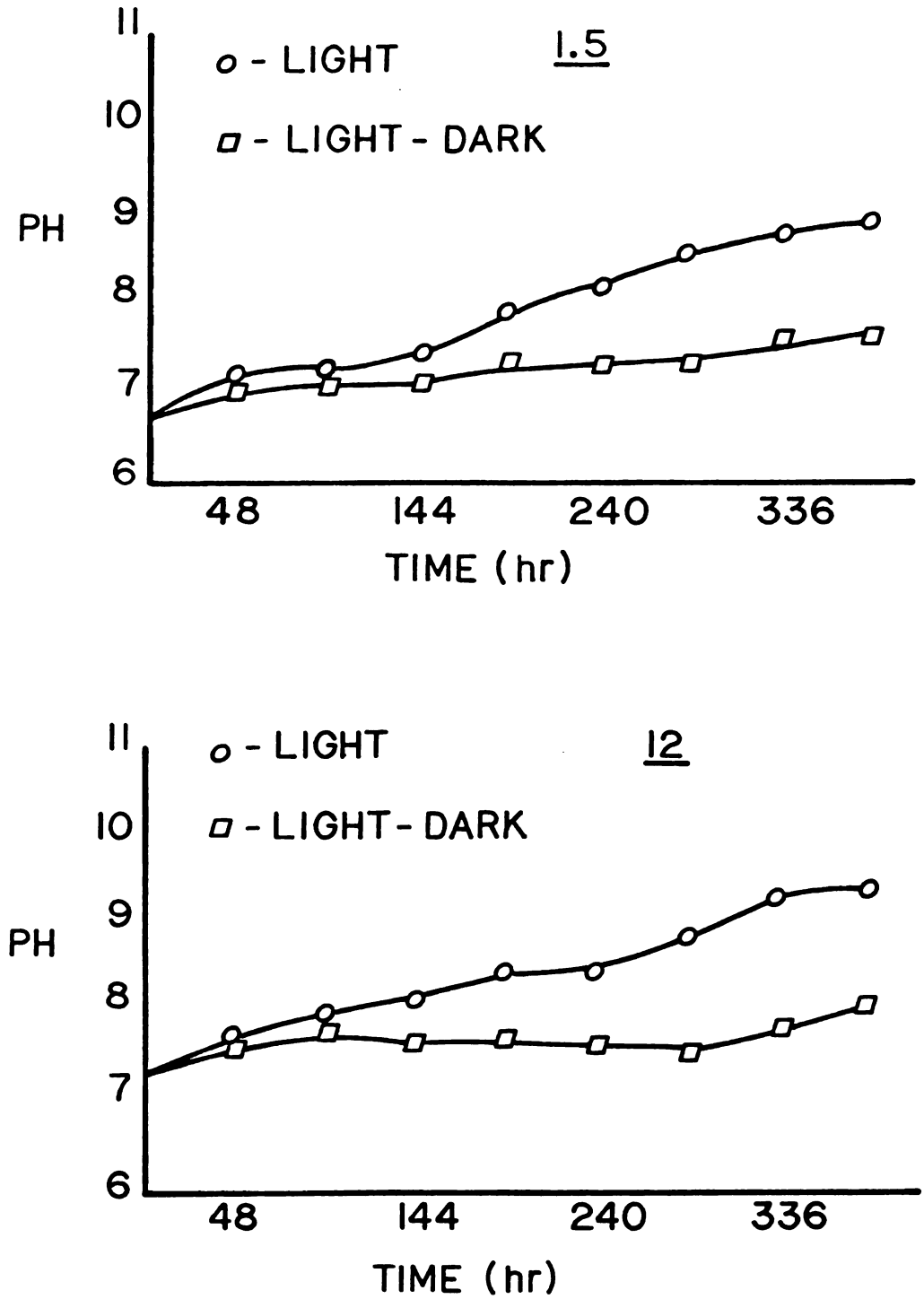


Figure 6. Time related pH response of Chlorella vulgaris at 16 foot candles illumination and varied alkalinity for both light and light-dark microcosms.

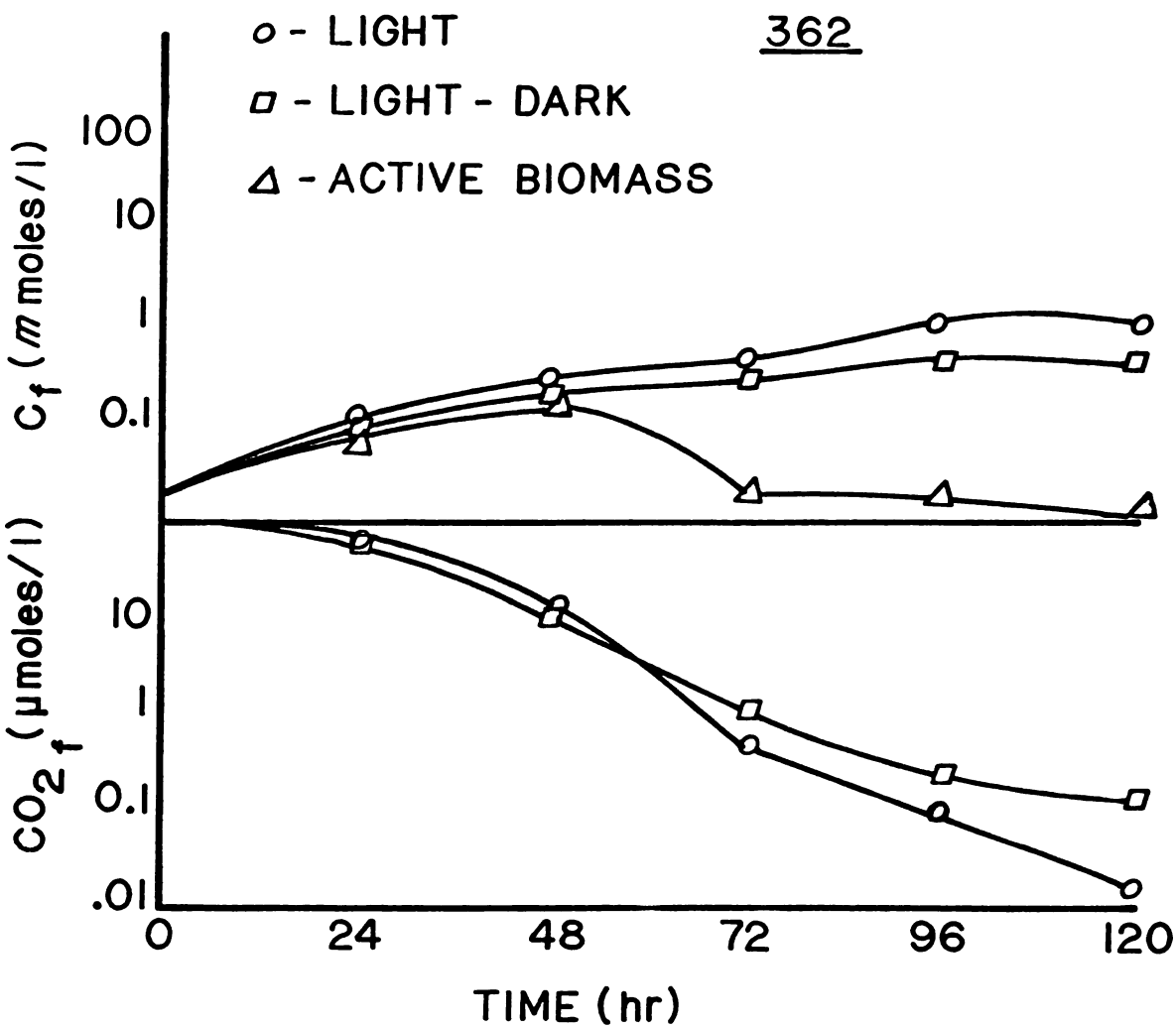


Figure 7. Time related accrual of carbon fixed, active biomass and CO_{2f} quit (Cq) by Chlorella vulgaris at 360 foot candles illumination and 2.0 meq/l alkalinity for both the light and light-dark microcosms.

with 2 meq. alkalinity/l fixed more carbon and attained a higher pH than the microcosm pair with 0.5 meq. alkalinity/l.

Algal growth was measured as carbon fixed and calculated with equations 1 and 2. Carbon fixed by the algae was determined at 24 hour increments over the growth period for microcosms subjected to light intensities of 360 and 240 foot candles and at 48 hour increments for microcosms illuminated at 50 and 16 foot candles. These calculations were made from data obtained from both the light and light-dark microcosms. Figures 7-10 represents the results of these calculations.

The carbon fixed curves in Figures 7-10 illustrate that under all light intensities the algae in the light microcosms fixed more carbon than did the algae in the light-dark microcosms. In addition, these preliminary data indicated, generally, that as illumination decreased CO_{2f} values at which photosynthesis stopped within a particular microcosm increased. This indicated that the concentration of CO_{2f} required for algal photosynthesis is a function of light intensity. At low light intensity, and thus higher required CO_{2f} concentration or carbon dioxide quit (Cq) values, less carbon is available from the alkalinity system and thus less carbon is fixed. This reduction in final biomass concentration caused by decreased light followed the same trends noted by King and King (1974) and Zeisemer (1974).

The difference in productivity within a particular light, light-dark bottle pair demonstrated the effect of the sinking

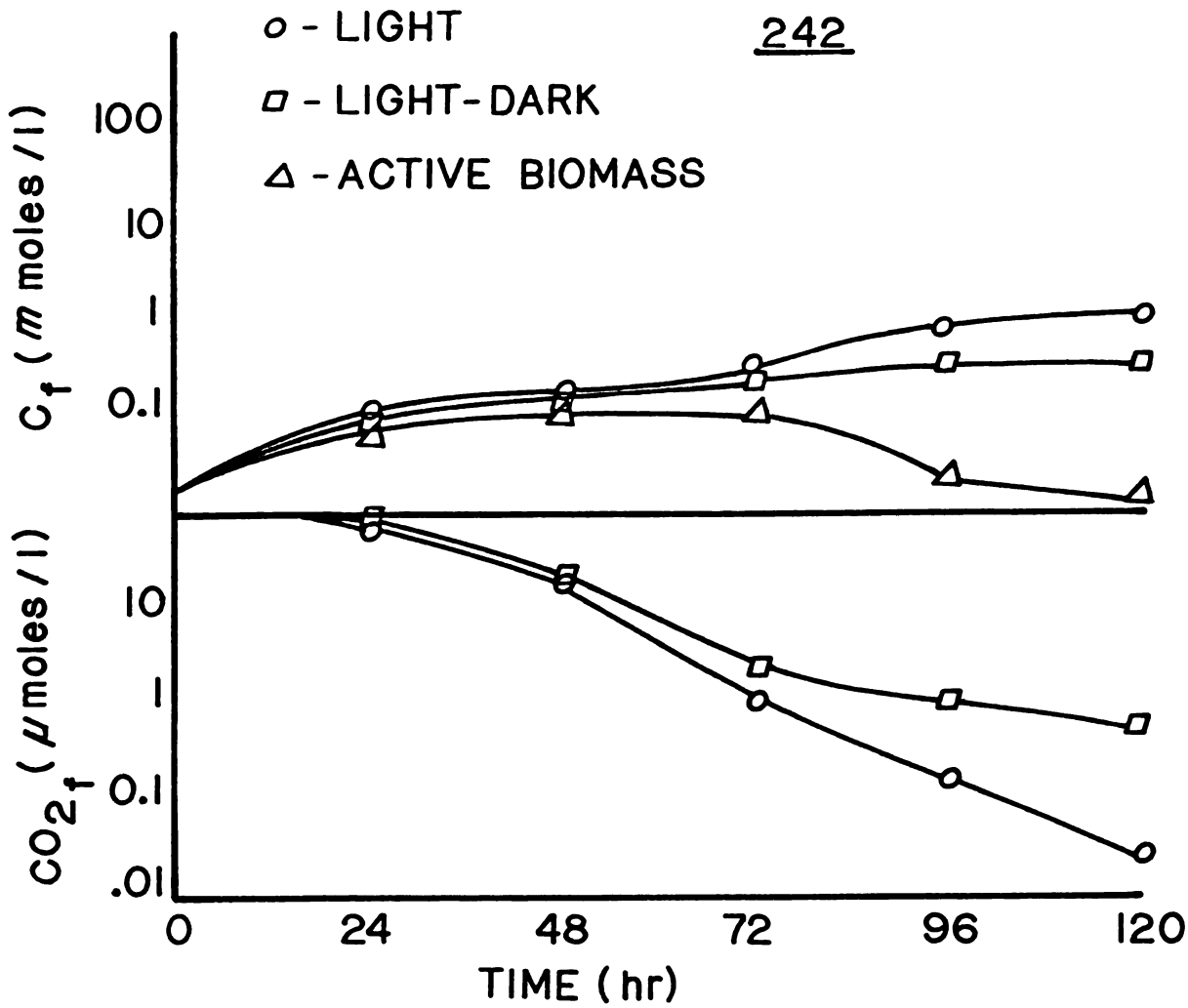


Figure 8. Time related accrual of carbon fixed, active biomass and existing CO_{2_f} concentration for Chlorella vulgaris at 240 foot candles illumination and 2.0 meq/l alkalinity for both the light and light-dark microcosms.

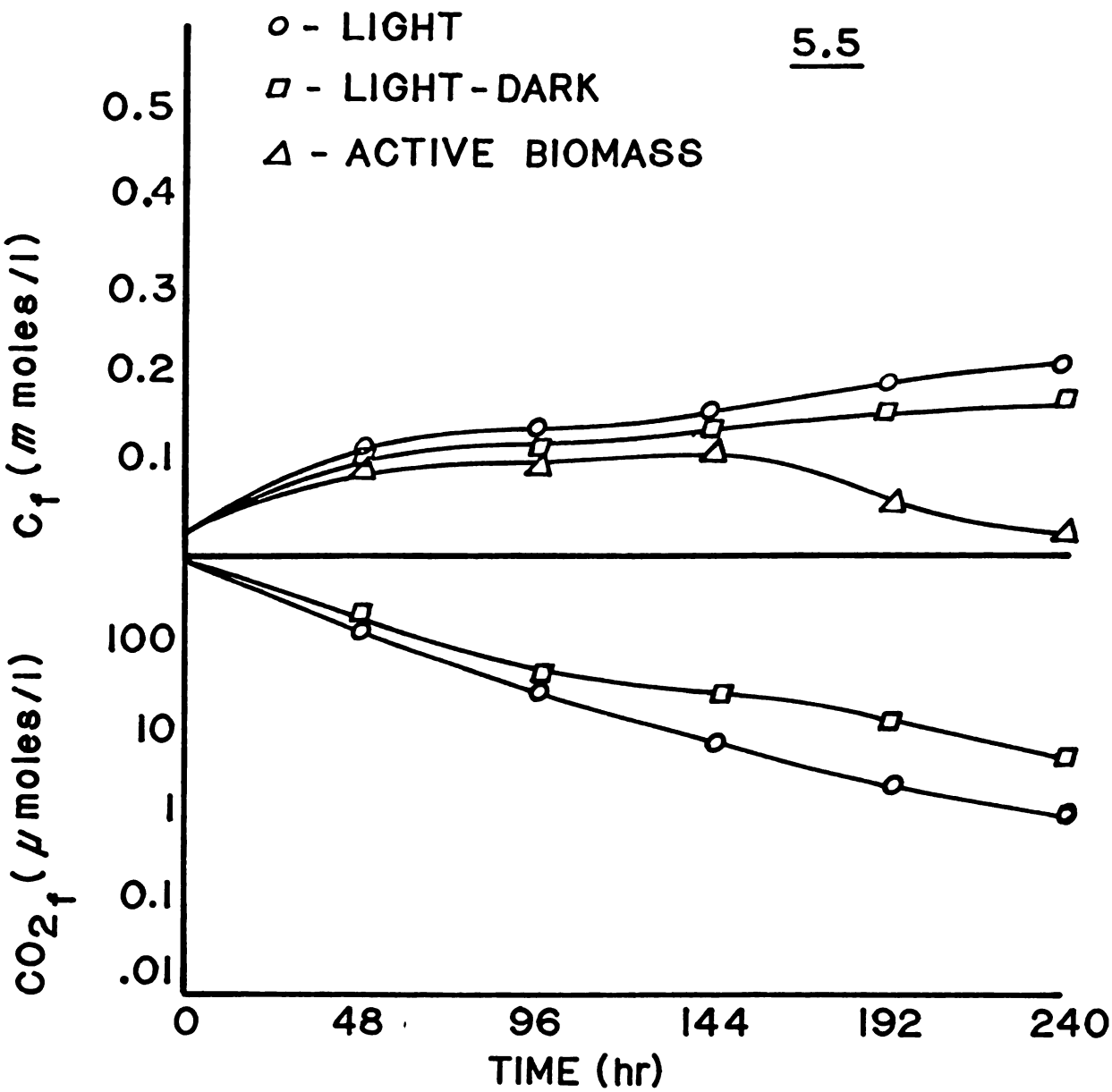


Figure 9. Time related accrual of carbon fixed, active biomass and existing CO_{2_f} concentration for Chlorella vulgaris at 50 foot candles illumination and 0.50 meq/l alkalinity for both the light and light-dark microcosms.

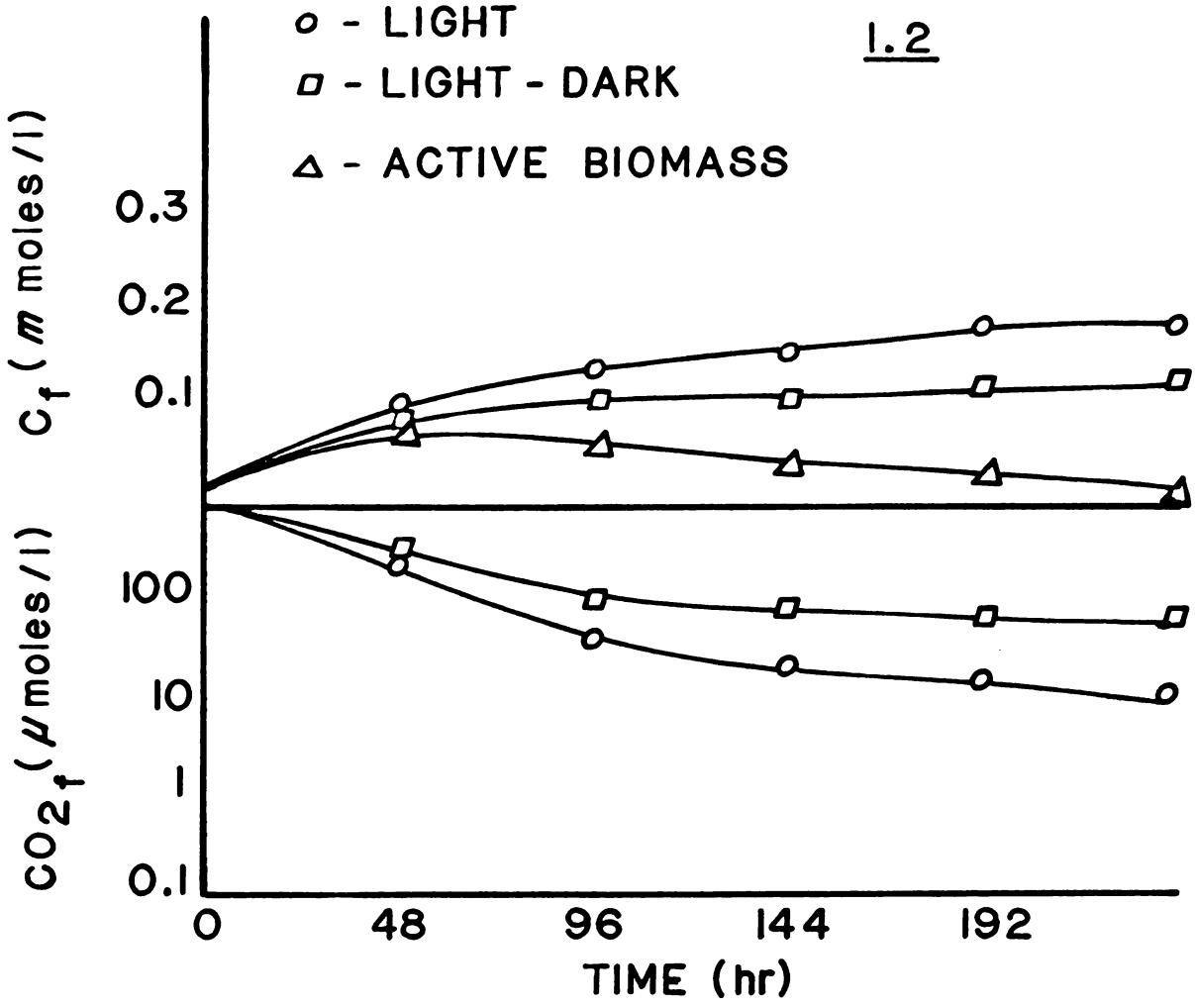


Figure 10. Time related accrual of carbon fixed, active biomass and existing CO_{2_f} concentration for Chlorella vulgaris at 16 foot candles illumination and 2.0 meq/l alkalinity for both the light and light-dark microcosms.

of algae as a parameter affecting overall algal production. All algal cells in the light bottles remained in the photic zone throughout the growth period even when they sunk to the bottom of the microcosm. Therefore, the minimum CO_{2f} in the light microcosm (Cq) is an index to the maximum carbon fixation from the alkalinity for a particular set of experimental conditions. In the light-dark bottles algal sinking removed biomass from the photic zone and photosynthesis stopped when the algae entered the dark portion of the flasks. The minimum CO_{2f} in light-dark bottles reflected the maximum carbon fixation from the alkalinity for an experimental condition in which the algae sunk from the photic zone.

Active biomass or planktonic biomass values were calculated for the light-dark microcosms using equation 6. The active biomass curves are presented in Figures 7-10. Comparison of active biomass curves to total carbon fixed curves for light-dark microcosms further illustrates the effect of algal sinking on overall algal production. The difference between the active biomass curve and the total carbon fixed in the light-dark macrocosms is biomass lost to the dark portion of the bottle.

Algal Growth

The specific growth rate (μg) values were calculated for the light bottles with equation 5 and are presented in Figure 11 as a function of CO_{2f}. A comparison of these specific growth rate curves for the light bottle systems indicated an interrelationship between CO_{2f} and light.

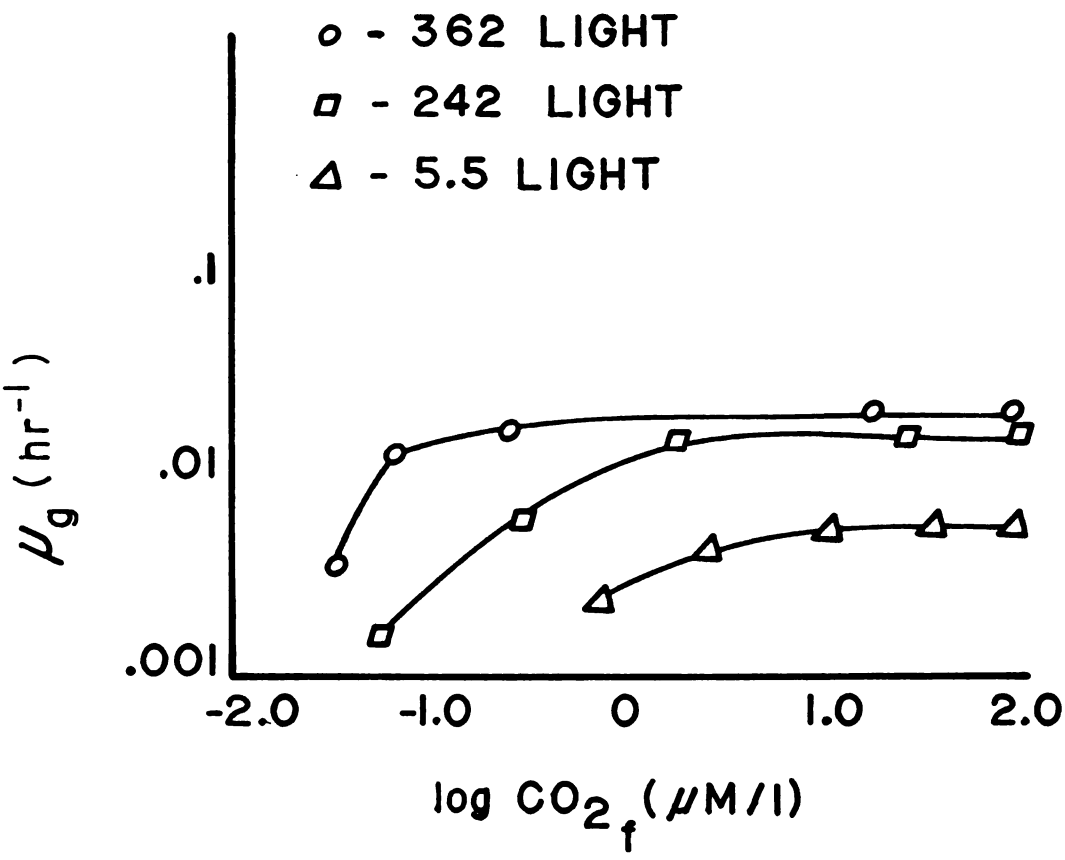


Figure 11. Variation in specific growth rate with free carbon dioxide for Chlorella vulgaris in light microcosms.

The specific growth rate at any CO_{2f} concentration was not constant but decreased markedly with decreasing light intensity. While the CO_{2f} threshold concentration (Cq) increased significantly with decreased light intensity.

Algal Sinking

In Figure 12 specific sink rate (μs) (calculated from equation 9) and specific growth rate (μg) are plotted as a function of time. Clearly, as Zeisemer (1974) reported, under those conditions where μs < μg the system is accumulating biomass and μab is positive. When μs > μg the system is losing biomass to the non-photic bottom of the light-dark microcosm faster than it is being replaced and μab is negative. When μg = μs the system is at equilibrium and the biomass being produced equals that sinking.

In Figure 13 specific sink rate (μs), specific growth rate (μg) and specific biomass accrual rate (μab) are plotted as functions of CO_{2f} for a culture grown under 360 foot candles and an alkalinity of 2 meq/l. From Figure 13 it can be seen that as CO_{2f} decreased due to algal photosynthetic removal, the specific growth rate (μg) decreased while the specific sink rate (μs) increased until they were equal, yielding a μab of zero at a CO_{2f} concentration of approximately 0.01 μ M CO_2/l. Therefore, it can be seen that at low carbon values the rate of decline of planktonic biomass increased markedly.

The data derived from the first phase of the investigation corresponds well with results reported by Zeisemer (1974)

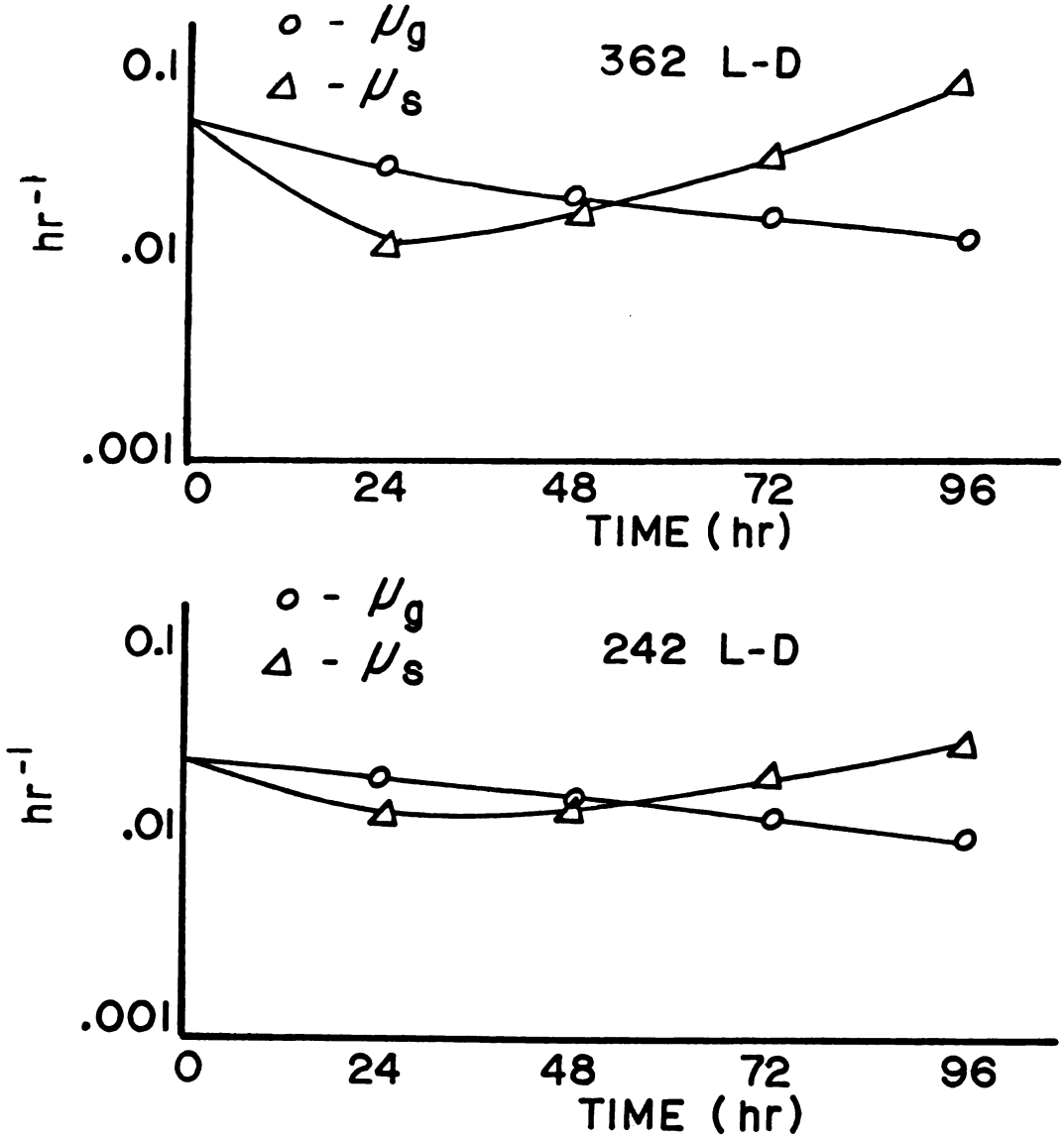


Figure 12. A comparison of specific growth rate to specific sink rate on a time basis for Chlorella vulgaris at 360 and 240 foot candles illumination and 2.0 meq/l alkalinity in the light-dark microcosms.

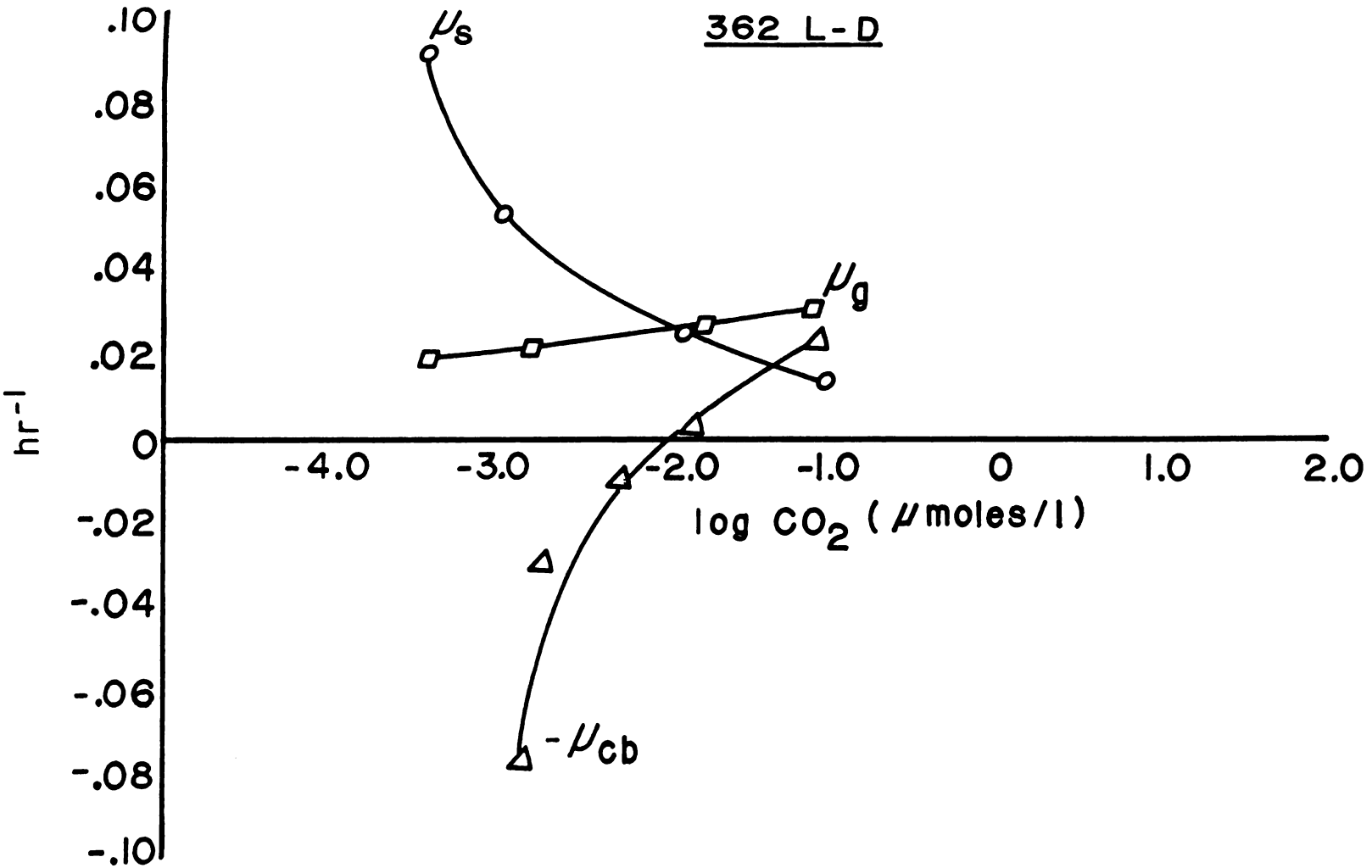


Figure 13. Variation in specific growth rate, specific sink rate and specific active biomass accrual rate with free carbon dioxide for Chlorella vulgaris at 360 foot candles illumination and 2.0 meq/l alkalinity in the light-dark microcosm.

primarily in that as limiting parameters were altered, the μg/μs ratio experienced a corresponding alteration. A reciprocal relationship was seen to exist between μg and μs and thus at a constant light intensity as carbon became limiting μg decreased and μs increased and μab decreased.

Experiment 2

Experimental Conditions

The experimental errors and difficulties discussed in part one of this investigation were overcome in the second phase with several modifications of the method. The first alteration was the selection of one alkalinity of approximately 2 meg/l for all the microcosms. This provided duplicate pairs of light and light-dark microcosms for each light intensity. Table 3 shows the nomenclature and experimental lattice used in the second part of this investigation.

A second modification in the method was the use of 12 inch hypodermic needles to remove samples from the microcosms. These needles served a two fold purpose. Over the period of the study water levels were drawn down in the microcosms as samples were removed such that near the end of the sampling period it was difficult to remove samples with 3 inch cannulas. The 12 inch needle eliminated this problem by reaching nearly to the bottom of the light bottles. A second, and most important, feature of the long cannulus was that they extended to the top of the dark portion of the light-dark bottles which made it possible to scrape the sides of the microcosms. This scraping combined with a gentle swirling technique prevented periphytic growth in all of the light-dark microcosms.

Table 3. Experimental lattice, coding procedure and initial data for the second phase of the investigation.

Microcosm*	Alkalinity (meq/l)	Light Intensity (Ft. Cd.)	Biomass Seed (m moles C/l)
360 A L	2.04	360	0.015
360 A L-D	2.04	360	0.015
360 B L	2.04	360	0.015
360 B L-D	2.04	360	0.015
240 A L	2.04	240	0.015
240 A L-D	2.04	240	0.015
240 B L	2.04	240	0.015
240 B L-D	2.04	240	0.015
50 A L	2.04	50	0.015
50 A L-D	2.04	50	0.015
50 B L	2.04	50	0.015
50 B L-D	2.04	50	0.015
16 A L	2.04	16	0.015
16 A L-D	2.04	16	0.015
16 B L	2.04	16	0.015
16 B L-D	2.04	16	0.015

*The two or three digits assigned to each microcosm indicates the light intensity. The A or B distinguishes duplicate microcosms and L or L-D indicates light or light-dark bottles respectively.

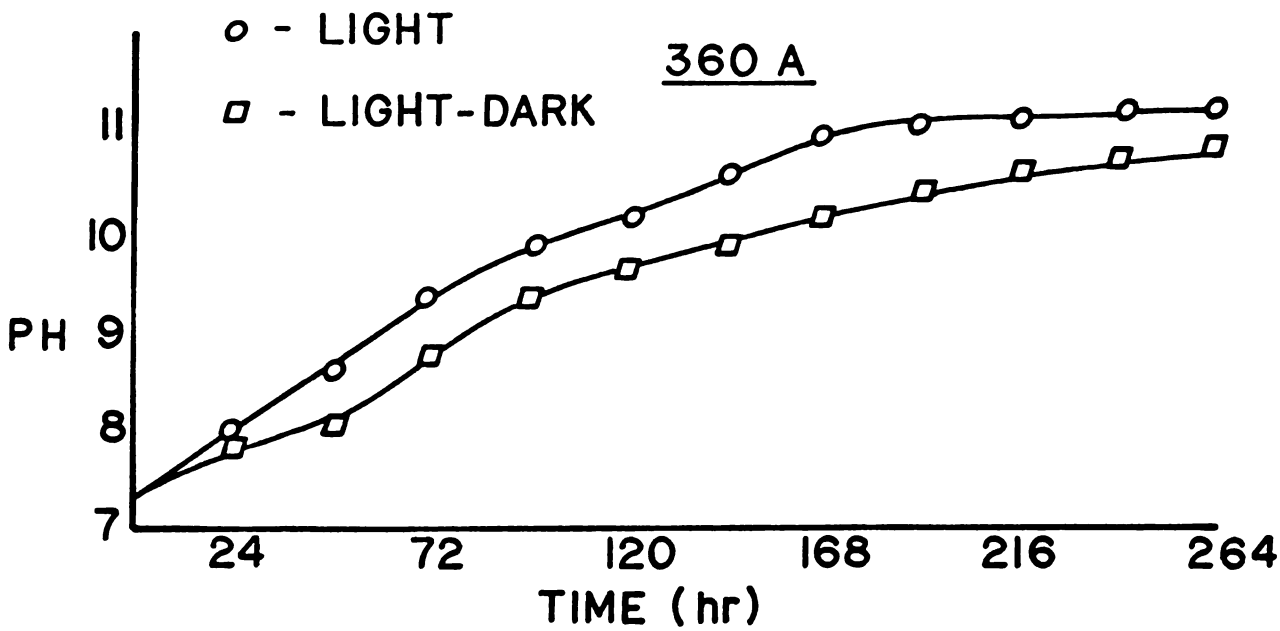
As a result of the large initial biomass seed (.03 mMC/l) introduced into each microcosm in part one, the growth rate was rapid and too few data points were obtained. In the second part of the study the initial biomass seed was cut in half and this produced more than twice as many data points, particularly in the microcosms subjected to the higher light intensities.

Overhead laboratory lights were removed from the proximity of the microcosms. This insured that no extraneous light sources influenced algal response. The same light intensities as used in part one were used in the second phase of this investigation.

pH and Carbon Fixation

The effects of a reduced biomass seed and duplicate microcosms are reflected in the pH curves shown in Figures 14-17. More data points were obtained from the microcosms in the second phase as a result of halving the biomass seed. Duplicate microcosms provided replicate pH data which permitted valid comparisons between microcosms subjected to different light intensities as well as indicating the reliability of the data. The algae in the light-dark microcosms subjected to a light intensity of 16 foot candles could not sustain a steady pH rise under such reduced light conditions and only the first two data points were used in further calculations.

Carbon fixed and active biomass values for the light and light-dark bottle pairs are presented in Figures 18-23. As in part one of this study, the sum of the carbon fixed was determined at 24 hour increments for microcosms subjected to a light



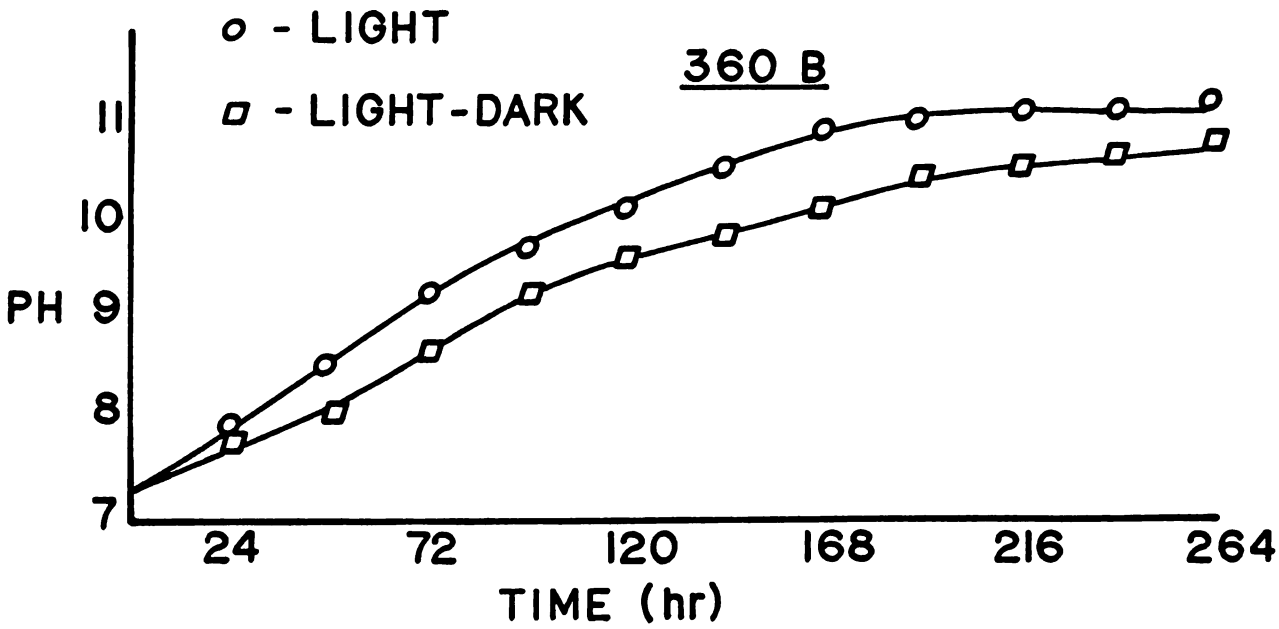


Figure 14. Time related pH response of Chlorella vulgaris at 360 foot candle illumination for duplicate light and light-dark microcosms.

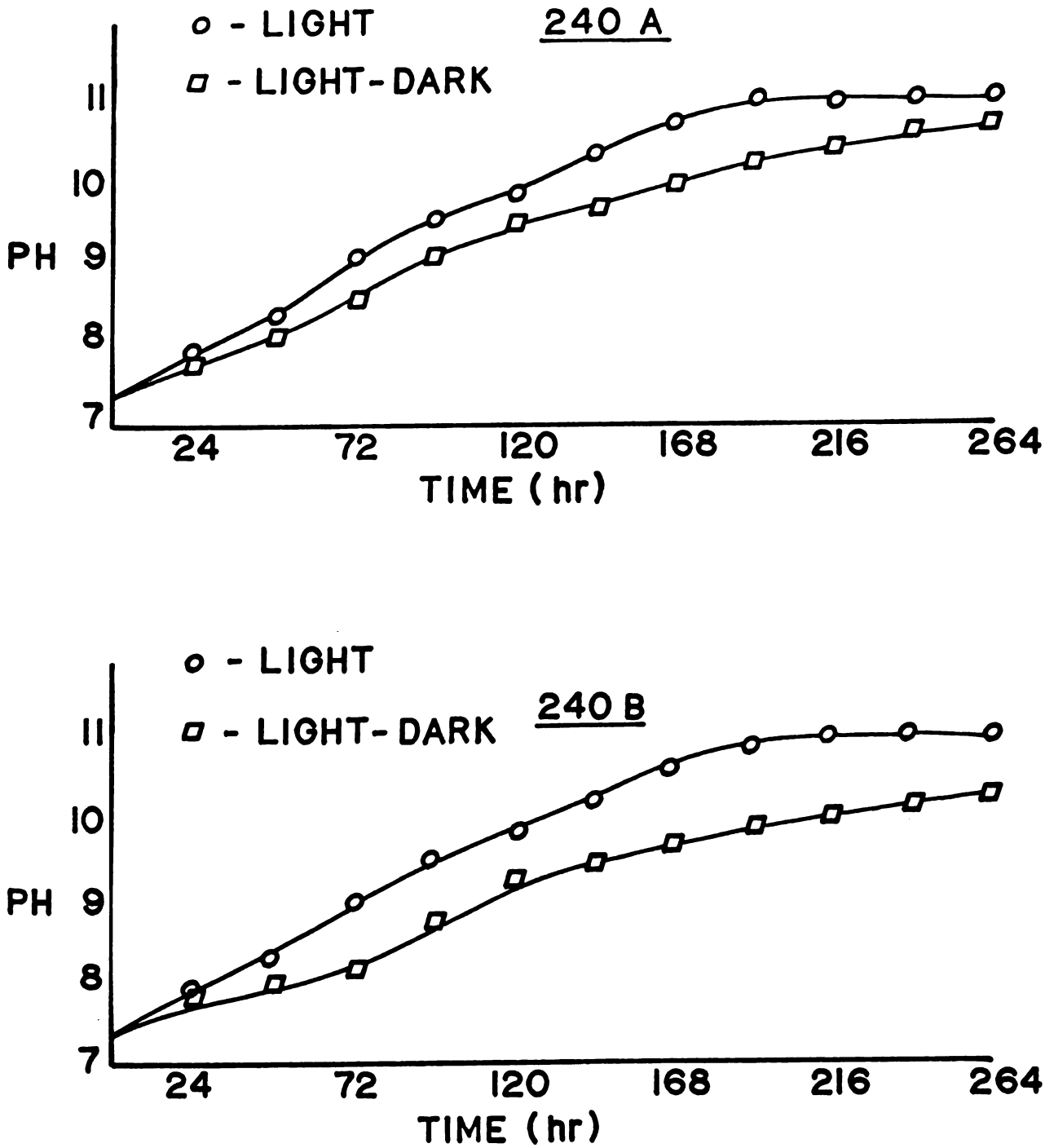
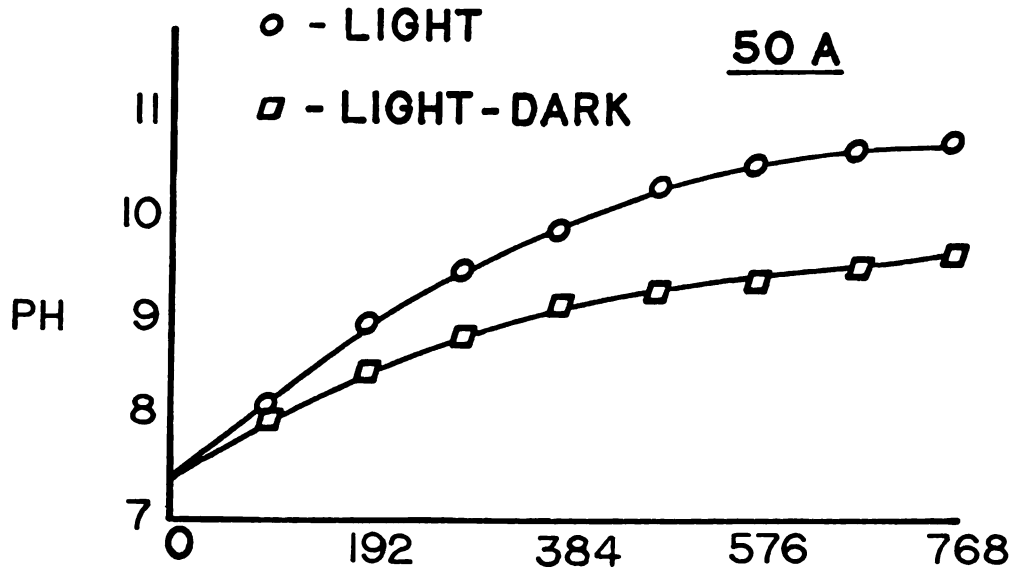


Figure 15. Time related pH response of Chlorella vulgaris at 240 foot candles illumination for duplicate light and light-dark microcosms.



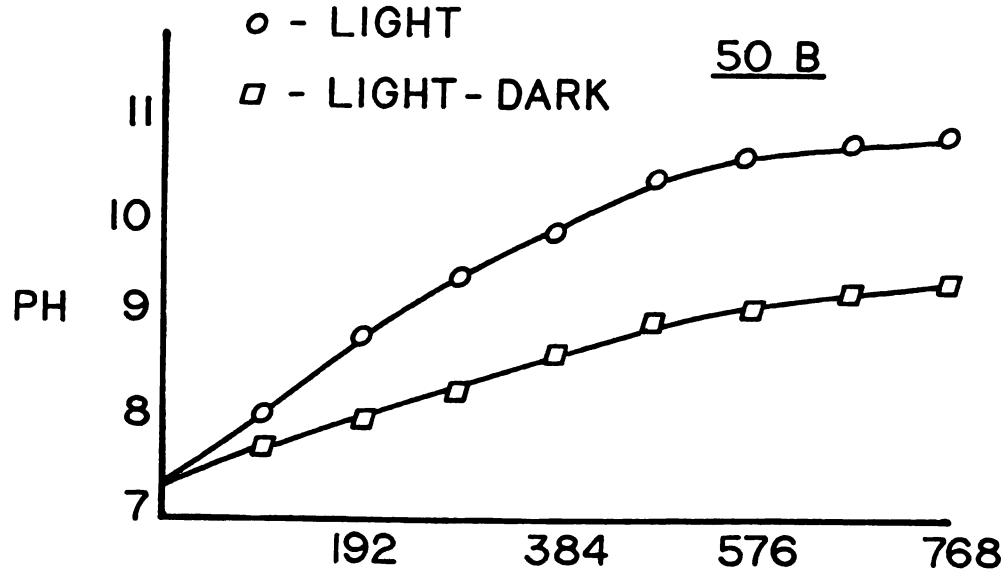
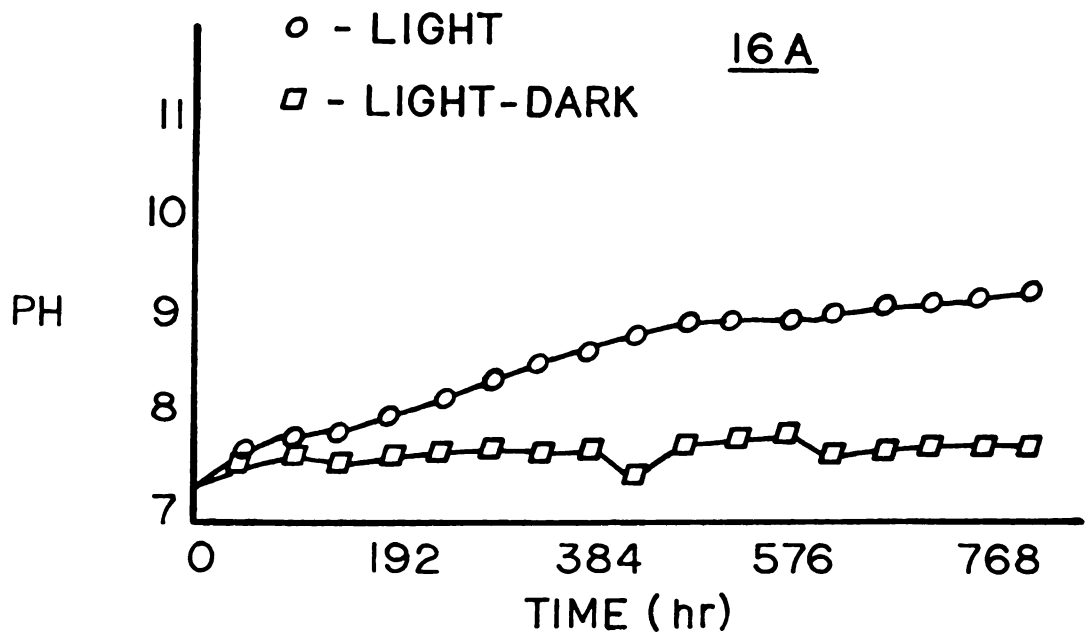


Figure 16. Time related pH response of Chlorella vulgaris at 50 foot candles illumination for duplicate light and light-dark microcosms.



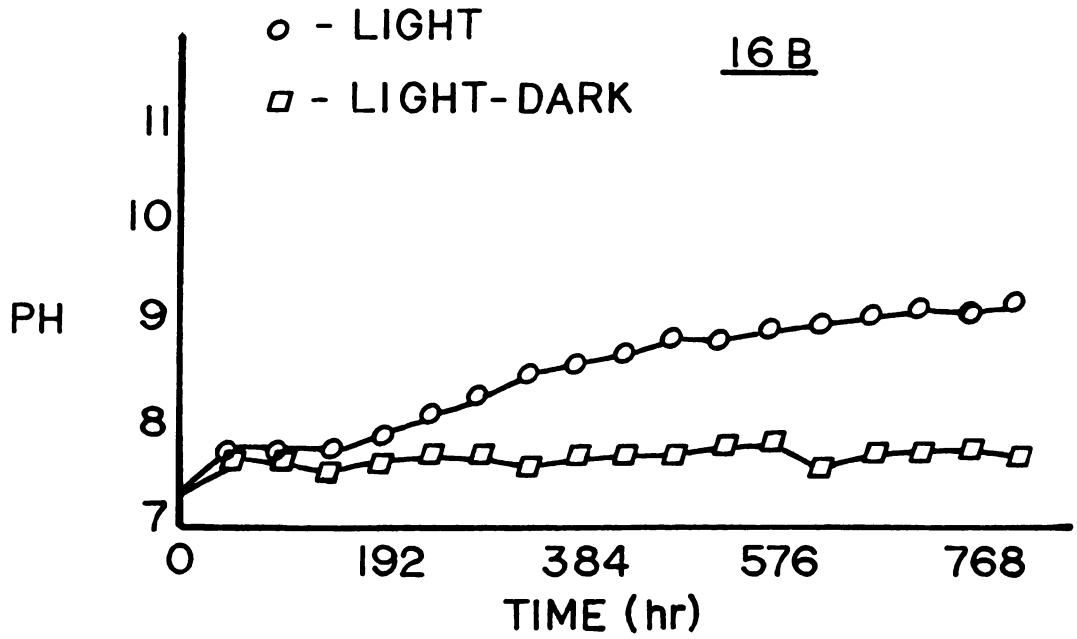


Figure 17. Time related pH response of Chlorella vulgaris at 16 foot candles illumination for duplicate light and light-dark microcosms.

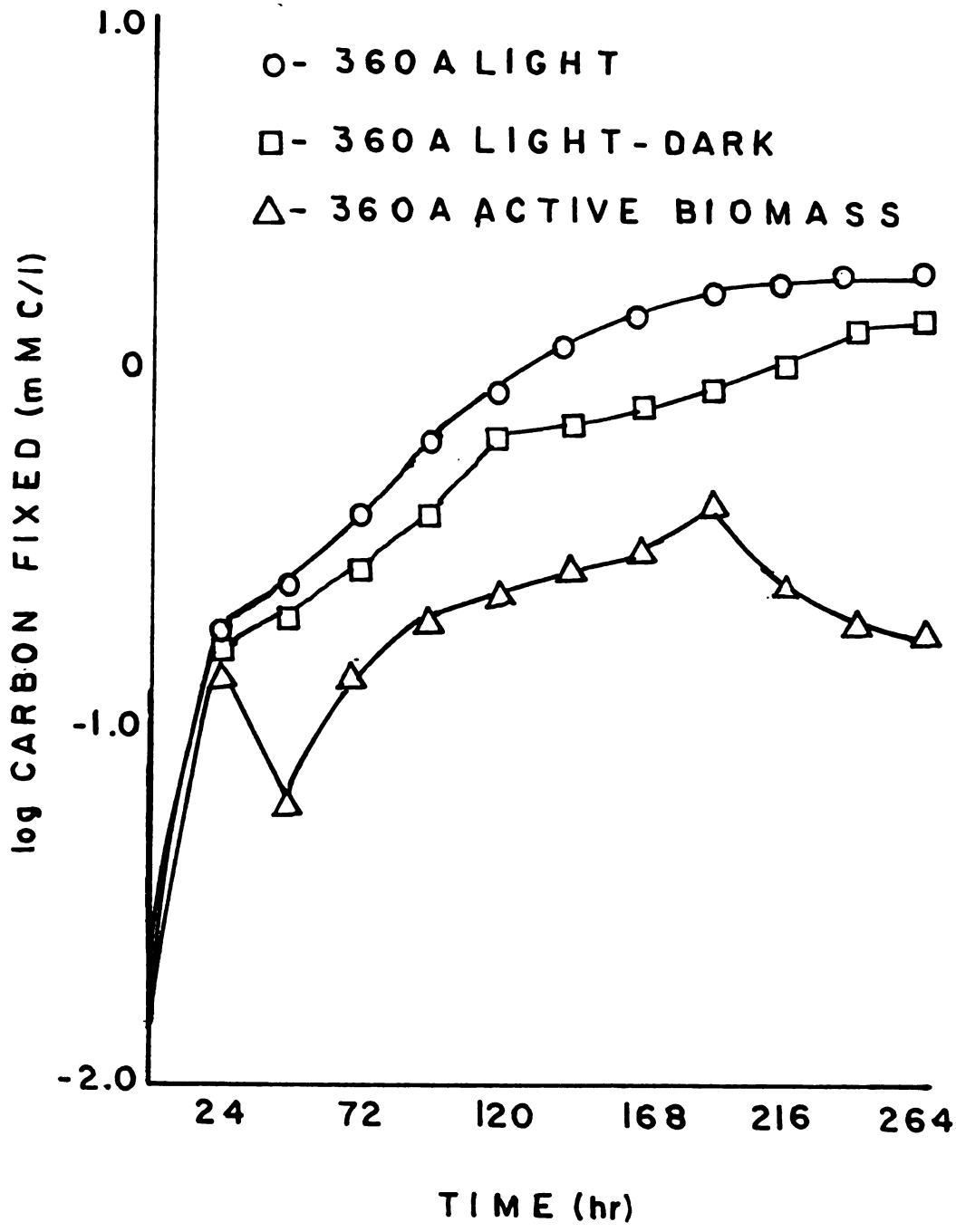


Figure 18. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 360 foot candles illumination for both the A light and light-dark microcosms.

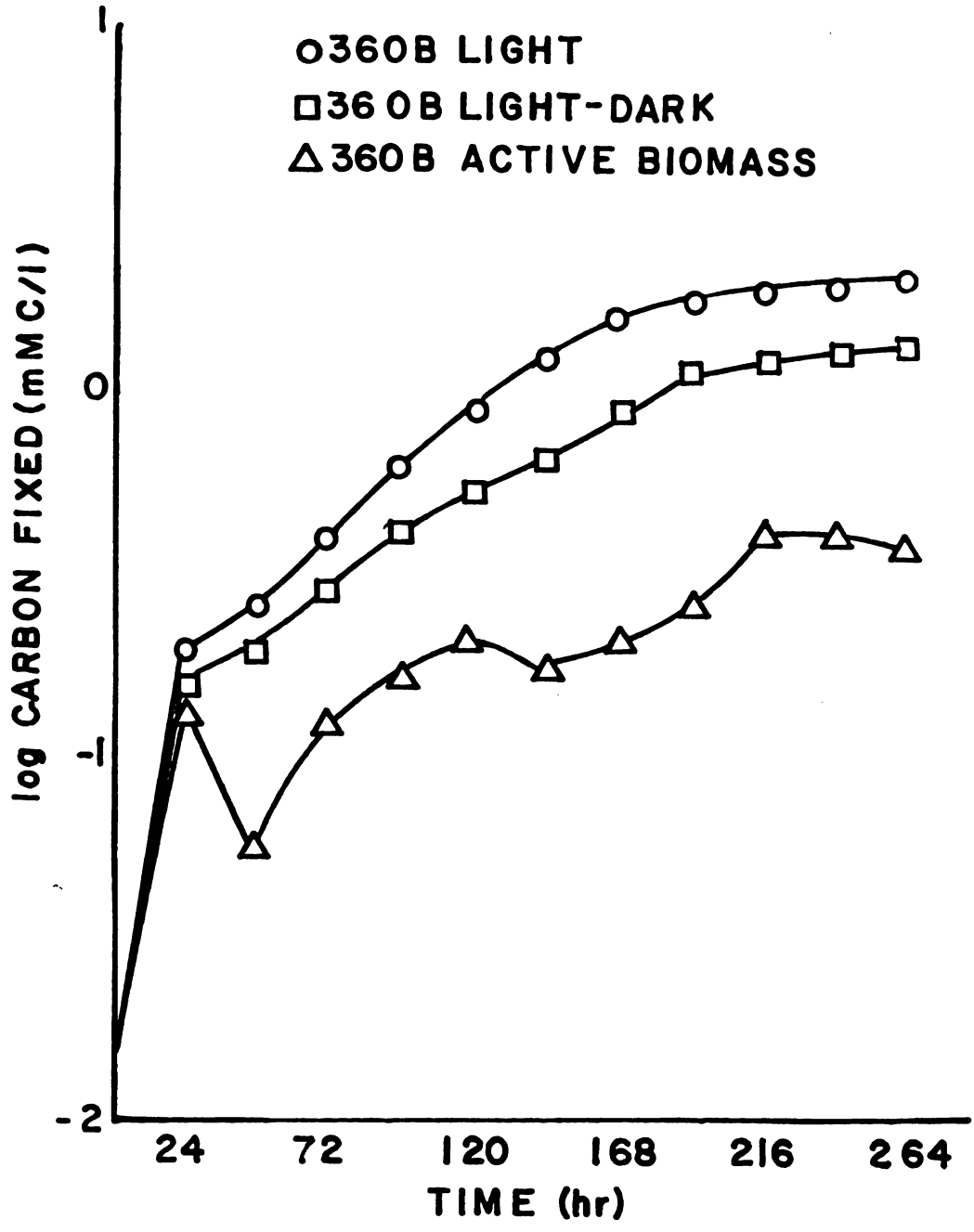


Figure 19. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 360 foot candles illumination for both the B light and light-dark microcosms.

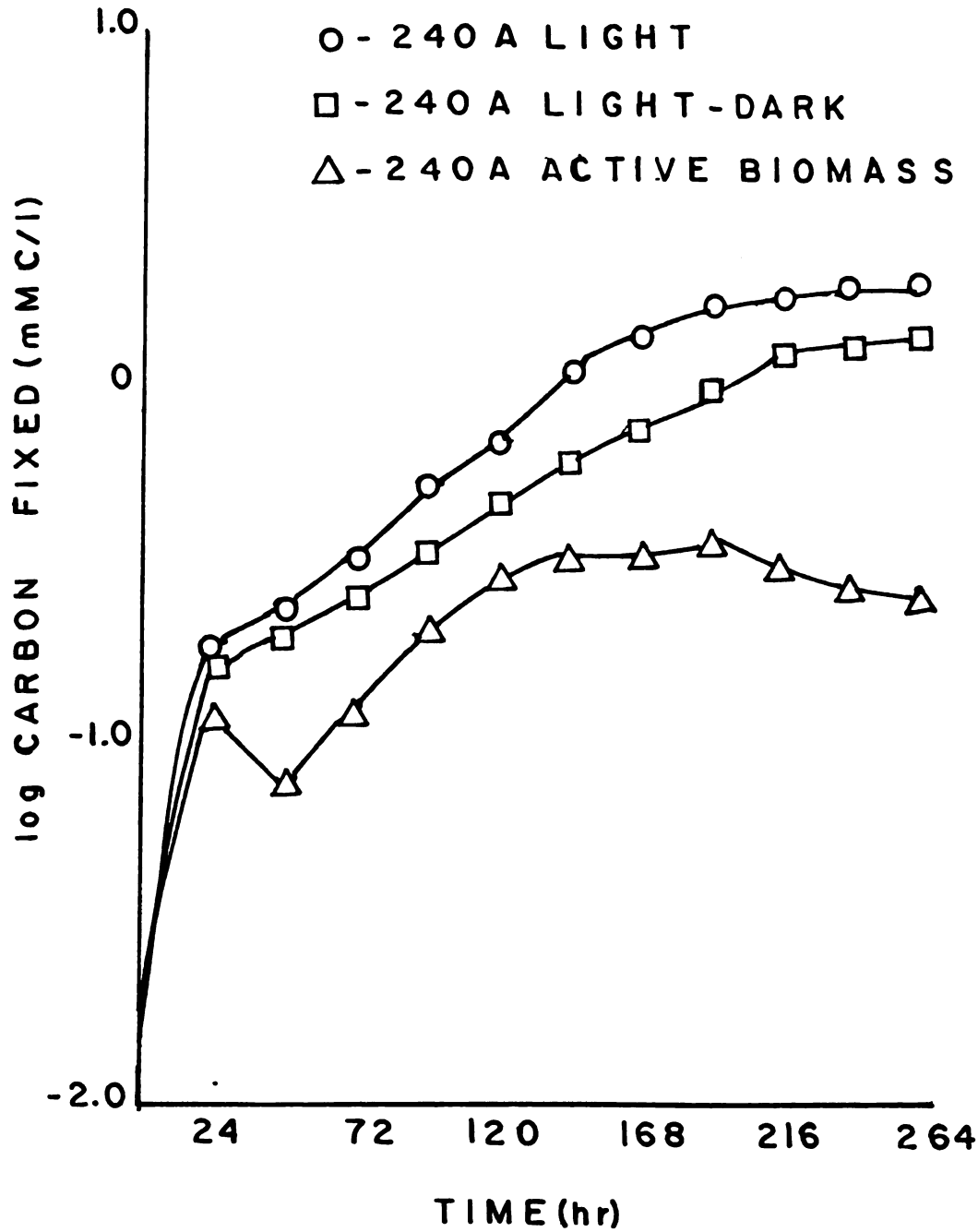


Figure 20. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 240 foot candles illumination for both the A light and light-dark microcosms.

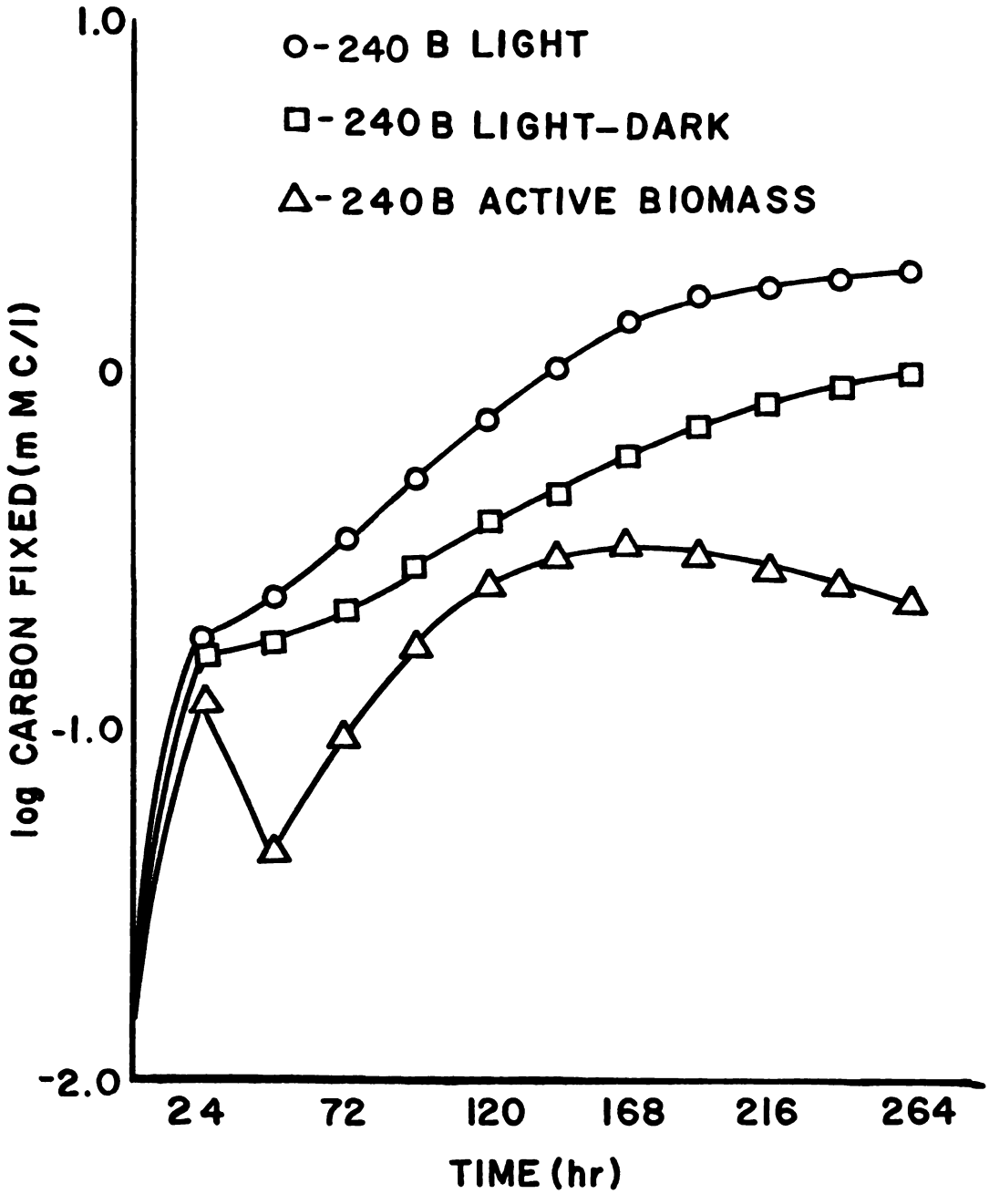


Figure 21. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 240 foot candles illumination for both the B light and light-dark microcosms.

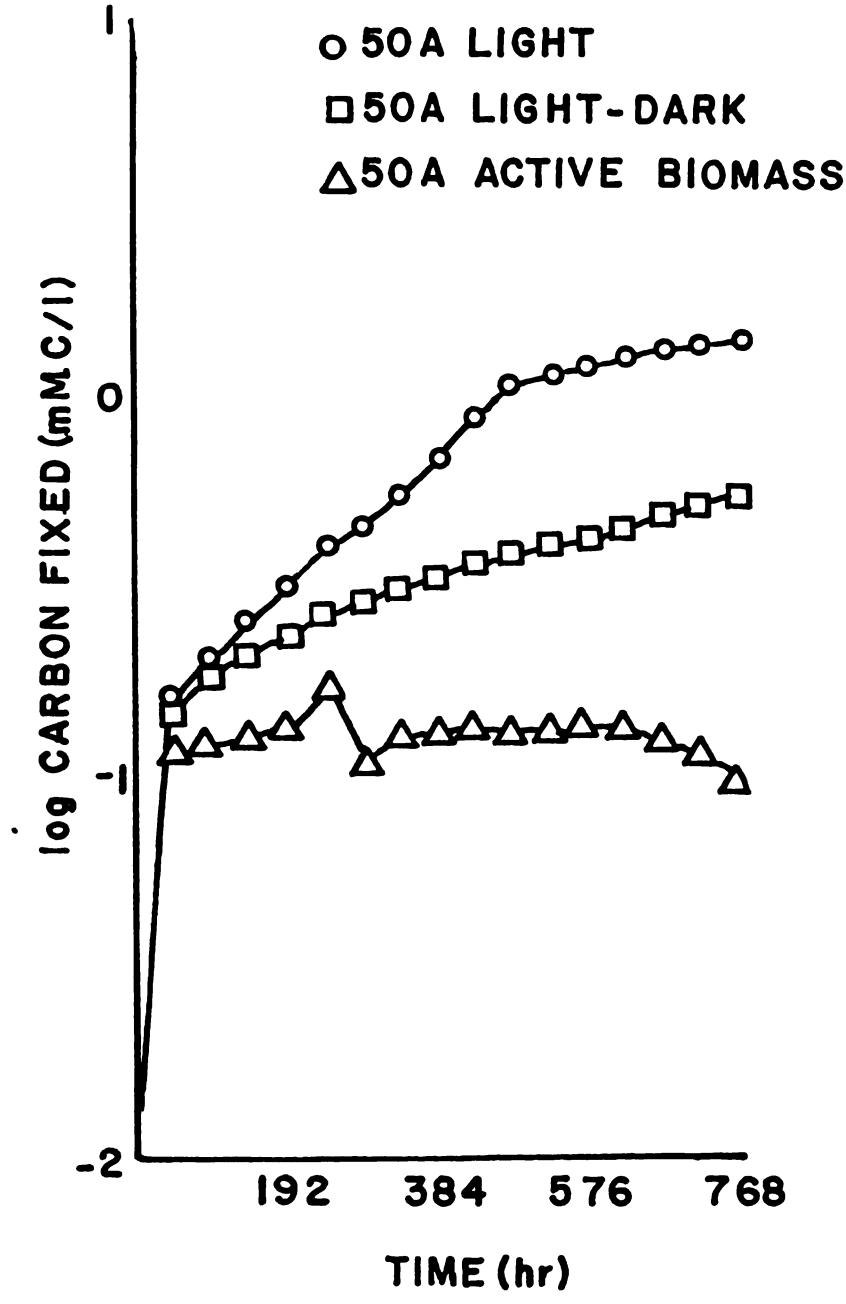


Figure 22. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 50 foot candles illumination for both the A light and light-dark microcosms.

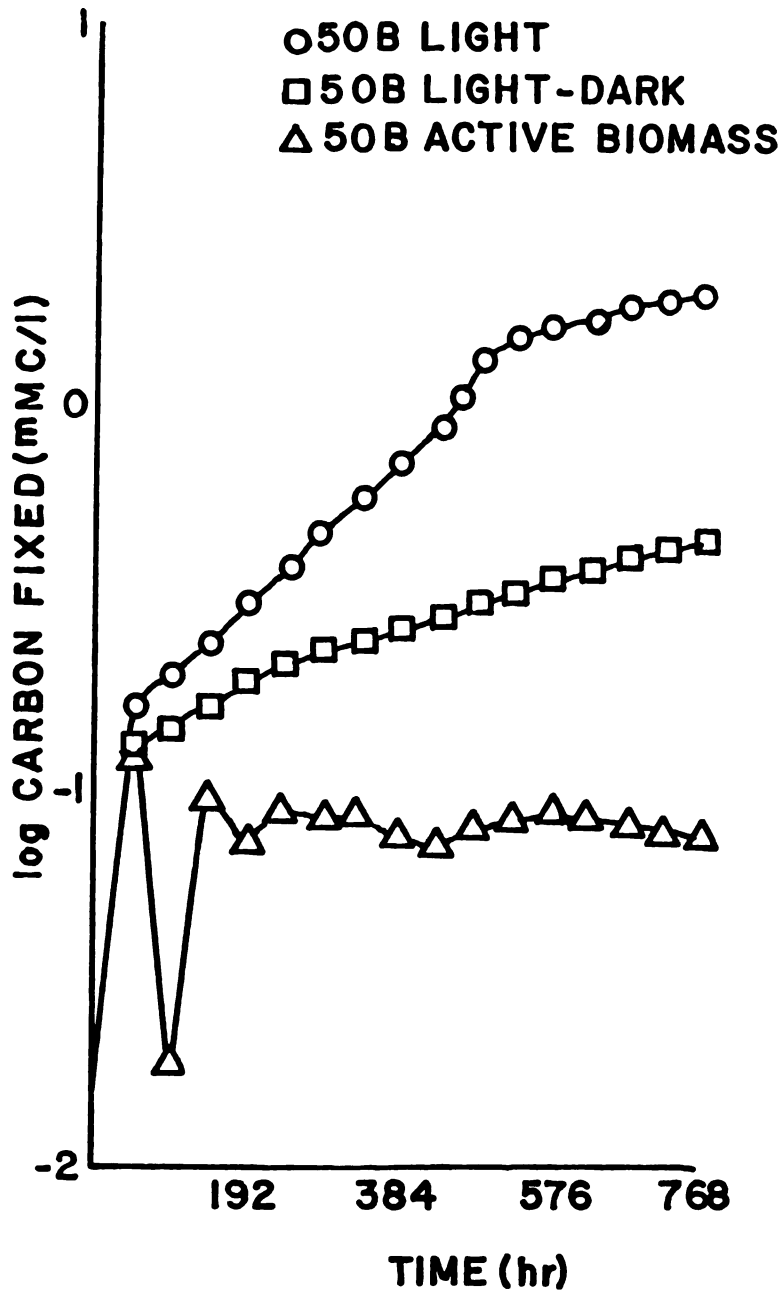


Figure 23. Time related accrual of carbon fixed and active biomass by Chlorella vulgaris at 50 foot candles illumination for both the B light and light-dark microcosms.

intensity of 360 foot candles and at 48 hour increments for microcosms under 50 and 16 foot candles. Carbon fixed was calculated with equations 1 and 2 and active biomass values were calculated with equation 6.

From Figures 18-23 it can be seen that carbon fixation in the light microcosms represented the total carbon fixed from the alkalinity under a particular light condition or the physiological maximum for Chlorella. The effect of Chlorella's specific sink rate (μs) on overall productivity is the difference between the carbon fixed curve in the light microcosm and the total carbon fixed curve from a light-dark microcosm. During the initial growth period, the active biomass approached that of the total carbon fixed and the specific sink rate (μs) was very low. This situation was temporary and lasted only as long as carbon limitations were of marginal importance. However, as the carbon limits became more important the algae became stressed and the percent that the active biomass comprised of the total biomass began to decline, indicating an increasing sink rate.

Algal Growth

Figure 24 is a plot of alga specific growth rates (μg) against CO_{2f} concentrations for the light microcosms. The most obvious trend in this figure is that μg decreased markedly with reduced light and there were three orders of magnitude difference in the maximum observed specific growth rates for algal cultures subjected to a light intensity of 360 foot candles and the cultures grown under a light intensity of 16 foot candles.

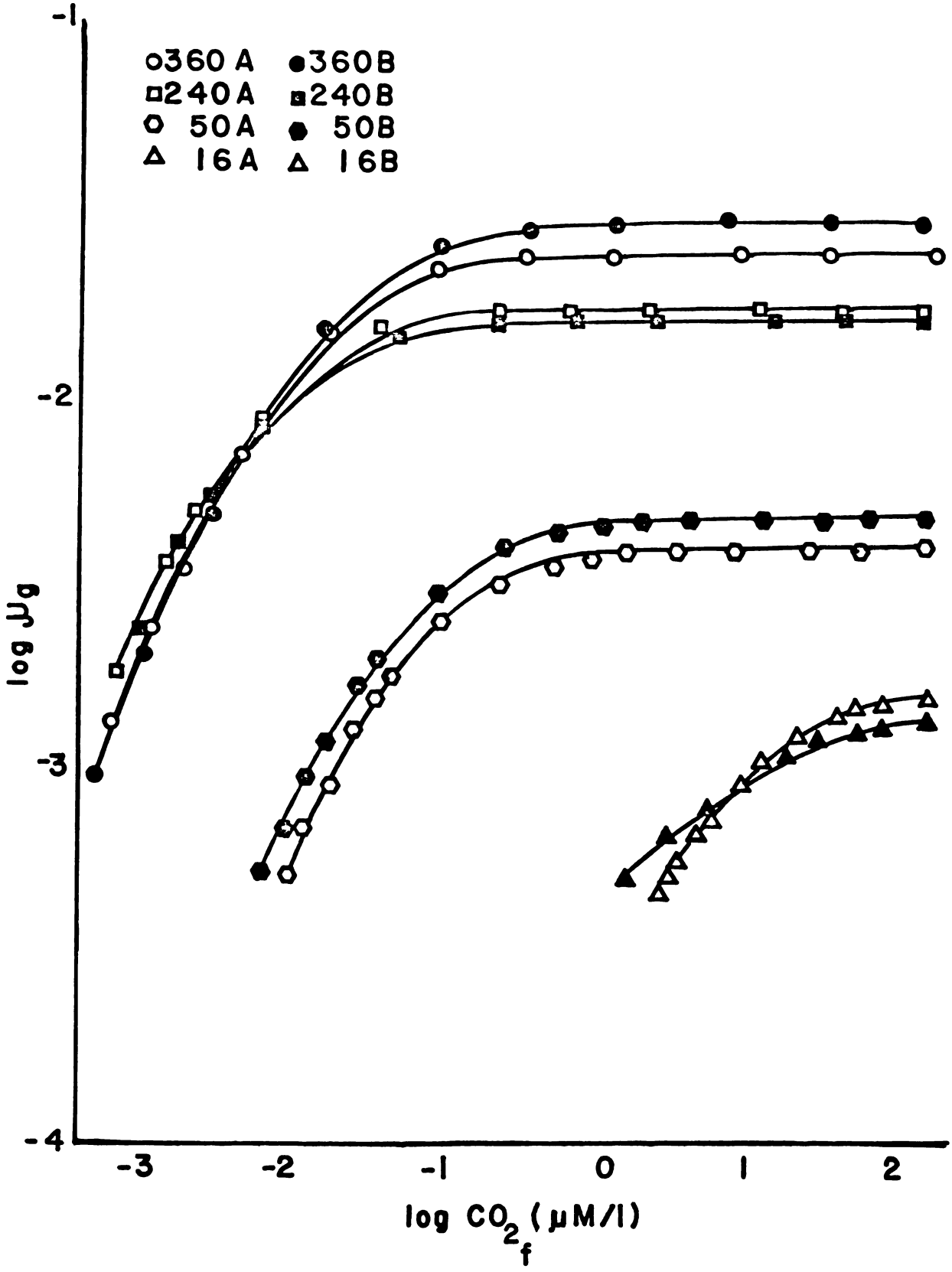


Figure 24. Variation in specific growth rate as a function of free carbon dioxide for Chlorella vulgaris in duplicate light microcosms.

It can also be seen from Figure 24 that as light intensity was reduced the carbon quit (Cq) value increased. This would suggest that the Cq value represents a threshold concentration of carbon dioxide for Chlorella. Below this carbon concentration, at a given light, the algae cannot survive. For instance, the algae subjected to 16 foot candles light intensity could not continue photosynthesis at CO_{2f} concentrations below approximately 2.50 μ M CO_2/l while algae grown under 360 foot candles light intensity continued photosynthesis to about 0.0008 μ M CO_2/l. Therefore, it can be seen that carbon and light interact to control both rate and extent of growth of Chlorella.

King and King (1974) investigated the interactions imposed on the specific growth rate of algae by simultaneous limitation of carbon availability and light intensity and approximated this multiplicative relation by the equation

$$\mu g = \mu max \left(\frac{C}{K_c + C} \right) \left(\frac{L}{K_L + L} \right) \qquad (11)$$

Where: μg = Specific growth rate, hr^{-1}

μmax = Maximum specific growth rate, hr^{-1}

C = Free CO_2 concentration, μ M/l

K_c = Half saturation free CO_2 concentration, μ M/l

L = Light intensity, ft. cd.

K_L = Half saturation light intensity, ft. cd.

This equation is only a first approximation and the more extensive light data of this investigation indicated a linear association between maximum specific growth rate (μ max) and light intensity over the range of light intensities considered (Figure 25). The linear regression calculations for Figure 25 resulted in an equation that described μ max at any light intensity.

$$\mu max = a + bL \qquad (12)$$

Where: μmax = Maximum specific growth rate, hr^{-1}

a = Intercept = 7.11×10^{-5}

b = Slope = 4.66×10^{-4}

L = Light intensity, ft. cd.

This equation was then substituted into the Monod formulation to yield.

$$\mu g = a + bL \left(\frac{C}{K_c + C} \right) \qquad (13)$$

Equation 13 describes the multiplicative interaction of carbon and light in limiting growth when Cq is not considered. However, both Cq and K_c varied as a function of light intensity as seen in Figure 26. This figure is a semi-log plot of Cq and K_c values for each light bottle pair against light intensity. Cq and K_c decreased in a parabolic fashion as light intensity increased. This relationship was resolved with a parabolic curve regression in which $y = a_o + a_1 x + a_2 x^2$ and the a's are

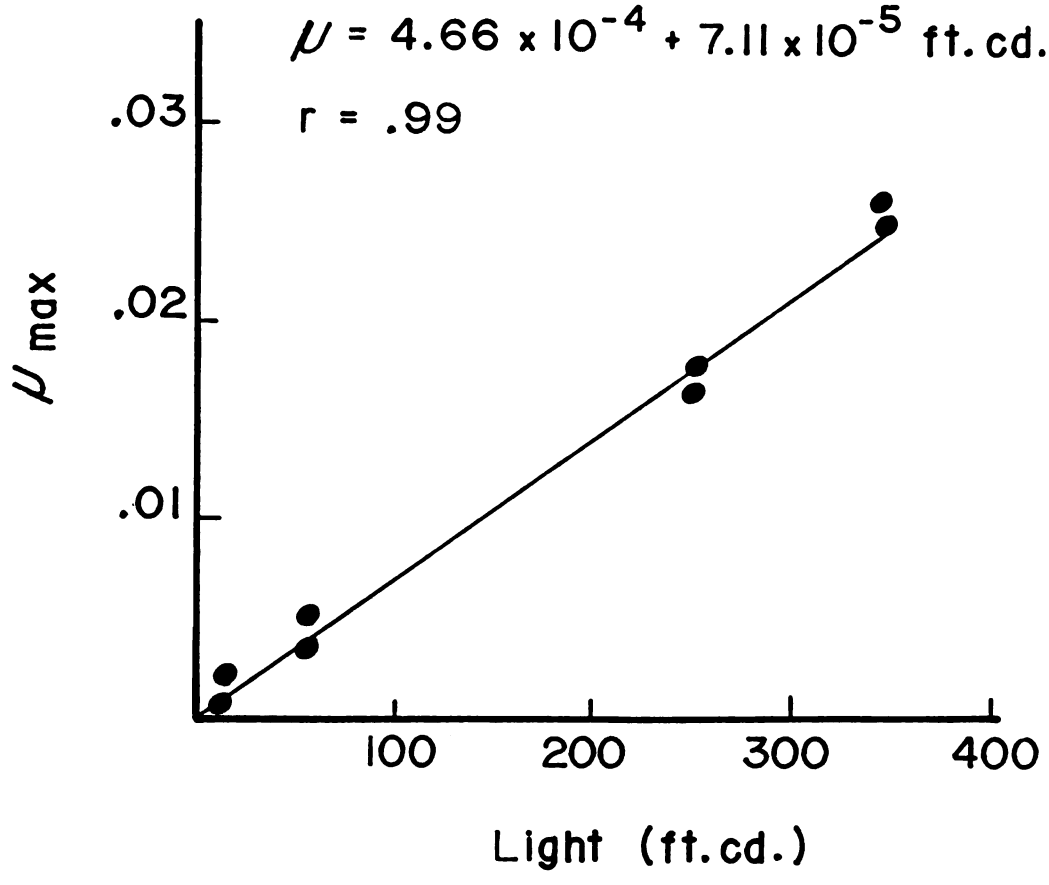


Figure 25. Variation in maximum specific growth rate as a function of light intensity for Chlorella vulgaris in light microcosms.

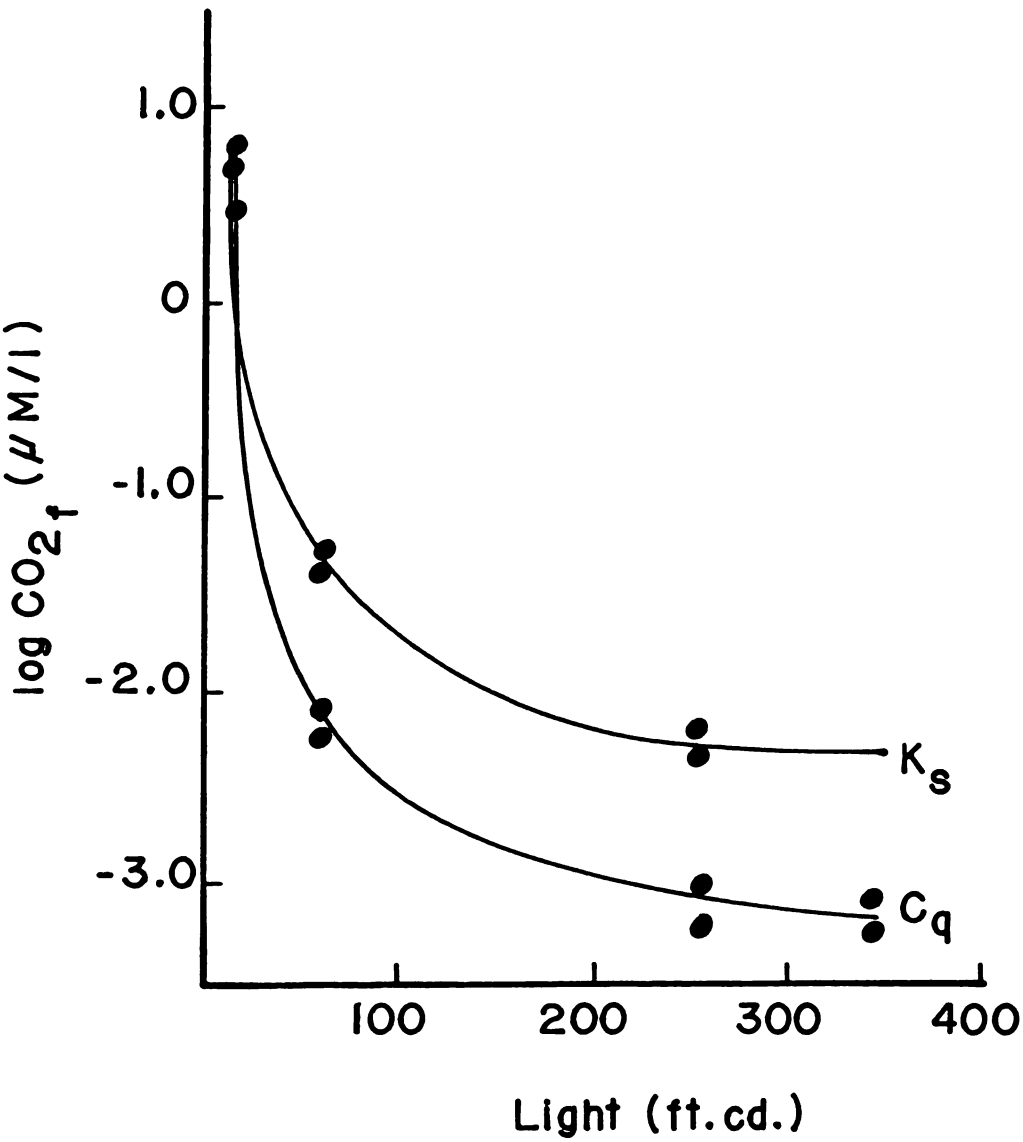


Figure 26. Variation in log free carbon dioxide values of K_S and Cq with light intensity for Chlorella vulgaris in duplicate light-dark microcosms.

constants. The parabolic equations describing Cq and K_c as a function of light are:

$$\log Cq = 11.867 - 12.829 \text{ Light} + 2.719 \text{ Light}^2 \quad (14)$$

$$\log K_c = 9.796 - 10.080 \text{ Light} + 2.098 \text{ Light}^2 \quad (15)$$

Therefore, Cq and K_c were constants for a given light condition and were incorporated into equation 13 to yield equation 16.

$$\mu g = a + bL \frac{C - f_1L}{(f_2L - f_1L) + (C - f_1L)} \quad (16)$$

Where: μg = Specific growth rate, hr^{-1}

$a + bL$ = Linear regression of μ max vs. Light

C = Free CO_2 concentration, μ M/l

f_1L = Parabolic regression of log Cq vs. Light

f_2L = Parabolic regression of log K_c vs. Light

Equation 16 quantifies the effect of carbon and light interaction to limit algal growth and represents (1) the physiological maximum growth of Chlorella and (2) what one might expect from a system in which algae cannot sink from the photic zone--in effect what would be expected from a chemostat.

Algal Sinking

Specific sink rate (μs), specific growth rate (μg) and specific biomass accrual rate (μab) values for each of the

light-dark microcosms were calculated with equations 10, 5 and 7 respectively. These calculations yielded μs, μg and μab values as functions of CO_{2f} for the different light intensities employed in this investigation.

In Figure 27, the light-dark microcosm 360B is used as an example of the relation of μg, μs, and μab to CO_{2f}. This figure reflects the reciprocal relationship between μg and μs that Zeisemer (1974) described; as μg decreased, μs increased and μab decreased rapidly. The CO_{2f} concentration where μab = o (or μg/μs = 1) was 0.24 μ M CO_2/l. This would suggest that at CO_{2f} levels higher than 0.24 μ M CO_2/l plankton biomass would remain in the photic zone (μg > μs) whereas at CO_{2f} concentrations below 0.24 μ M CO_2/l there would be a net loss of plankton biomass from the photic zone (μg < μs).

Consequently, the CO_{2f} concentration at which μab = o in the light-dark microcosms represents the minimum concentration of CO_{2f} required for accrual of planktonic biomass in those systems where the algae can sink from the photic zone. Figure 28 is a plot of μab values against CO_{2f} for each of the light-dark microcosms. It can be seen in Figure 28 that the CO_{2f} concentration at which μab = o increased as light intensity was reduced. This suggests that Chlorella's competitive ability to remain in the photic zone was reduced by the interaction of carbon and light in that the algae required increasingly larger CO_{2f} concentrations to maintain a positive μab and thus to remain in the photic zone as light intensity decreased. The limitation of μab by the interaction of carbon and light

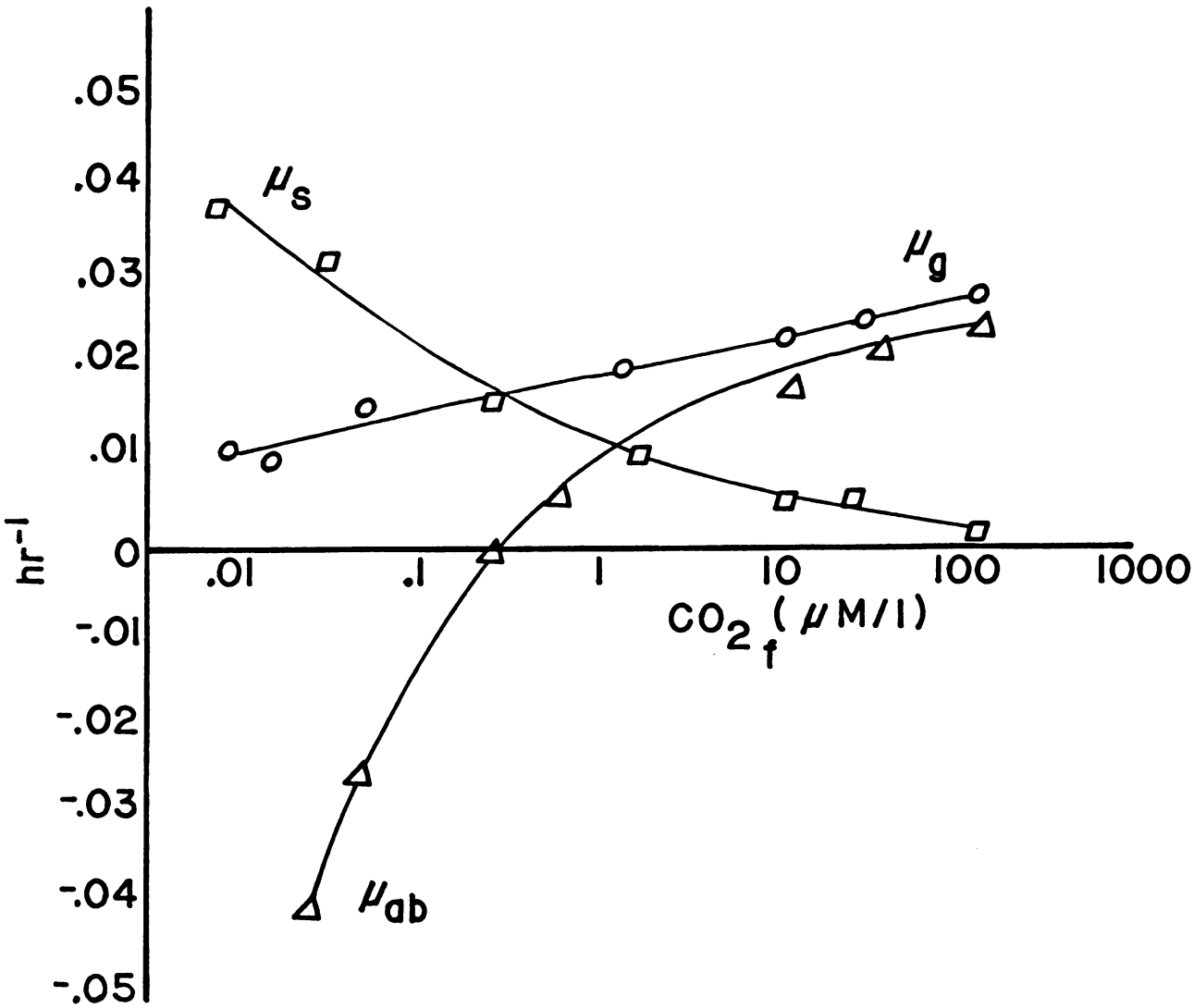


Figure 27. Variation in specific growth rate, specific sink rate, and specific active biomass accrual rate with free carbon dioxide for Chlorella vulgaris at 360 foot candles illumination in the B light dark microcosm.

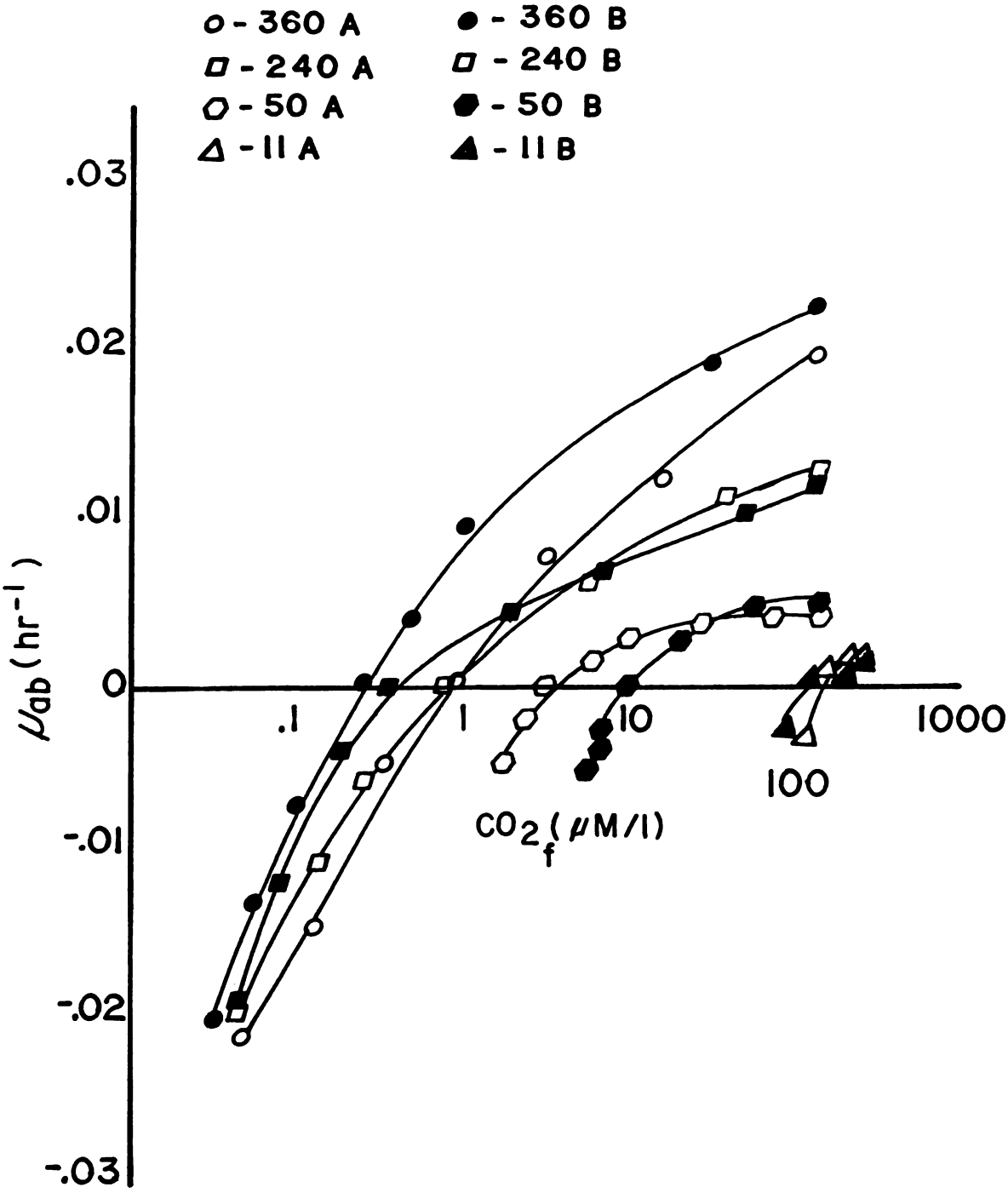


Figure 28. Variation in specific active biomass accrual rate with free carbon dioxide for Chlorella vulgaris in duplicate light-dark microcosms.

indicates that μg and μs were likewise affected in the manner shown in Figure 27.

Figure 29 is a plot of μg vs. μs values for the light-dark microcosms which includes a line for μab = o values. Carbon decreases toward the right in this figure for any given light intensity. Figure 29 shows that as the light-dark microcosms were subjected to decreasing light intensity, μg and μs decreased as did μab. However, the change in μg as a function of changing carbon levels was considerably smaller than was the change in μs.

The point illustrated in this figure is that an algal culture grown in a light-dark microcosm under a light intensity of 360 foot candles exhibited a larger μg and μs value than algae grown in lght-dark microcosms under a light intensity of 16 foot candles. Therefore, carbon and light interacted in such a manner as to limit Chlorella's specific growth rate (μg), specific sink rate (μs) and specific active biomass accrual rate (μab).

Polymer Excretion and Algal Sinking

In effect, growth limitation by carbon and light stresses the algae and Chlorella's physiological response to stress is for the cells to leak organic polymers. Pritchard et al., (1962) found that algae excrete extracellular substances at low CO_2 concentrations and Hellebust (1965) and Nalewajku et al., (1963) reported algal excretion under low light conditions. Ward et al., (1976) wrote that excretion by Chlorella is

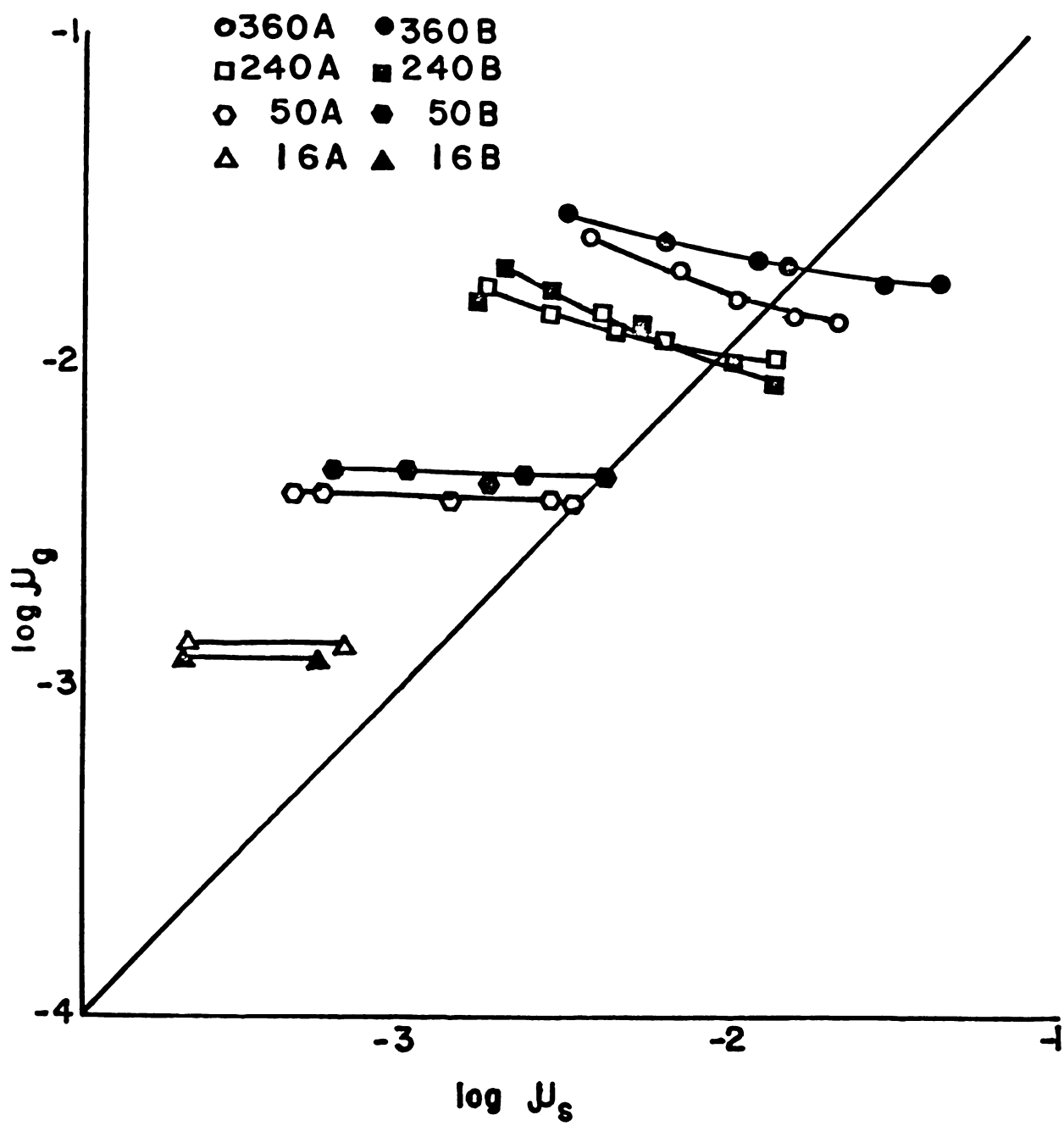


Figure 29. Variation in specific growth rate with specific sink rate for Chlorella vulgaris in duplicate light-dark microcosms.

dependent upon several factors, the most important of which are light intensity, CO_2 concentration, and population density.

These extracellular algal products were described by Pavonii et al., (1971) as four categories of organic polymers: polysacchorides, proteins, RNA and DNA. Davis et al., (1970) and Adams et al., (1975) described and listed these polymers in greater detail.

Investigators have looked at numerous environmental factors that stress algae and cause polymer excretion, but the overall effect of these extracellular polymers is to cause biological flocculation and, thus, algal sinking (Pavonni et al., 1971).

Pavonii et al., (1971) found that at high pH there was a direct correlation between polymer excretion and algal cell flocculation. This was interpreted as a surface coverage phenomenon in which polymers electrostaticly or physically bond and subsequently bridge algal cells in the dispersion into a three dimensional matrix of sufficient magnitude to cause sinking of algal biomass.

The actual bridging mechanism was described by O'Melia (1969). When a polymer molecule comes into contact with a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out into the solution. If a second particle with some vacant adsorption sites contacts these extended segments, attachment can occur. A particle-polymer-particle is thus formed in which the polymer serves as a bridge.

In this study Chlorella vulgaris was stressed with carbon and light limits. Presumably, at various points where the carbon concentration and light intensity interacted to stress the algae, organic materials were leaked as a function of the degree of stress (Pritchard et al., 1962; Hellebust, 1965; Nalewajko et al., 1963; and Ward et al., 1976).

These leaked organics serve as polymers which flocculate phytoplankton (Pavonii et al., 1971). The specific growth rate (μg) of Chlorella vulgaris decreases as a function of increased stress on the algae induced by carbon and light limits. Since specific sinking rate (μs) is generally reciprocal to specific growth rate (μg), μs appears to be a direct function of the degree of stress. This suggests that as algae are stressed, the rate at which polymer forming materials are leaked increases with increased stress and that flocculation of the algae, and thus μs, increases as a direct function of the degree of stress. As such, carbon and light induced limitation of algal photosynthesis lead to a decreased μg, an increased μs and thus a decreased μab.

Figure 30 is a plot of CO_{2f} concentration vs. light intensity with a curve describing the CO_{2f} concentration where μab equals zero for the light-dark microcosms and curves describing K_c and Cq values for the light microcosms. The curve for the CO_{2f} concentration where μab equals zero in this figure illustrates the effects of carbon and light limitations and polymer excretion on algal growth and sinking.

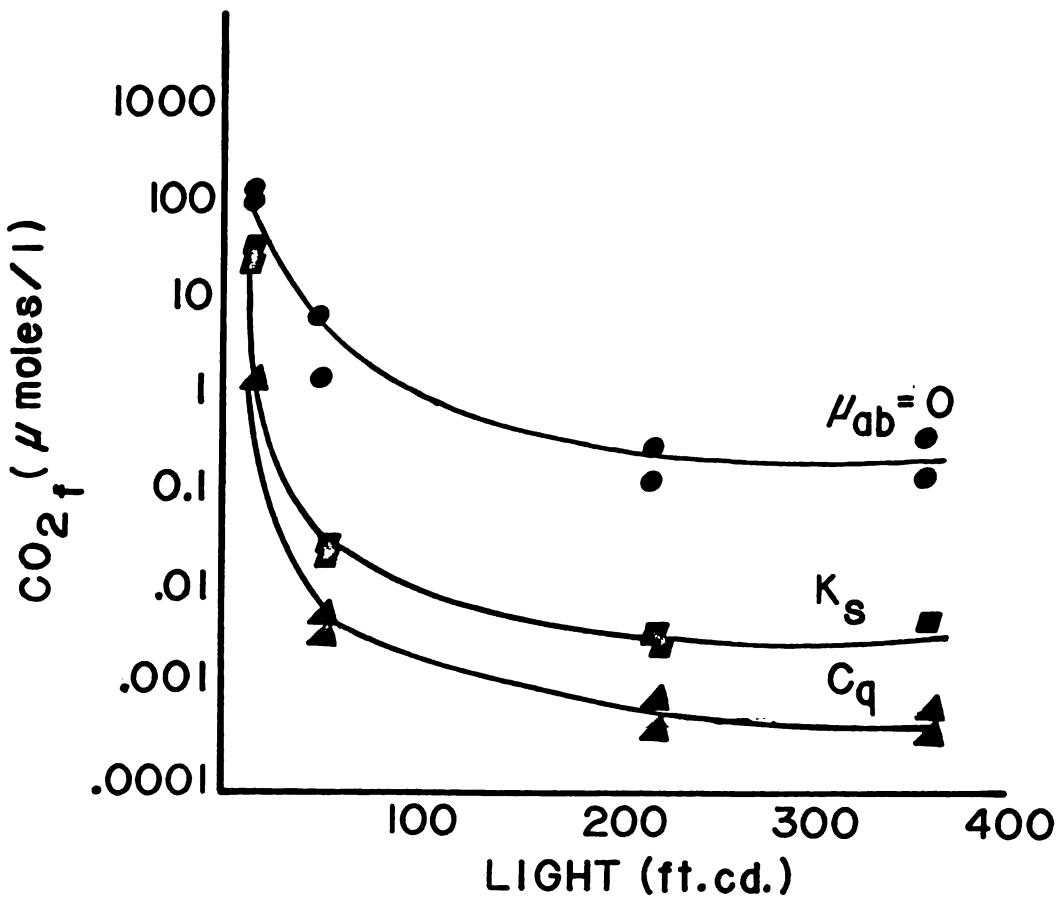


Figure 30. Variation in free carbon dioxide values of μ_{ab} = 0, K_s and Cq with light intensity for Chlorella vulgaris in duplicate light-dark microcosms.

Biological flocculation is dependent upon (1) the rate of particle formation and (2) the rate of polymer formation. In turn, both of these rate functions are dependent upon Chlorella's physiological response to CO_{2f} concentrations and light intensity. For instance, an algal culture in a light-dark microcosm subjected to a light intensity of 16 foot candles was stressed ($\mu ab = 0$) at a CO_{2f} concentration of about 150 μM CO_2/l as seen in Figure 30. Because there was not much carbon available from the alkalinity system prior to the sinking of biomass μg and thus the rate of particle formation was low. Correspondingly, the rate of polymer formation was small and μs was likewise low when algal cells were bridged and caused to sink.

On the other hand Chlorella grown in light-dark microcosms under 360 foot candles light intensity were stressed ($\mu ab = 0$) at a CO_{2f} concentration of approximately 0.40 μM CO_2/l as seen in Figure 30. Consequently, there was a large quantity of carbon available to the algae from the alkalinity system prior to the sinking of biomass and the rate of particle formation and μg was high as was the rate of polymer formation. Therefore, μs was greater than that observed in light-dark microcosms under lower light intensities.

The curve describing the CO_{2f} concentration where μab equals zero in Figure 30 represents the competitive ability Chlorella would exhibit in a lake as a function of carbon and light limits. The area above the curve reflects an environment in which Chlorella can successfully compete for carbon and light resources because $\mu g > \mu s$. However, there are varying

degrees of success. For example, Chlorella's productivity is greater in a high light - high carbon area of the curve because it takes the algae longer to reach the stress point than in areas of the curve where there is low light and low carbon. At CO_{2f} concentration where μab equals zero, Chlorella is only surviving, neither growing nor sinking because μg = μs. In effect this represents a steady-state point.

If carbon and light levels in a lake were to fall such that their intersection was below the point where μab equals zero, Chlorella could not remain competitive and would sink out of the photic zone because μg would be less than μs. Chlorella's ecological maximum competitive ability in nature as a function of carbon and light would appear to be described by the interaction of CO_{2f} concentration and light intensity at the point where μab equals zero.

The Cq curve in Figure 30 represents the alga's physiological maximum competitive ability, as seen in the light microcosms, and is what chemostat studies describe. There are three orders of magnitude difference in these curves which indicates a large margin of error when steady-state systems are used to describe an alga's ecological response to environmental conditions.

The light-dark microcosms employed in this investigation approximate light conditions found in lakes in that there is a non-photic zone into which algae can sink when they can no longer be competitive. Consequently, when the algae where stressed by carbon and light interactions the effect of polymer

formation was seen as an increase in specific sink rate (μs) and a resultant loss of competitive ability at a CO_{2f} concentration three orders of magnitude above that seen in light microcosms.

Chemostats, like the light microcosm in this study, are designed to measure algal response to limiting conditions but do not provide an environment in which algae can sink. Since the algae in a chemostat cannot sink, the effects of stress induced polymer formation on their ability to remain planktonic cannot be seen. Consequently, steady-state systems only assesses an alga's absolute ability to function in various limiting conditions. Whereas light-dark microcosms allow evaluation of the point where algae actually lose their competitive ability at substrate levels orders of magnitude higher than those associated with the absolute physiological limit.

CONCLUSIONS

1. The specific growth rate of Chlorella vulgaris at any free carbon dioxide concentration is not constant but decreases markedly with decreasing light intensity.

2. The threshold free carbon dioxide concentration required to allow photosynthesis by Chlorella vulgaris increases significantly with decreased light intensity.

3. A reciprocal relationship exists between specific growth rate (μg) and specific sink rate (μs) of Chlorella vulgaris. At a constant light intensity as carbon becomes limiting μg decreases, μs increases and the specific active biomass accrual rate (μab) decreases.

4. The effect of carbon and light interaction to limit the specific growth rate of Chlorella vulgaris in light microcosms is described by the following equation.

$$\mu g = a + bL \left[\frac{C - f_1 L}{(f_2 L - f_1 L) + (C - f_1 L)}\right]$$

5. The specific growth rate of Chlorella vulgaris decreases as a function of increased stress on the algae induced by interactions between carbon and light limits.

6. The ability of Chlorella vulgaris to compete in natural systems appears to be limited at free carbon dioxide

concentrations three orders of magnitude greater than the free carbon dioxide concentration required to sustain photosynthetic carbon fixation.

7. Application of results from chemostats studies of plankton algal kinetic response to environmental limits incorporates a significant error associated with the sinking of the algae in natural systems.

LITERATURE CITED

LITERATURE CITED

Adams, D. V., R. Rink, P. Cowan and D. Porcella. 1975. Naturally occurring organic compounds and algal growth in an eutrophic lake. Utah Water Res. Lab., College of Eng., Utah State Uni. 140 pp.

Bella, D. A. 1970. Simulating the effect of sinking and vertical mixing on algal population dynamics. Jour. Water Poll. Control Fed. 42(5):R140-R152.

Davis, E. M. and E. F. Gloyna. 1970. Algal influences on die-off rates of indicator bacteria. Proc. 25th Indiana Waste Conf., Purdue Uni.

Dowd, J. E. and D. S. Riggs. 1965. A comparison of estimates of Michales-Menton kinetic constants from various linear transformations. Jour. Biological Chem. 20(2):863-869.

Fogg, G. E. 1966. Algal cultures and phytoplankton ecology. Uni. of Wisconsin Press. P. 11-32.

Gavis, J. and J. F. Ferguson. 1975. Kinetics of carbon dioxide uptake by phytoplankton at high pH. Limnol. and Oceanog. 20:211-221.

Goldman, J. R., W. J. Oswald and D. Jenkins. 1974. The kinetics of inorganic carbon limited growth. Jour. Water Poll. Control Fed. 46:544-549.

Harvey, H. W. 1957. The chemistry and fertility of sea water. 2nd ed., Cambridge Univ. Press, Cambridge, England.

Hellebust, J. A. 1965. Excretion of some organic polymers by marine phytoplankton. Limnol. and Oceanog. 10:192-206.

Kalantyrenko, I. I. 1972. Calculation of the concentration of the blue-green alga biomass upon settling. Jour. of Hydrobiology. 4(2):104-107.

King, D. L. and J. T. Novak. 1974. The kinetics of inorganic carbon-limited algal growth. Jour. Water Poll. Control Fed. 46(7):1812-1816.

King, D. L. and R. E. King. 1974. Interaction between light and carbon dioxide availabilities as a control of algal species succession. A report to the Missouri Water Resources Res. Center, University of Missouri, Columbia, Mo.

King, D. L. 1972. Carbon limitations in sewage lagoons. Nutrients and Eutrophication special symposia, Limnol. and Oceanog. Vol. 1:98-110.

King, D. L. 1970. The role of carbon in eutrophication. Jour. Water Poll. Control Fed. 42(12):2035-2051.

Luebbers, R. H. and D. N. Parikh. 1966. The effect of algal concentration, luminous intensity, temperature and diurnal cycle or periodicity upon growth of mixed algal cultures from waste stabilization lagoons as determined on the Warburg apparatus. Proc. 21st Indust. Waste Conf., May 3, 4, and 5. Part I. Eng. Ext. Series No. 121. Eng. Bull. of Purdue Uni. P. 348-367.

Nalewajk[illegible]., N. Chowdhuri and G. F. Fogg. 1963. Excretion of gly[illegible] acid and the growth of a planktonic Chlorella. Studi[illegible] on microalgae and photosynthetic bacteria. University of Tokyo Press, Tokyo, Japan. 275 pp.

O'Melia, C. R. 1969. A review of the coagulation process. Public Works. May. P. 87-97.

Park, P. K. 1969. Oceanic CO_2 system: evaluation of ten methods of investigation. Limnol. and Oceanog. 14:179-186.

Pavonii, J. L., M. W. Tenney and W. F. Echelberger, Jr. 1971. The relationship of algal exocellular polymers to biological flocculation. Eng. Bull, Purdue Uni. Proc. 26th Indust. Waste Conf. Eng. Ext. Series No. 140, Part II. P. 947-974.

Pritchard, G. G., W. J. Griffin and C. P. Whittingham. 1962. The effect of carbon dioxide concentration, light intensity and isonicotinyl hydrazide on the photosynthetic production of glycolic acid by Chlorella. Jour. Exp. Botany. 13(38): 176-184.

Smayda, T. J. 1974. Some experiments on the sinking characteristics of two freshwater diatoms. Limnol. and Oceanog. 19(4):628-635.

Standard Methods for the Examination of Water and Wastewater. 1973. 13th Ed. Publ. jointly by the Amer. Public Health Assoc., Amer. Water Works Assoc., and Water Poll. Control Fed., Washington, D.C. 874 pp.

Ward, C. H. and J. M. King. 1976. Fate of algae in laboratory cultures. Ponds as a wastewater treatment alternative. Water Res. Symp. No. 9. Center for Research in Water Resources. Uni. of Texas, Austin. Ed.: E. F. Gloyna, J. F. Malina and E. M. Davis.

Young, T. C. and D. L. King. 1973. A rapid method of quantifying algal carbon uptake kinetics. Limnol. and Oceanog. 18(6):978-981.

Zeisemer, C. 1974. The influence of carbon and light variation on Chlorella vulgaris and Anacystis nidulans ability to maintain planktonic populations. Unpublished M.S. Thesis, University of Missouri, Columbia, Missouri.

APPENDIX A

Composition of Inorganic Nutrient Medium

Medium	Composition
$NaHCO_3$	84mg/meq/l
KNO_3	114 mg/l
$CaCl_2$	43.2 mg/l
$FeCl_3$	4 mg/l
$MgSO_4 \cdot 7H_2O$	20 mg/l
Na-EDTA	1.2 mg/l
KH_2PO_4	3 mg/l

Microelement	1 mg/l
H_3BO_3	2.86 g/l
$MnCL_2 - 4H_2O$	1.81 g/l
$ZnSO_4 \cdot 7H_2O$	0.22 g/l
$(NH_4)_6MO_7O_{24}$	0.18 g/l
$CuSO_4$	0.05 g/l
$Co(NO_3)_2 \cdot 6H_2O$	0.49 g/l