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# MASS CULTURE OF A BLUE-GREEN ALGA

# IN CONTROLLED LIGHT-DARK REACTOR SYSTEMS

by
Gordon Richardson Pyper

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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## ABSTRACT

### MASS CULTURE OF A BLUE-GREEN ALGA IN CONTROLLED LIGHT-DARK REACTOR SYSTEMS

by
Gordon Richardson Pyper

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This thesis describes the results of an investigation into conditions for effective light utilization in growing algae in mass culture. Mass culturing of algae is related to water pollution control as a potential tertiary treatment for extraction of nitrates and phosphates from wastewaters. Dark-light systems were used to determine effective growth patterns pursuant to the light and dark reactions of photosynthesis, and to determine whether light integration of any practical significance occurs in light-dark reactor systems.

An algal culture was circulated through a 1.1 liter, 10 cm. deep, light cell by means of a motor-driven impeller. Six dark-time detention variations were provided by fillers, total volume three to twelve liters, dark-light ratios of two to ten.

An artificial light source consisted of a bank of special fluorescent lamps; this source provided continuous culture surface light intensity of 2500 fc. Culture density was automatically controlled by photovoltaic cells and solenoid valves. Continuous growth records were maintained by collecting the overflow produced from medium addition to maintain constant density.

The diluting medium was a non-limiting nutrient mixture which approximated a strong sewage plant effluent. Medium nutrients were

augmented by additions of carbon dioxide to provide carbon and control culture pH at 7.5.

The dominant study organism was the blue-green alga Schizothrix calcicola.

Observable system response variables were: pH; nitrate-nitrogen; nitrite-nitrogen; ammonia nitrogen; orthophosphate; alkalinity; and algae growth rate as determined by both algal count and algal dry weight. Physical control variables were light intensity, temperature and velocity.

Several special studies were performed to more completely define particular observations. Two were mass culture growth at zero dark time and titratable alkalinity changes in batch cultures using nitrate and ammonia nitrogen media. Media studies are recorded in the Appendix.

Culture density and decreasing dark-light detention ratios produced growth effects comparable to those obtained by varying light in intensity. Dark-light detention ratios were most advantageous for high light intensity algal culturing. This was evident under continuous light conditions where growth rates were highest but light energy was not utilized efficiently.

Velocity demonstrated pronounced effects on growth and light efficiency. This system had a critical mean effective velocity of 0.8 ft/sec. Velocity above 0.8 ft/sec produced a light integration effect at a dark-light ratio of 3.5 under high, mean effective light conditions. This indicated that in mass culture high velocities are desirable, decreasing flash time, if light energy is to be used effectively.

Improved yield was demonstrated in areas where possible integration effects were observed. Mean effective light intensity was evaluated by means of probability expressions.

Total inorganic nitrogen (TIN) removal was a linear function of density with a slight removal increase up to 1 ft/sec. Most favorable TIN removal appeared to be obtained with five to six second dark time. Orthophosphate removal was predominately a linear function of density.

Dark-light culturing produced predictable algal growth characteristics; percent transmittance and algal dry weight correlated well. This indicated that dark-light conditions are advantageous in high light intensity algal culturing. Algal cells from dark-light systems demonstrated variation from those produced in continuous light.

The thesis contains seventeen tables, sixty-four data curves, four appendices and one hundred and sixty-eight references.

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## SYMBOLS

b -- Thickness of absorber, light penetration distance (cm)

c -- Algal concentration (no./ml)

d -- Unit algal size (cm)

$d_t$ - Dark time (min)

D.F.-Dilution factor

D.T.-Detention time

E -- Extinction coefficient (l/mg-cm)

i -- Number of shading organisms

$I_b$ - Light intensity at point b

$I_c$ - Intensity of continuous light

$I_f$ - Intensity of intermittent light

$I_M$ - Mean or integrated light intensity

$I_o$ - Initial light intensity

k -- Growth rate or specific growth rate, natural log base ($days^{-1}$)

k' - First order reaction rate constant ($min^{-1}$)

L -- Algal length

m -- Number of layers of shading cells

n -- Number of times shaded

N -- Number of algal cells

$N_o$ - Number of algal cells at t = o

$N_t$ - Number of algal cells at time t

$r_n$ - Fraction of total cells shaded

t -- Time (days)

$t_d$ - Dark time (sec)

$t_f$ - Light time (sec)

$t_T$ - Total time (sec)

$V_o$ - Overflow volume (liters/24 hrs)

$V_s$ - Culture volume (liters)

$\Gamma$ -- Single cell light transmission fraction for unit depth of algal cells

$\gamma$ -- Shape factor

$\rho$ -- Algal density (mg/l)

$\tau$ -- Average fractional transmittance, 400-700 mμ light wave length

$\bar{\tau}$ -- Average light intensity fraction

# INTRODUCTION

Eutrophication is a natural process involved in the aging of streams and other bodies of water. In the natural state the transition of a stream or lake from a clear, cool trout water to a turbid, more fertile, warmer, shallow, coarse-fish water may take many thousands of years. However, the transformation is accelerated significantly when waste loads are imposed on the stream or lake.

The discharge of raw wastes results in the most severe eutrophic acceleration. However, most wastes are--or will be in the near future--subjected to extensive treatment to reduce their oxygen demand, and thus their oxygen consuming impact on the receiving water. Nevertheless, effluents from conventional waste treatment processes tend to be balanced fertilizers which contain nitrates, phosphates, hormones, vitamins and many other trace elements.

The nutrient compounds of greatest concern are nitrogen and phosphate. Dilution of these nutrients in receiving waters might reduce their concentrations to a limiting level at any one point of addition, but the total contributing quantity from multiple sources tends to negate any dilution advantages. Also, low concentrations may still contribute materially to algal blooms in lakes and ponds because such bodies of water tend to trap and hold nutrients, and any increase above the natural levels tends to produce pronounced acceleration of the aging process.

One possible approach to this problem of overfertilization of lakes and other receiving waters lies in using the natural nutrient extraction process involved in stimulating algal growth to reduce the concentrations

of these materials in waste discharges. If algae can be readily grown under properly controlled environmental conditions, it may be possible to effectively extract nutrients from waste effluents and, at the same time, produce a useful by-product--algae; this might have important food applications.

Several previous studies have been directed toward more completely developing these concepts (76,77,150). Most of these studies have been made with respect to the problems of mass culturing. Some have involved investigation of nutrient removal from waste effluents, nutrient limitation levels, and depth-density studies. Much information necessary for future development of the nutrient extraction process has been gained by these studies. However, one factor which has heretofore received little attention is that of light integration effects in mass culturing of algae for nutrient extraction. It would appear that if an effective and economical treatment is to be obtained by this approach, some answers must be obtained with respect to the nature and effects of light integration in mass cultures.

The "flashing light effect" is a promising approach to light integration and is primarily a function of relative light and dark exposed times, light intensity, and rate of transition from complete dark to light and light to complete dark conditions. Regarding intensity-time relationships, high intensities and exceedingly short exposure times have appeared to be most advantageous. However, this type of experimental result has usually been obtained in very shallow, low-density algal systems. Such short exposure times and extremely high light intensities are exceedingly difficult to obtain in a mass culture system. However, it is important to know how it may be possible to begin to approach such conditions.

Another aspect related to the effectiveness of the "flashing light effect" involves the dark period, during which time light is not necessary. There have been indications that, if integration is to be obtained, two factors are of particular importance relative to the dark period. The first is the duration of the dark period and its relationship to the light period (72). In general, this duration must be a relative function of the light time. Longer dark time must be provided for longer light time (71); ratios of 4-10 have been suggested. If these time periods and the actual light exposure conditions are to be well defined and easily evaluated, transition from light to dark must be as sharp as possible. Poor demarcation could contribute to longer effective light periods, or shorter dark periods, than used in evaluation calculations. An example would be that increased growth could be attributed to being a result of noncalculated (noncredited) light exposure. The second factor is that the dark system must be absolutely dark, as even low levels of light intensity may reduce the efficiency of this part of the process (72). High light intensity depresses the enzymatic and photochemical processes; dark periods reactivate it (59). Without a completely dark period there would not be complete reactivation.

As a result of these implications it was thought that studies should be conducted to obtain growth and yield information with respect to: (1) variable dark time with a constant light exposure time, velocity constant, providing a sharp demarcation between light and dark periods; and, (2) the influence of variable velocity in the controlled dark-light system on the effect of probable light exposure.

These investigations should provide information which will contribute to the engineering solution of energy utilization problems related to

growth of algal cultures associated with treatment of wastewaters for removal of nutrients. Information concerning light integration potential at low light intensity in mass culture will provide a significant contribution toward this end.

## CHAPTER I

## EUTROPHICATION AND ALGAE

The extremes of water fertility have been described as oligotrophic versus eutrophic (20). An oligotrophic body of water is least productive and thus not particularly conducive to supporting fish life. It tends to have relatively uniform oxygen distribution, clear water and a pleasing appearance (24). Its main attraction would be for drinking water supplies, irrigation and recreation. Such waters will, with time, change to the eutrophic state. Usually, this change from oligotrophic to eutrophic is a slow process, but the man-made stimulants may cause marked changes as recorded in Lake Washington (23) and other bodies of water.

It would appear that an increase of fertility might be advantageous for purposes of biologic productivity, such as fish production (11). This has been done with chemical fertilizers and could be accomplished with wastes if they have been treated to remove undesirable oxygen consuming fractions and pathogenic bacteria. Lackey (24) indicated that possible beneficial aspects of fertilization are mineralization, improved re-oxygenation and the possibility of increased fish production as a result of increased algal growth. However, such advantages may not be apparent as the disadvantages often appear to be greater (12,24). Over fertilization may produce excessive algal growths which create a poor appearance in water as well as producing tastes and odors. Dead algae produce B.O.D., and toxin producing algae may also be stimulated (24). Re-oxygenation may be accomplished more readily during photosynthesis when algae are

present than by normal atmospheric transfer. This has been considered to be a desirable benefit of having algal growth under pollution conditions (9,29,45,87,94). Oswald and Gottas (87) indicated that 1 gram of algal growth should produce 1.6 or more grams of oxygen. This re-oxygenation capability may not always be desirable, because supersaturation may occur during sunlight periods and oxygen depletion may become severe as a result of algal respiration during dark periods (24). Algae may produce growth as the result of waste effluent fertilization, a condition which might be called secondary pollution (33) in that waste treatment is provided to initially eliminate the possibility of the occurrence of low dissolved oxygen and yet the low dissolved oxygen condition may develop as a result of algal respiration (22) or decomposition of deal algae (24). This has produced delayed oxygen sag curves in streams, sags which are less predictable than those caused by direct overloading with organic material (27).

Quantitative evaluations have been made to determine the influence of algae on dissolved oxygen in streams. McDonnell and Kountz (27), in their study of Bald Eagle Creek in Pennsylvania, reported a 24-hour variation of dissolved oxygen of 1.5 mg/1 to 16.5 mg/1. Sawyer (25), in reporting on conditions in Spring Creek, Pennsylvania, indicated observed variations of dissolved oxygen of 20% to 140% of saturation. Such extremes of oxygen concentration are not desirable and few animals will inhabit such an area.

Many attempts have been made to try to define what is a desirable extent of eutrophication. One approach has been to define it as primarily a problem of nutrient concentration (11). Sawyer (26) indicated that concentrations of 0.01 mg/1 of inorganic phosphorus and 0.30 mg/1 of

inorganic nitrogen could produce conditions where agal blooms could be expected. These concentrations may originate from many sources, such as natural surface drainage, drainage from agricultural areas and by direct domestic and industrual waste discharges.

At times, when nutrient concentrations are equal to or above the indicated border-line levels, pronounced algal growth may not develop. Light, temperature, nutrients and other factors must be just right before growth may develop. An algal bloom has been defined (22,120) as a concentration greater than $0.5 \times 10^6$ organisms per liter. Bloom levels have also been defined (35) as greater than 10 mg/20 qt. sample. Lackey reported on a bloom in an Oak Ridge settling basin (38) which started with one Chlorella per ml. ($1 \times 10^3$/1) and in three days multiplied to 20,000 per ml. ($20 \times 10^6$/1). Dry weight is possibly a better indicator of algal growth and bloom conditions as long filamentous algae may be present and counts would not represent the true amount of cellular material. In water supply work, bloom conditions have been indicated to have a range of 500-1000 standard units/ml. (39) (0.5-1.0 units/1). A standard unit is 400 sq. microns in plan area/ml.

The concentrations of nutrients in natural and agricultural drainage are difficult to evaluate but at times they have been reported to be very high. Sawyer (25) indicated typical surface run-off values as 0.3 mg/1 $NH_3$ - N, 0.2 mg/1 $NO_3$ - N, 1.5 mg/1 Org. - N, 0.2 mg/1 $PO_4$ - P, 0.6 mg/1 Org. - P.

The immediate concern with respect to accelerated eutrophication is the nutrient contribution from both treated and untreated domestic and industrial wastewaters. These waste effluents generally contain nitrates, phosphates, hormones, vitamins, and many other trace elements (24). Of

these, nitrogen and phosphorus compounds appear to be of greatest concern because they are the most important and readily measurable components required in plant nutrition. Their concentrations in effluents are usually quite high. It has been indicated that raw domestic wastes may have concentrations in the order of 15-35 mg/l of nitrogen (26) and 2-15 mg/l of phosphorus (26,8). Biologic treatment will reduce this, depending upon the type and degree of treatment. The effluent from the normal complete treatment process will show a reduction to about 7-15 mg/l of nitrogen (28) and 2-4 mg/l of phosphorus (8). McDonnell and Kountz (27) reported plant effluent concentrations of 30 mg/l total nitrogen and 24 mg/l of $PO_4$. Sawyer (28) reported stream concentrations of 0.8 mg/l total nitrogen and 0.3-1.2 mg/l of $PO_4$.

Efforts are presently being made to define parameters which will indicate the eutrophic potential of receiving waters. Lee (22) defined the basic measure of eutrophication as the standing crop of organisms sustained by photosynthesis. Oligotrophic conditions yield small crops and great distribution of types. Eutrophic conditions produce large crops with a small number of types. Sawyer (20), in discussions of this topic, included this approach along with six other methods. It has been indicated that vertical oxygen distribution characteristics (15,20) are also indicative of eutrophication. Another approach has been algal bio-assay (19). However, these methods may not provide a complete answer as physical environment and other factors may be of equal importance.

Toxic algal growths may be stimulated by nutrient additions (24) and there have been many reported cases (30,31). While the characteristics of such toxicity have not been well defined, there have been reports of fish kills, cattle toxication, adverse effects on oysters, and water

fowl deaths as the result of compounds produced by algae, and the compounds have been isolated from both the algae and the waters in which the organisms were growing (9). At present, there has not been any indication that human deaths have resulted from such toxic compounds. Mackenthun and Ingram (9) listed 42 reported cases of human gastrointestinal, respiratory and skin disorders associated with algae. This could take on more serious proportions in the future as more and more waters tend to become highly enriched as a result of the need to discharge increasing quantities of wastes into receiving waters.

Another aspect of algal blooms or excessive algal growth is the production of large quantities of organic material. While algae are alive the oxygen balance of the water may remain satisfactory. It has been indicated that the death of large masses of algae creates high B.O.D. values and deoxygenated bottom deposits (24). The high B.O.D. may, under certain circumstances, force oxygen levels to concentrations of zero. Also the demand may be of long duration because of the nature of the cellular material. This will contribute to the production of benthic deposits. Such deposits have significance with respect to bottom ecology and also with respect to toxic potentials. Anerobic conditions may exist and have been indicated as potential endemic areas for botulism (24). If enough organic material is available and the conditions are just right, the exotoxin from botulism might be produced and dissipated in the water. In extreme situations this could possibly develop to detrimental concentrations since the lethal toxin level is very low. This is another reason for concern for eutrophication.

## ALGAL GROWTH

### General

Algal growth follows the same pattern as other green plants. Photosynthesis is the basic mechanism in such growth. Photosynthesis is the combining of carbon dioxide and water under conditions of light, by means of chloryphyll, to produce carbohydrates and oxygen (36). This may be expressed in the following general equation (36,40):

$$CO_2 + H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} (CH_2O)_n + O_2 \qquad [1]$$

The green chlorophyll serves as a photocatalyst, and provides a means of storing light energy for other reactions (41,46). Benson and Calvin (47) have shown, by experiments with Chlorella, that this process can store the necessary energy to convert $CO_2$ to sugar. They indicated that this conversion may take place after the photochemical process. However, there are algae which do not utilize carbon in this photosynthetic manner (48).

Equation 1 indicates a process proceeding in one direction. Most plants and algae develop in an equilibrium condition between photosynthesis and respiration and thus for any growth condition Equation 2 represents the growth process (36,40).

$$CO_2 + H_2O + \text{energy} \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} (CH_2O)_n + O_2 \qquad [2]$$

This indicates both respiration and photosynthesis occurring at the same time. When chlorophyll is subjected to light energy there will be both photosynthesis and respiration. When light is removed the reaction tends to go in one direction, that of respiration.

There are two fundamental concepts involved in the light stimulated direction of the reaction. These steps are separate from the two possibilities, photosynthesis or respiration. The photosynthetic direction may be divided into two parts referred to as the light reaction and the dark reaction (3). At times it has been reported to take place in three stages (41).

The light reaction is one which absorbs light energy and utilizes water. This light reaction may be expressed as (1):

$$H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} (H) + (OH) \qquad [3]$$

This photolysis must take place in the presence of light (81); the reaction is thus light dependent and the amount of light will have a significant influence on its rate and extent. It may occur in as little as $10^{-5}$ seconds (3).

The second part of the total photosynthetic relationship involves chemical reactions in fixing carbon dioxide, destruction of hydrogen peroxide and the synthesis of other organic compounds. These reactions may take place in either light or dark and thus they are frequently referred to as the dark reactions. The carbon fixation may be expressed by the following relationship (1):

$$CO_2 + 4\ (H) \longrightarrow (CH_2O) + H_2O \qquad [4]$$

The hydrogen peroxide breaks down to produce oxygen (3):

$$2H_2O_2 \longrightarrow 2H_2O + O_2 \qquad [5]$$

These chemical reactions will be temperature influenced and may be expected to proceed more rapidly in warmer temperatures and slower in colder temperatures. Also, since carbon dioxide is being fixed, the reaction characteristics tend to vary with respect to carbon dioxide concentration (36).

Light

The concepts of photosynthetic activity indicate that a major factor affecting algal growth is light energy. This energy influences growth in several ways. The major variables associated with light stimulation are intensity, wave length, and frequency of exposure.

Under constant light conditions there is an increase in photosynthetic activity as light intensity is increased. If we consider the photosynthetic activity of an organism as starting in an environment of zero light, there will be an increase in this activity as the light increases; this increase will be arithmetically linear from zero to some point at which the relationship becomes curvelinear (121). The linear range, in which, if all other conditions are constant, plant activity will increase in direct proportion to an increase in light intensity, is referred to as a light-limiting range.

Within the linear range, just above zero, there is a point of interest related to the evaluation of algal activity. This point is the compensation point (121). This is a level of activity where the rate of photosynthesis is just equal to the rate of respiration. In continuous light this level of intensity has frequently been observed and reported to be in the order of 20-40 ft.c.

Above the linear range, the photosynthesis-light relationship goes through a transition of a nonlinear nature to a relatively constant value. At this point the reaction is light saturated. This means that additional light energy will have little, if any, effect in stimulating further activity. The continuous light saturation level for many algae has been indicated to be in the range of 100-400 ft.c. (42). The saturation level may vary with the particular alga. Cramer and Myers (97) report saturation

levels for Euglena gracilis of 1500 ft.c. with Chlorella giving saturation effects at 600 ft.c.

Inhibition of photosynthetic activity tends to occur at light concentrations of 1000-2000 ft.c. (42). This means that as light is increased above this range there would tend to be a reduction in photosynthesis and thus in algal growth rates. Pronounced growth reductions have been shown to occur in Chlorella (43) with pronounced inhibition above a level of 3000-4000 ft.c. Similar saturation and inhibition characteristics have been reported for laboratory and field studies (44) for a marine diatom Chaetoceras affinis. Full sunlight will tend to produce these light-inhibiting effects if algae are exposed to the full intensities. Sunlight intensities have been recorded in the range of 1000-10,000 ft.c. (42).

Growth characteristics of microscopic organisms may be related to effective light intensity according to Equation 6 (62).

$$I_b = I_0 e^{-Ebc} \qquad [6]$$

This is a general expression applicable to many radiation and radiant energy relationships. Equation 6 relates the intensity ($I_b$) at a point for a given initial intensity ($I_0$) after an absorbing material with a light path, b, has been placed between the source and the point. In this equation the term, E, is the extinction coefficient expressive of the amount of absorption per unit concentration of absorbing material and C is the concentration, in this case, of algae. Some extinction coefficients for algal systems have been reported to vary in the range of $2.0 \times 10^{-3}$ to $3.9 \times 10^{-3}$ $cm^2/mg$ (42).

In continuous light, plants and algae appear to be able to use a limited range of intensity at full efficiency (50), with efficiencies

reported in the range of twenty to possibly thirty percent. In studying algal light efficiency under conditions of growth in oxidation ponds, using sewage as a source of nutrients, Oswald (51) indicated a function of detention time as well as intensity. He reported that Chlamydomonas agloiformis, Chlorella pyrenoidosa, Scenedesmus obliquus showed efficiencies of about 1.5-2.5% at detention times of 1.5 days with a rather sharp decrease in efficiencies on either side. In the same study (51) Oswald indicated a lower percent (0.75%) efficiency for Euglena gracilis at 5 days of detention time. The efficient use of light energy with respect to intensity was reported to be of an inverse nature for studies made on Chlorella pyrenoidosa, starting at a maximum efficiency value of about 8.5% and at an intensity of about 5 cal/l/min.

Efficiency and growth curves into the light saturation range will tend to vary with respect to pre-study growth conditions. Taylling (44), in reporting on growth characteristics of Chaetoceras affinis, reported that preconditioning did not change the saturation intensity of about 10,000 lux. However, he did report that cultures grown at high intensity, stored in the dark, and then used for studies, produced higher photosynthetic rates than cultures grown in moderate light intensity and then used immediately for study purposes. This might be the result of increased chlorophyll content. Emerson (57) reported similar curves for experiments with both high and low chlorophyll content in Chlorella vulgaris. High chlorophyll content gave higher rates of photosynthesis than low content, for the same light intensities. However, the light saturation intensity was the same in both cases. This relative intensity relationship has also been reported for both ice and water organisms in antartic sea studies (53). However, epontic diatoms, which grow under the ice continuously,

tend to show saturation intensities at about 1000 lux (93 ft.c.) with rather pronounced inhibition at higher values. This appeared to show shade adaptation for some types of organisms (53).

Growth or photosynthetic characteristics of algae have been expressed as a linear relationship with respect to intensity, until saturation is reached. This was verified by Varma and Wilcomb (45) as they indicated that their studies demonstrated the rate for photosynthesis tended to increase as light intensity increased. Their studies were made in the light intensity range of 480 to 1280 ft.c.

In mass culture studies, an important measure of photosynthetic activity is the rate of growth with respect to selected system parameters. Growth rate in algal systems can generally be best described by a rate expression of the first order,

$$\frac{dN}{dt} = kN \qquad [7]$$

where N is the number of algal cells at any time, t, and k is the first order rate constant, commonly referred to as the specific growth rate (90). The integrated form of Equation 7 is

$$\text{Ln} \frac{N_t}{N_o} = kt \qquad [8]$$

The specific growth rate may be used as a parameter with which to evaluate the effect of light intensity. The relationship between the specific growth rate, k, and light intensity shows the expected linear increase in k up to a point of light saturation where the growth rate tends to become constant. Myers reported a saturation value for k of 1.96 for Chlorella pyrenoidosa at 100 ft.c., 25°C (54). This value is important because the organism has one of the highest algal growth rates at 25°C (54). However,

algae tend to have much lower growth rates than other organisms. Escherichia coli is reported to have a k value of 60.0 (54,95).

Attempts have been made to overcome light saturation effects in continuous light. Myers and Graham (90) reported yields with Chlorella ellipsoidea of 230-260 mg/day with cone light diffusers compared to yields of a maximum of 140 mg/day for the same system without cones. These experiments were done with light intensities about 8000-10,000 ft.c., equal to full sunlight.

Increasing light concentrations has demonstrated an increased growth rate up to saturating intensity. The saturating range has been reported to be as low as 400 ft.c. (56) to as high as about 1000 ft.c. Relatively constant growth rates have frequently been reported between saturation and about 2000-3000 ft.c. (43,56).

High Light Intensities

Previous discussions have shown that generally there will be decreases of photosynthetic activity or of growth rates with light intensities of about 25 Klux (53) or 2000-4000 ft.c. (56,43).

The inhibition effect has been indicated by Kok, in experiments with Chlorella, to be a time and intensity related relationship (58). The experiments showed that the rate of photosynthesis was higher under light saturation conditions at 38°C than under the same light conditions of 22°C, which agree with other data. It has also indicated that the rate of photosynthesis was reduced below the maximum saturation value in about 3 minutes when exposed to continuous saturation of light intensity. When exposed to light intensities somewhat less than saturation ($0.3I_s$) the time for a reduction in the maximum rate to appear tended to increase,

up to 8 minutes. Thus, this indicated that as intensity increases near the saturation level, and especially above saturation, the time for inhibitory effects to take effect tends to become shorter.

Nielson (59) indicated that the photosynthetic rate at low light intensities was limited by the photochemical process and at high intensities enzymatic processes tended to limit. This was demonstrated by growing Chlorella vulgaris, previously grown in light intensities of 3 Klux and 30 Klux, under varying light intensity conditions. The algae grown in 3 Klux intensity showed a saturating intensity of about 5 Klux and a relative photosynthetic rate of 40 as compared to algae grown in 30 Klux which reached a saturation intensity of about 11 Klux, relative photosynthetic rate of about 100. This indicated that pregrowth sensitization to tenfold light conditions only stimulated about twice the saturation photosynthetic rate as compared to the lower light intensity sensitized organisms. The results of these discussions indicated that both the light activated photochemical process and the enzymatic process were depressed as a result of exposure to high light intensities. Dark periods tended to reactivate both of these processes (59). Nielsen (60) had shown similar relationships in experiments on Cladophora insignis using 3 and 100 Klux light intensities.

Similar reactions have been reported in a general way for higher plants. Burkholder and Johnston (61) reported that in higher plants light intensities tended to destroy or inactivate important plant growth substances. Some of the first work by Meier (62) concerning light effects on algae, using Stichococcus bacillaris and Chlorella vulgaris, indicated that for continuous illumination low light intensities were more favorable for growth and also that in higher intensities intermittent light was

better than continuous light. These studies also included wave length considerations and it was shown that chlorophyll formation appeared to occur in all light ranges between 3700-6000 A. Formation appeared to be best when the wave lengths were in the blue-violet range. Multiplication of the algal cells appeared to be best in the red and infra-red range of 0.6 to 1.4 microns.

Light Wave Length

Continuous light growth studies have usually assumed that the light has suitable wave characteristics to produce algal growth. However, Meier (62) indicated that wave length had an effect on chlorophyll production and growth rate. Effective light wave lengths were in the range of the visible light spectrum, 400-700 mu. Krouse (48) reported that the effective visible spectrum range was between 3800 and 7600 A (380-760 mu) and that less than 40% of sunlight energy is in this range. This is significant because the effectiveness of wave length is a function of the absorption in chlorophyll. Light absorption for chlorophyll A appeared to be most pronounced in the range of 380-450 mu, peak about 430 mu, and 640 to 680 mu with a peak at about 658 mu (36,63,40). Chlorophyll B showed a similar absorption spectra with a band width of 420-480 mu and a peak of about 460 mu and another band width of 625-660 mu and a peak of about 640 mu. Both types of chlorophyll had very little relative adsorption between the given ranges or on either extreme.

Wave length absorption is of considerable significance as shown by Rabinowitch and Govindjee (41) in reporting on the Emerson effect. Their report showed the curve of quantum use, for the given spectrum distribution, paralleling the absorption curve. They also indicate that

both chlorophyll A and B were necessary (41) for full photosynthetic efficiency. The light absorption stimulation process in one chlorophyll was enhanced by the wave length absorption in the other chlorophyll. This was shown to be especially noticeable in the red light range (700 mu).

Light wave length below 375 mu (3700 A) was not effective with respect to photosynthesis because of the lack of absorption in chlorophyll. However, lower wave lengths of light, in the ultra-violet range, have been shown to be toxic to Chlorella vulgaris (64). The most severe damage appeared to be in the range of 2600 to 2800 A. Moderate damage occurred in the range of 2250 to 2600 A. with toxic effects ceasing at about 3025 A. Time of exposure at various wave lengths made a difference in the degree of toxic effect.

It appears that sensitivity to ultra-violet radiation varies considerably, depending upon the type of organism. McLeod and McLachlan (65) indicated that at 2537 A. diatoms tend to be the most sensitive. Scenedsmus quadricauda and other green marine algae were used in their studies and the green algae Chlorella vulgaris and Ankistrodesmus falcatus showed the least damage at this wave length. They indicated that this wave length had the most effect during logarithmic growth. Photosynthetic fixation of $CO_2$ is also responsive to wave length. The utilization of $CO_2$ is relatively constant in the range of 425-725 mu. Very little $CO_2$ is photosynthetically utilized in a light environment of less than 400 mu or greater than 740 mu (36,66).

Intermittent Light

In considering the growth of higher plants or algae, then, two basic reactions are of importance: the light and the dark reactions (3). In discussing intermittent light relationships it is desirable to consider the effect of high light intensities. This may be demonstrated by exposing plants to longer and longer periods of high light intensity, with carbon dioxide fixation serving as the criterion for measuring photosynthesis. A curve is developed which has the typical linear phase and a transition to a saturation phase where longer periods of high light intensity will not produce increased activity (36,67). The dark reaction fixation of carbon dioxide is composed of three parts, the active light sensitized portion, a decay portion from light stimulation to continuous darkness and fixation at a low level in the dark. Increasing light intensities above the saturation level only tends to make the reaction reach its maximum in a shorter period of time.

This relationship suggests that the dark reaction limitation could be more efficiently handled if algae were exposed to light for just the right amount of time and then allowed to react in the dark for an appropriate amount of time (48) and thus, this serves as the basis for the concept of light integration. This is of interest because natural sunlight tends to have too high an intensity for maximum growth potential (56,50). However, in intermittent applications it is necessary to consider the characteristic of the initial light response ($CO_2$ fixation) curve with respect to time (69).

Flashing Light

Algae will tend to show more evidence of photosynthetic activity ($O_2$ produced, $CO_2$ utilized, increased growth rate) per unit of light if light exposure occurs as short flashes with fairly long dark time between light exposures. This improvement is a function of flash frequency and light intensity. Long flash times and/or low intensities tend to have less effect than short time and/or high intensities (82).

The work of Burk, Cornfield, and Schwartz (70) indicated that it should be possible to obtain high photosynthetic efficiencies in mass cultures, at high light intensities, as well as in laboratory systems. Their work with Chlorella indicated that the photochemical relationship tended to be obscured by continuous illumination and became more recognizable as a highly efficient operation when the organism was subjected to rather short light and dark times. The back chemical reaction in the dark tended to be the limiting relationship in ranges of high light intensities. It was found that for a high intensity light flash, constant duration and intensity, increasing oxygen quantities could be produced for increasing dark periods, up to a point termed the maximum dark time. This was reported to be the time required for the completion of the dark reaction. Intermittent applications of low light intensities did not indicate any effect on yield efficiency. They (70) indicated that light yield efficiency could be increased up to very high levels regardless of the magnitude of the light intensity. This relationship would only be limited by the amount of oxygen necessary to return the process to its original state in the back reaction. They indicated a very practical light and dark system using a saturating intensity with one second exposure. Desirable dark times would be in

the range of 12 to 30 seconds. Effective dark times could possibly be longer if a saturating flash time could be insured since they indicated the dark time should be expressed as shown in Equation 9.

$$0.63\ d_t = \frac{1}{k'} \qquad [9]$$

Practical values of k', reported in Equation 9, were 5-20 minutes$^{-1}$.

Phillips and Myers (71) in their work on Chlorella in flashing light further defined the concept of light integration. They indicated that the relationship for the condition of no integration influence was:

$$\text{Zero Integration Growth Rate} = 2.1 \times \frac{\text{"flash time"}}{\Sigma(\text{"flash time"} + \text{"dark time"})} \qquad [10]$$

Perfect integration would be a condition where the growth rate which occurred would be equal to the value which would be obtained in continuous light. They did not report on data for long flash and dark times. However, they did report a tendency to produce a growth rate gain per flash with increasing flash time and a dark time to flash time ratio of about 4 to 7. In flashing light work on algae Kok (73) indicated that the maximum flash yield (about 6.3 units of $O_2$ per unit of chlorophyll) was independent of temperature but was a function of dark time, flash time equal to 0.48 m.sec. and dark time varying up to 240 m.sec. In these studies experiments were reported with long flashes in the order of 1.9 m.sec. flash time, 50 m.sec. dark time. Integration effects were indicated by the results of the flash yield versus flash time being above the sloping base line representing the equivalent yield in continuous light under light saturation conditions. These curves tended to be parallel with the continuous light saturation line after about 4 to 6 m.sec. Higher yield values were indicated per

unit of flash time as the light intensity approached the saturation value. Long light-flash times, in the order of 0.2 sec. and dark times in the order of 0.4 sec., gave results which were similar to the shorter flash period results. This indicated that even longer values of flash time and dark time could produce effective light integrating growth.

In flashing light studies (71,73) the sharpness of transition from dark to light and from light to dark has been carefully defined and controlled to insure that each cell receives the same light and dark exposure. This sharp demarcation was felt to be especially important (71) for the dark period, particularly when the periods tend to be long.

Kok, in other work (72), reported similar results in growing Chlorella pyrenoidosa using about 7000 ft.c of light in the wave length of 400 to 600 mu. In all of these studies (71-73) highest efficiencies have been indicated to occur with flash time values in the order of 3 to 6 m.sec. Kok (72) indicated that maximum efficiency could only be obtained when the ratio of dark time to flash time was equal to or greater than the ratio of the incident light intensity to the saturation light intensity. Saturation light intensity has been defined, for flashing light conditions (71-73), as the light intensity at the intersection point where the maximum yield line and initial slope of the light dependent line meet. The point of intersection for these two lines (73) defines a situation where it is impossible to exceed, by any arrangement whatsoever, this limit of efficiency or rate.

Most algal studies using oxygen evolution or algal growth rates as indicators of environmental effects have indicated consistent results without reporting any difficulties with adaptation. Semenenko (74) has indicated that Chlorella pyrenoidosa needs a considerable amount of time,

possibly 10 days, to become acclimated to growing under intermittent light. The intermittency used in the studies was in the order of 10 flashes per second with a flash time of about 25 u.sec. and dark times of about 0.1 sec. Other differences in adaptation have been reported (71-73) indicating that with micro second flash times adaptation was required, with milli second (25 to 200 m.sec) flash times this adaptation did not appear to be pronounced. Phillips and Myers in their work (55,71) insured that adaptation had been accounted for in any of their growth rate expressions by plotting accumulated overflow with respect to time (55). Constant values indicated that the growth rates were valid.

Most experimental work in flashing light studies has involved very small laboratory systems and relatively shallow algal cultures. When mass algal culturing has been considered it has been on the basis of extending micro systems to macro systems. One of the conjectures has been that it would be possible to obtain the flashing light effects in dense algal cultures by rapid mixing. Theoretical aspects of this were discussed by Fredrickson et al. (75) on the premise that the rate of photosynthesis could be improved by rapid mixing but could efficiency (light integration effects) be improved? The results of this theoretical analysis indicated that it should be possible to improve efficiency of light utilization but that the amount and characteristic of the mixing action would be a very critical relationship. It was thought that a partially stirred culture might exhibit higher efficiency than one which is completely stirred.

Gates (76) in his studies with induced turbulence in mass culture indicated that more active growth rate could be stimulated by active

stirring but there was no indication of light integration or greatly improved light utilization efficiency. Echelberger (77), in his studies on higher light intensities and controlled depth-density relationships, indicated similar growth results, improved growth rates and yield, but no indications of light integration or integration efficiency. Fredrickson et al. (75) presented theoretical calculations which indicated that it should be possible to calculate and design such a facility. However, in all cases one factor was neglected. This was necessity for sharp demarcation between light and dark periods. Low light intensities during the dark might produce undesirable inhibition of the photosynthetic process and thus light integration might not occur (72).

Miller et al. (78) indicated that, in their experiments with Chlorella, efficiency was improved with mixing. This work was done with a dense culture of Chlorella pyrenoidosa in a centrally stirred cylindrical culture chamber surrounded by photoflood lamps as a source of light. They (78) showed that for varying rotation speeds there would be increased theoretical photosynthesis since the flash time would become shorter and shorter. They also indicated an increase in photosynthesis for any given flash time. The ratio of flash time over the sum of flash time and dark time was changed from a value of one-half to a value of one-quarter, in other words, the dark time was increased to about twice its value. Their experimental results were close to a ratio of flash time to total time of one-quarter. The results indicated that they were operating in a saturation range of light intensity, and their highest efficiency of light utilization was in the range of their lower intensities. In considering overall efficiency, light plus stirring power requirements, highest efficiency tended to occur at speeds in the order of 600 rpm while light efficiency tended to favor

the higher rpm, approximately 1800 rpm. This work was not compared directly with the light integration work of Phillips and Myers (71).

In mass culturing of algae there might be major problems with algal respiration, especially under conditions where long dark periods are involved. Some problem areas have been mentioned in work by Allen (79) in which he indicated that free oxygen in the substrate was not necessary for photosynthesis. However, it was found that anerobic conditions tended to inhibit the photosynthetic rate in Chlorella. From this work Allen concluded that the inhibition might be the result of metabolic products of anaerobiosis. Allen and Franck, in their work with Scenedesmus (80), found that in an anerobic system some of the mechanism associated with oxygen production could become inhibited if exposed for a prolonged time. The effect of this inhibited condition was reduced by a sequence of two short flashes. The sequence appeared to have two possibilities, a short flash and then a longer flash, or possibly a single short flash superimposed on a low light energy background. Their conclusion was that in a flashing system the number of quanta of light was not the only consideration but the time relationship of each flash was important.

## Temperature

Photosynthetic activity and algal growth are influenced by temperature in rather selective ways. In considering the effect of light and temperature there are two main areas of influence. One process involves the photosynthetic portion of any plant (photosynthizable) system and the other involves the optimum physiological temperature necessary to establish an environment desirable to the organism.

At low light intensities, photosynthesis has not been shown to be affected by changes of temperature and thus the reaction has been indicated to be purely light dependent in the lower portion of the linear range (36,82,48). However, at high light intensities, in the light saturation range, temperature has demonstrated noticeable effects on photosynthetic activities. At high intensities increased temperature has tended to raise the light saturation level (36,82). This indicates that the algae can use higher and higher light intensities when temperature is increased (78).

The effect of temperature on photosynthesis in algae under low light intensity was studied by Eddy (89) in work using Dunaliella bioculata. He developed growth curves for temperature conditions of 25°C, 30°C and 35°C. The light intensities used in these experiments were relatively low, 100 and 400 ft.c. The results for the two conditions produced similar growth curves with a tendency for the curves to separate at higher densities. In the higher light intensity (400 ft.c.) the cultures reached a higher density. Similar results have been reported by Sorokin and Krauss (56) in their studies on several algae, including Chlorella pyrenoidosa. These studies were made with light varying from low intensities up to saturation and beyond, even to light inhibition. Light intensities of 7500 to 8000 ft.c. showed growth results which indicated that there was a pronounced increase in growth rates at temperatures of 39°C as compared with 25°C. Chlorella showed an increase of about three times, at an intensity of about 1200 ft.c. in increasing temperatures from 25°C to 39°C. Light saturation intensity was found to be about 600 ft.c. at 25°C temperature and it increased to about 1400 ft.c. at 30°C.

Nielsen (60) reported similar temperature response characteristics, increased photosynthetic activity, in studies on Cladophora intenstinalis. These observations were made with temperatures of 15°C to 25°C. After being exposed to high light intensity and higher temperature, a decrease was noted in photosynthesis when returned to a 15°C environment. This rate of decrease was below the original 15°C rate. This was reported to indicate that photosynthesis, in bright light, was dependent upon temperature since the rate in high intensity light was chemical reaction limited and one or more of the enzymes associated with photosynthesis were impaired in action as a result of exposure to high light intensity.

The phenomena of increased photosynthetic capability at saturating light, with increased temperature, indicated that algae used higher and higher light intensities as the temperature increased, so long as the particular organism could physiologically withstand the increased temperature. Thus thermophylic organisms would probably have advantages with respect to growth and light utilization.

Dyer and Gafford (92) reported that a blue-green alga Synechococcus lividus was able to maintain exponential growth at a rate of nine doublings per day at 52°C and 1500 ft.c. of light intensity. In optimum temperatures and light saturation studies on this organism, it was shown that there was an increase in overall growth when temperature and light saturation values were increased. At a temperature of 48°C light saturation was 400 ft.c. but when the temperature was raised to 52°C light saturation was 1500 ft.c. Growth conditions in the 52°C, 1500 ft.c. light intensity environment, produced doublings per day which were twice the value for the 48°C, 400 ft.c. environment.

Tamiya et al. (99) demonstrated the effect of temperature on the saturation level in which a typical linear growth rate curve developed with increasing light intensity. In these experiments Chlorella was the indicator organism in a 7°C environment. Saturation occurred at this temperature at about 1 Klux (approximately 100 ft.c.). Higher temperatures, 15°C, light saturation occurred at 4 Klux (about 400 ft.c.) and a 25°C environment produced light saturation at a much higher light intensity, about 25 Klux (2500 ft.c.).

Temperature has had some significant implications with respect to fluctuating or flashing light effects. Whittingham and Bishop (83) indicated that in their work with Ankistrodesmus there was a reaction of thermal significance between two flashes of light. This was observed for temperature conditions of 4°C and 20°C. At 20°C the desirable and effective flash time spacing was about two seconds. In the 4°C environment the optimum spacing was about 60 seconds but there was about a 25% reduction in yield, compared with the 20°C condition. They concluded that the dark reaction was enhanced and thus appeared to be effective if there was a short space of time between flashes. Kok (73) in working with Chlorella in flashing light conditions indicated that the temperature of the culture did not affect the maximum flash yield, for constant flash time and variable dark time. In plotting the rate of oxygen production per flash versus the dark time (m.sec.), the initial slope of the curve was the same as obtained for saturation conditions in continuous light. The slope of this curve was steeper for the 30°C temperature than for the 15°C value, as would be expected for a saturation condition. Also increasing flash time and constant dark time, higher temperatures produced increased flash yield as the temperature increased from 15°C to

30°C. In other flashing light studies Kok (72) showed that Chlorella demonstrated a lowering of the light saturation level with decreasing temperature confirming that saturation light intensity tended to decrease with a decrease in temperature. This temperature dependent decrease of the saturation level produced decreased light utilization efficiency. The most pronounced changes took place in the 1-10 m.sec. flash time. There was little change in the efficiency in the 30 m.sec. to 100 m.sec. flash time range. Setlik (91) studied the periodic characteristics of photosynthesis in several blue-green algae. These studies indicated that temperature was exponentically related to the oscillation period, having a high oscillation period of about 90 sec. at a temperature of about 10°C and an oscillation period of about 4 sec. at 40°C, using Oscillatoria as the study organism. These oscillations were produced by short, dark intervals, using light approximately ten times the saturation intensity, for the temperature conditions being studied.

Miller et al. (78) reported on temperature effects and flashing light on Chlorella pyrenoidosa. They obtained flashing effects in a rotating system. Rotor speeds of 2000 rpm showed a doubling (oxygen production units) from 17°C to 27°C and another doubling from 27°C to 37°C. At 400 rpm the increase in oxygen production was in the order of 1.5 times, going from 17°C to 27°C, and about 1.9 times going from 27°C to 37°C. The 17°C relationship was relatively constant over the whole range of rotor speeds. The 27°C and 37°C curves increased rather markedly with increasing temperature. This indicated improved photosynthetic capability at elevated temperatures with more rapid mixing. This demonstrated that the system was being affected by light limiting factors rather than by factors of mass transfer. Increasing rotor speed

in this system improved the flashing light pattern.

Temperature has other implications on algal growth than just the effect on the photosynthetic process. Temperature will affect the physiology and the physiological response of the organisms in that there tends to be an optimum temperature for any organism. This temperature tends to vary depending upon the particular organism as well as its general environment. This type of response has been recorded by Cairns (162). He reported that the three most common organisms in clean waters, diatoms, green algae and blue-green algae, generally showed favorable growth characteristics in different temperature ranges. Diatoms grew best at 18°C to 30°C, green algae tended to grow best from 30°C to 35°C, and blue-green algae showed a preference for temperatures of 35°C to 40°C. Cairns also indicated that there was a considerable amount of temperature overlap for satisfactory algal growth, but the temperature ranges indicated tended to show dominance of one type of organism over the others at certain temperatures.

The maximum range of temperature for algal growth has been indicated (34,84) as being from 0°C to 70°C. This wide range of temperature tolerance was also indicated by Bush (101). He indicated that Chlorella has demonstrated maximum growth characteristics between 68°F (20°C) and 86°F (30°C). Most Chlorella are inhibited by temperatures below 41°F (5°C) and above 95°F (35°C). However, there are strains of Chlorella which grow well at temperatures of 102°F (39°C).

This discussion indicates that there are three general classifications of algae with respect to their temperature preference, physiologically. These ranges are the mesophylic (normal range), thermophylic (high temperature), and psychrophilic (cold temperature) range algae. Most

common algae are probably in the mesophylic range. Marre (84), in reporting on the effects of temperature on algal growth, indicated that most fresh water algae favor growth temperatures of about 25°C to 30°C, the mesophylic range. If they do grow above these temperatures they tend to show poor growth characteristics. A common example of an alga which grows in the mesophylic range (a green alga) is Chlorella. Maximum growth for this organism is in the range of 25°C to 30°C (48). Eddy (89), in working with Dunaliella bioculata reported that temperature had a considerable amount of influence on the maximum growth characteristics of this organism. Generation times (hours) were reported for a range of temperature from 25°C to 35°C. For these temperature conditions 25°C gave a generation time of 13.5, 30°C gave a generation time of 9.8, 35°C gave a generation time of 12.2. This indicated that the most favorable temperature was about 30°C because this temperature gave the lowest generation time. It was noted that during these studies the maximum population density tended to decrease from the 25°C to the 35°C cultures. Milner (98) confirmed the favorable temperature conditions for growth of Chlorella under saturating light conditions. He also reported that at 30°C Chlorella showed a 25-30% increase in growth rate at 30°C if diurnal light variation was utilized and temperature reduced from 30°C to 20°C during the dark time.

There have been indications that in any particular group of organisms, such as the green algae, there may be some strains which will grow better in higher or lower temperatures. Sorokin (100) indicated some values of growth characteristics for low and high temperature strains of Chlorella. The low temperature strains appeared to grow better at 25°C to 26°C giving a maximum of 3.1 doublings per day. The high

temperature strains grew better at a temperature of 38°C to 39°C with a maximum of 9.2 doublings per day. Careful environmental control might have increased this to 10 doublings per day. The low temperature strain represented an eight-fold increase of cellular material per 24 hours over normal strains, and 10 doublings per day represented a thousand-fold increase. In general, under natural environmental conditions, the growth rate of algae doubled every 20°F (11°C) rise in temperature (34).

Thermophylic algae are algae which show a preference for the higher temperatures of the algal growth range. There are many strains of thermophylic algae but the majority are blue-greens. Most of the blue-greens favor a growth temperature of 35°C to 40°C but some high temperature algae have been found in hot springs (84). Fogg (93) indicated that blue-green algae are more capable of withstanding extremes of temperature than most other algal forms. Phormidium oscillatoria has been found living under conditions of temperature as high as 89°C. He indicated that Nostic muscoreum had developed a maximum growth rate value, k, of 1.97 at a temperature of 32.5°C. An alga identified as having one of the highest growth rates is Anacystis nidulans, which grows at 41°C. This has been reported to have a k value (growth rate) of 8.2 ($days^{-1}$); a generation time of about two hours. Tamiya (85) reported on studies of A. nidulans at 41°C and he obtained a k value of 9.6 under conditions of continuous illumination. The growth rate obtained in these studies involved a sixteen-thousand-fold increase in cellular material per day. A rapid growth alga Chlorella ellipsoidea has been indicated to have a specific growth rate of 1.2 at 38°C for a first order rate expression in $\log_{10}$ units, or a value for k of 2.75; this gave a sixteen-fold increase in cellular material in that period of time (85). The thermophylic green alga Chlorella

tx 71105 was reported by Myers and Graham (90) to have produced at maximum yield, about 50% (grown at 35°C), greater yield than Chlorella ellipsoidea (Gerneck) grown at 25°C, its most favorable temperature. This maximum yield was obtained in conjunction with light diffusing cones projecting into the culture. It was found that the maximum yield of the high temperature strain was obtained with lower cell concentrations than for the lower (25°C) strain.

Some algae tend to grow best at extremely low temperatures. Burkholder and Mandelli (53) confirmed low temperature growth possibilities in their report of important algal productivity under extremely cold conditions, in the order of 0°C or even lower. Some low temperature algae have been found growing on the surfaces of snow (84).

Efficiency of algal growth and light utilization tends to be influenced by temperature. Tamiya (85) studied several strains of Chlorella in mass culture. Under low light intensity conditions, the highest light energy conversion efficiency, obtained at a temperature of 25°C, was approximately 24% at 2 Klux light intensity. Lowering the temperature under these light conditions reduced the efficiency to about 8% at 7°C. Under conditions of 50 Klux the same organisms gave efficiency values of 7.9% at 25°C and almost zero efficiency at 7°C. This sharp reduction could be expected because at high light intensities the optimum temperature for growth has been rather sharply defined (82). Algal yield also ($g/M^2$/12 hr) increased as the temperature increased from 7°C to 25°C and growth rate tended to increase linearly as light intensity was increased. Growth rate tended to be highest when efficiencies were the lowest. This indicated that temperature alone, under any particular constant light intensity condition, would not be the only controlling effect on efficiency. Goleuke (96)

confirmed this in his finding that both temperature and detention time affected light conversion efficiency in mixed algal cultures of Chlorella and Scenedesmus. It was shown that maximum efficiency values of about 18% were obtained at a temperature of 30°C and a detention time of one day. Longer detention times tended to produce higher efficiencies at temperatures lower than 30°C when compared with the value found in the one day, 30°C condition. Similar temperature effects, but somewhat reduced efficiencies, were reported by Oswald (51) for Chlorella pyrenoidosa from studies on light energy efficiency conversion in sewage. The temperature induced efficiencies were about 0.4% at 7°C, about 3.8% at 20°C and about 3% at 30°C. The maximum efficiency should possibly have been at a temperature of about 25°C but in the sewage the turbidity was high at this temperature and this tended to limit light conversion.

Under high light intensities, above saturation level, light tends to produce growth inhibiting conditions. Temperature plays an important part in this inhibition process in that higher temperatures will tend to produce greater rates of inhibition than lower temperatures. Kok (58), in reporting on inhibition effects on Chlorella, indicated that the inhibiting action followed a first-order relationship with respect to the photosynthetic rate and time. Two temperature conditions were studied, 38°C and 22°C. The higher temperature tended to show a slight increase in the inhibition rate at the highest light intensities when compared with the 22°C conditions. At lower light intensities the rate of inhibition was about the same regardless of temperature. In all cases the rate at which inhibition occurred was greater with high than with low inhibiting light intensities.

In many algal studies the algae have been processed extensively without much concern for the conditions preceeding certain studies. Such handling might have effect on the results and temperature could be a significant part of this. Temperature condition was reported to be very important by Clendenning and Brown (88) in algal studies where centrifugation was employed in some part of the study or processing. It was found that detrimental effects could be observed in centrifuged algae as a result of excess heat and lack of oxygen during centrifugation. Most damage appeared to be the result of too high a temperature, but the high temperature also contributed to a high respiration rate. The alga Chlorella did not show any damage under very high centrifuge values, 145,000 g., when performed at 0°C. Higher temperatures did show damage even in the temperature range of 20°C.

Under natural conditions temperature may produce significant effects other than normally expected with respect to growth, photosynthesis, and physiological changes. This type of condition has been noted by McDonnell and Kountz (27). These authors indicated that stream temperatures in the range of 5°C to 24°C, tended to produce extreme oxygen deficiencies when algae were growing profusely. They concluded that temperatures and algal respiration requirements, may, under certain circumstances, be more critical with respect to the location of oxygen sag points than the external amount of B.O.D. discharged to a receiving water.

pH

It has been indicated that pH has a considerable effect on the growth characteristics of algae depending upon the type of algae, general environment and type of nutrition. The best pH level for many algae has been indicated to be in the order of 7-8 with some variations above and below these values.

The pH developed in any growth medium may or may not be best for a particular organism but the resulting pH will be the product of the interaction of the various components of the environment. Sawyer (25) indicated that during photosynthesis aquatic plants and algae tend to remove carbon dioxide from water and this carbon dioxide tends to be replaced from the atmosphere. The atmospheric exchange is a slow process and thus, the carbon requirements of the algae will more likely be obtained from bicarbonates present in the water. Since the carbonate, bicarbonate and hydrogen ions are in equilibrium, this process affects the pH of the water. Values as high as 10 for pH in natural waters have been recorded as a result of this process. This tendency to shift the pH to higher levels may favor blue-green algae. McKinney (7) indicated that desmids preferred low pH and soft water while blue-green algae preferred high pH and harder water. Gusev (108) reported variations for many strains of blue-green algae along with many of their characteristics. In his discussions the majority of the blue-green algae mentioned favored the alkaline pH range. However, he indicated an exception, Cyanidium calbarium, isolated from hot acid springs, which demonstrated maximum growth at pH 2.0 with death resulting at pH 5.0. However, some strains of this same organism survived at a pH as high as 7.0.

Toxicants and Trace Elements

Algae and toxic materials may be classified in several groups. The major areas of consideration may be classified as control agents, metabolic products, trace nutrients and other inhibitors.

There are many agents which have toxicity to algae and thus may be utilized as control material. Krauss (122) listed an extensive number of antibiotics as well as copper sulphate as control or inhibiting materials. Some of the antibiotic concentrations were: penicillin G, 0.1 to 1000 mg/1; terramycin, 2 to 1000 mg/1; copper sulphate, 1 to 2 mg/1. The effect of most inhibitory intoxicants tended to vary with pH. Hale (39) indicated values of toxic concentrations for many microscopic organisms. For copper sulphate the general range of toxic level was from 0.1 to 2.0 mg/1 with some values going as high as 10 mg/1. Chlorine tended to be somewhat more toxic for some resistant organisms, ranges in the order of 0.3 to 2.0 mg/1.

In laboratory cultures unwanted effects have been observed as the result of toxic agents leaching from culture container materials. Dyer and Richardson (127) reported on toxicity studies concerning materials frequently used in algal culturing. They performed these experiments using a thermophylic blue-green alga, Synechoccoccus lividus, and a green alga Chlorella pyrenoidsa. Their results, at pH 7.5 and in a nitrate medium, indicated that it was difficult to evaluate potential toxicity without actually making trial assays for each material, metal or nonmetal. Some copper containing materials showed strong inhibitory action, others did not show any inhibition. Silver, tin, cadmium, nickel and several types of stainless steel did not show inhibitory effects. In the nonmetal areas the polyvinyl chlorides tended to show strong inhibitory action. However, there were some forms which did not show inhibition. Synthetic

and natural rubber appeared to be undesirable materials to use as they tended to produce strong inhibition. Nylon, polyethylene and acrylic did not produce any undesirable effects. It appeared that toxic effects were a function of pH although there was not any data reported for studies under variable conditions.

Metabolic products have been of considerable concern in algal culturing because it has appeared that algae have produced toxic materials which tended to inhibit their own growth, kill off other algae, or have been toxic to higher forms of life. This is of special concern where water or food systems might be involved.

Krauss (122) indicated the possibility that some algae produce inhibitory material, especially Chlorella. Pratt (123) confirmed this tendency in growth experiments with Chlorella vulgaris as a growth inhibiting substance was produced which was capable of diffusing through collodion membranes. This diffusable material could be destroyed by heat but it did not appear to be affected by oxidation. It was subjected to alkaline and acid extraction and it appeared that it could be more easily removed from the alkaline system than from the acid one and it was thus concluded that whatever material the inhibiting agent was, it was some form of organic phase. In other studies Pratt (125) indicated that Chlorella appeared to produce an inhibitor which accumulated as the culture increased in density and even affected subsequent cultures when the old culture cells were used as innoculum. Jorgensen and Nielsen (126) found that filtrate taken from Chlorella vulgaris, as well as from several other green and glue-green algae, when used in growth studies of Staphylococcus aureus, showed that the bacterial growth could be either increased or decreased by the filtrate. There was evidence in some situations that two inhibitors were identified.

One agent appeared to be reduced in effectiveness by illumination and the other was absorbed to Norit.

Myers (54) indicated that algal produced bacterial inhibitors might be photo-oxidized fatty acids. However, no evidence of growth limitation was reported on algal cultures up to algal dry weight densities of 50 g/l. Talling (124) concluded from experiments with Asterionella and Fragellaria that there wasn't any evidence of these organisms producing any material which appeared to inhibit the growth of the other. Thus, it appeared that if such algal or bacterial inhibiting materials were produced it was highly selective.

Another area of toxic product interest is the type of toxicant that may be detrimental to higher forms of life. Specific algae have been studied in relatively thin, dominant cultures (31) in order to evaluate their importance in producing toxic products. In these studies (Gorham) the three organisms used were Microcystis aerouginosa, Aphanizomenon flos-aquae, and Anabaena flos-aquae. From these studies it appeared that the organisms produced a fast death factor and a slow death factor. The toxicity of the toxicants varied depending upon the higher animals. In studies on bacteria there did not appear to be any growth inhibition of Bacillus subtilis, Straphylococcus aureus, Escherichia coli, and Tseudomonas hydrophilia.

There have been many indications which have confirmed that algae have produced toxic materials to varying degrees. Most algae associated with toxic occurrences in higher animals have involved blue-green algae. The algae primarily associated with such conditions have been Microcystis, Aphanizomenon, Anabaena, Nodularia, Coelosthaerium and Glosohrichia. Water treatment may not always be effective in treating water containing the

toxic metabolite produced by algae in that this material may not be removed or denatured by normal coagulation, filtration, chlorination, or even by activated carbon. Lackey (24) indicated that fish and oysters have been affected by algal blooms. Some fresh water and ocean organisms have produced blooms of toxic nature such as Gymnodinum brevis, Prymnesium and blue-green algae. Prymnesium has been indicated to be an algal form which may possibly be stimulated by sewage pollution.

In algal nutrition discussions it usually has been indicated that there may be many trace materials necessary for proper algal growth. Krauss (43) listed the chemical requirements known to be necessary for the growth of photosynthetic micro organisms. The primary requirements were C, H, O, N, T, S, Mg, Ca, Co, Cu, Fe, Me, K, Cn. In addition there were some elements which were of significance in trace amounts such as B, Mo, Si, Na, Va, Sr, Rb. It may be noted that many of the nutrient chemicals have been listed in areas of toxicity with respect to control agents or inhibiting material. There frequently has been a very narrow margin between toxicity and nutritional requirements (128).

## NUTRITION

### General

Nutritional requirements of algae may be quite varied depending upon the particular organism or the particular growth conditions. However, there are many common requirements for most algae. McKinney (7) described algae as orthotropic, microscopic plants which utilized inorganic compounds during growth. Carbon is one of the primary compounds and is obtained most readily from $CO_2$. Nitrogen is generally indicated as a common requirement and is used as nitrate, nitrite and as ammonia

nitrogen. Phosphorus is essential and is usually most readily utilized as orthophosphate. Copper compounds frequently must be available and they should be in the sulphate form. Many trace elements are required such as sodium, potassium, calcium, magnesium, tin, cobolt, molybdenum. Some of the variations with respect to media preference may be exemplified by the Euglenophyta which prefer ammonia nitrogen. Pipes (94) indicated that the general classification of nutrients might be: (a) energy, (b) macro, (c) micro, (d) growth factors.

Of all the nutrients, carbon, nitrogen and phosphorus are probably the primary ones with respect to total quantity utilized for almost all algae. Stumm and Morgan (33) indicated that the theoretical C:N:P content in a medium should be in the order of 106:16:1 if the medium is to provide a balanced ratio to meet all requirements in supporting an algal mass. MacRenthun et al. (131) reported that blue-green algae, in a natural environment, were composed of C = 39%, N = 6.1% P = 0.64%. This gave a weight ratio of 61:9.5:1. These values tended to support the balance which Bogan (42) indicated to be the required C:N:P balance.

Most natural waters contain all the nutrients, vitamins and carbon dioxide required for supporting algal growth (10). Nitrogen and phosphorus are normally the only materials which may have variable concentration and may be deficient. However, sewage plant effluent may contain these and on discharge to a receiving body of water produce appreciable nutrient concentrations (27). Carbon might be limiting under some circumstances such as in a very soft water (25,168), since carbon dioxide concentration would tend to limit photosynthesis. In water containing bicarbonate pH would tend to rise as carbon dioxide was used and thus recarbonation from the air would be facilitated by this higher pH. Nitrogen may not always be

limiting since blue-green algae may fix nitrogen from air if it is not available from other sources.

# CHAPTER II

## EXPERIMENTAL SYSTEMS

### DEVELOPMENT BASIS

The fundamental concepts which serve as a basis for the development of this algal research are relationships of dark to light ratios, need for sharp delineation of light and dark periods, light moderating effects of density, the possibility of light integration, even with mass cultures and low intensities, and the concept of efficiency measured by yield per unit of lighted area.

Light integration has been the subject of much discussion (50) and it has been shown to be theoretically possible in small systems involving high light intensity and low algal density (71,72). If effective use is to be made of this concept, some method must be found to utilize the light integration capability in mass cultures. There have been many studies which have demonstrated various growth characteristics, such as Witt (150), Yates (76) and Echelberger (77) but there has not been any indication of light integration. This study has been made to try to delineate some of the problems which may be involved in obtaining light integration in a mass culture.

The first consideration in developing equipment and in studying dark and light period increments was the ratio of dark to light time, as well as the absolute value of dark and light time, as there have been many recommended variations of dark to light ratio. Burlew (50) and Davis *et al.*

(102) indicated that for Chlorella pyrenoidosa the dark to light time ratio should be about 10. There have been other indications that the dark-light ratio should be longer (50).

Some experimental work has reported dark to light ratios of 4.5 (72). The studies for most of this work were performed at dark to light ratios of three to ten.

Another important criteria in the development of the equipment and considered important in the research is the requirement that there is a sharp line of demarcation between the light and dark sections and also such a demarcation in the dark to the light (72). This indicates that light integration and effective light evaluation could not be accomplished if definite separation is not provided. The ideal light situation is thus graphically demonstrated by Figure 1 and poor light control is demonstrated by Figure 2.

Light integration has been shown to be possible and most effectively obtained at high intensity and short flash times (72). However, there have been indications that integration might be obtained with total dark-light periods as long as 30 seconds (142).

The basic experimental criteria for this research was developed on the basis of a fairly long light time, about 0.7 seconds (velocity about 0.85 feet per second), with provisions for varying this time and velocity. Variable dark to light ratios were established in the range of three to ten and continuous light intensity was provided at about 2500 ft.c. Culture density variation was established as being desirable from very dense (170 mg/l) to very light (30 mg/l) because density had an effect on the mean effective available light per cell as a result of mutual shading (50,72).

Fig. 1. Ideal light control--flashing light.

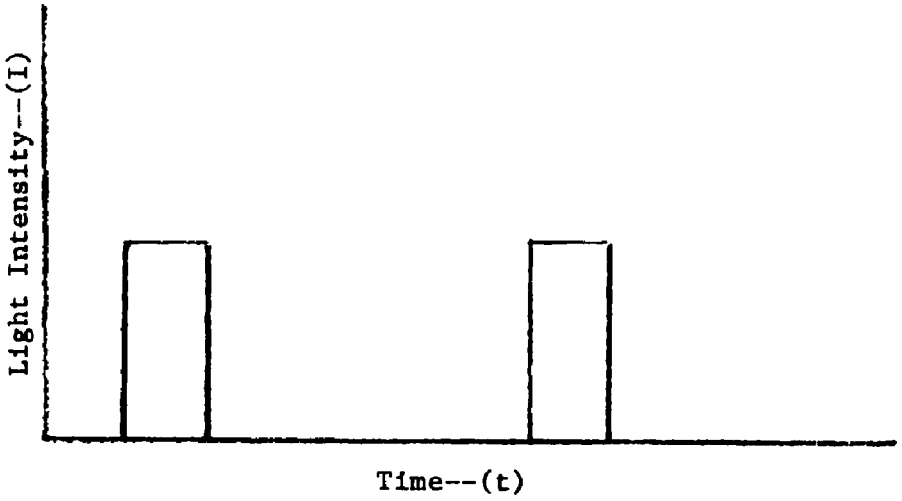
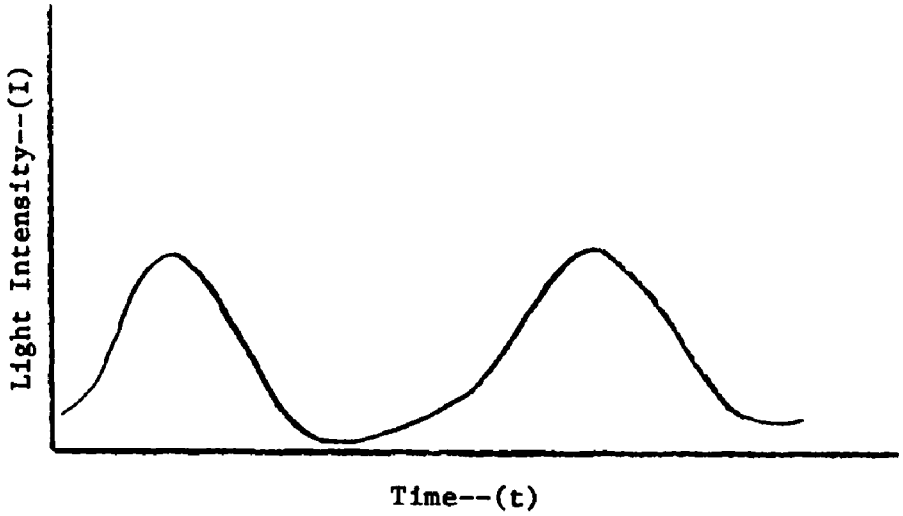



Fig. 2. Poor light control--flashing light.



## TANKS

The cultural systems used in this research were primarily of two distinct tank configurations. All tanks used were constructed of acrylic resin. The tanks were completely black except for the light window area which allowed light to enter the light cell. In most cases this light cell had a capacity of 1.1 liters.

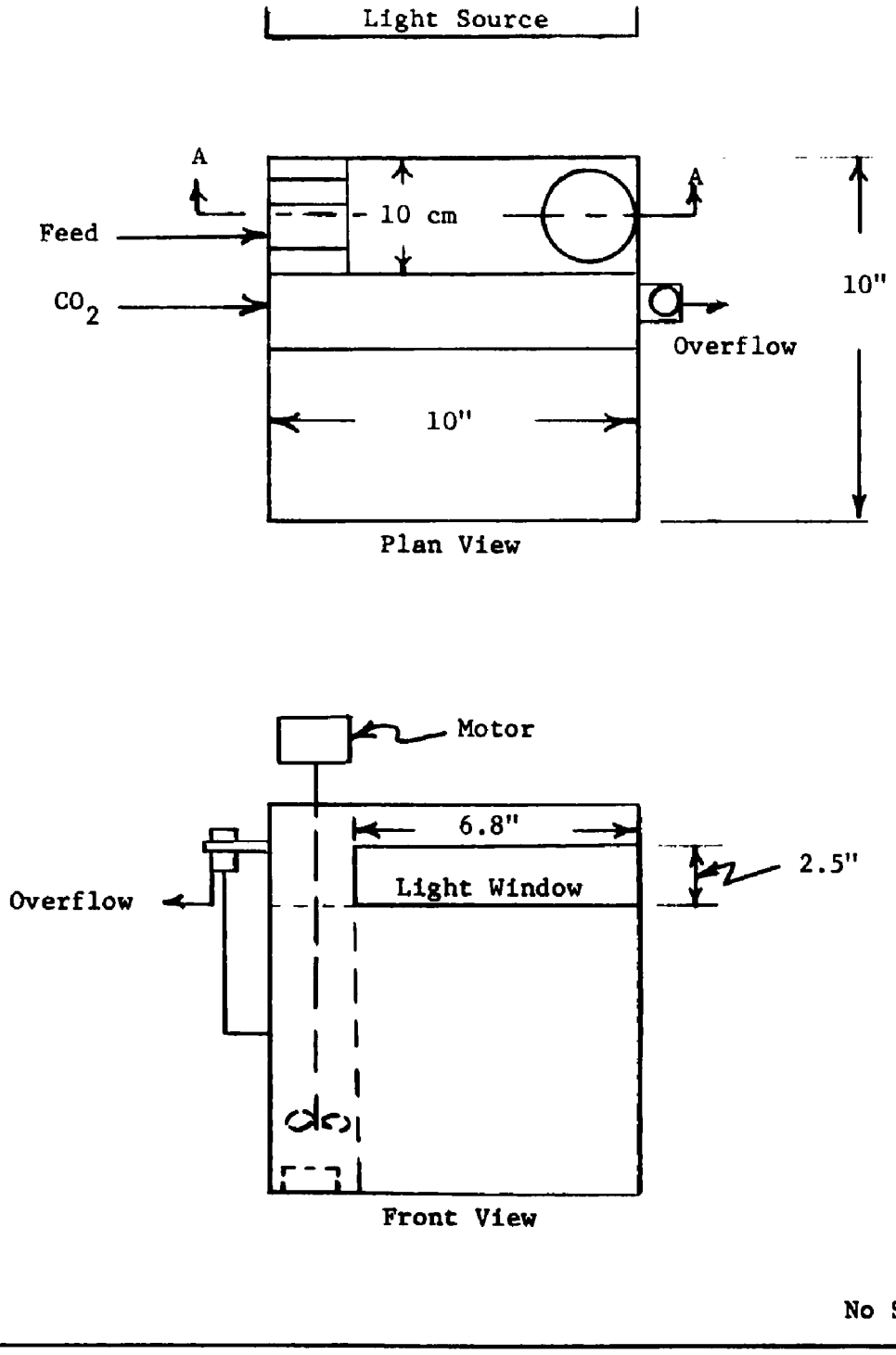
The basic culture tank system was a tank with a capacity of about 12 liters. This tank was provided with fittings shown in Figures 3 and 4 which provided control conditions for variable dark capacity and a constant light volume. For special studies the light volume was reduced to 0.56 liters with a filler block of acrylic resin.

The basic configuration consisted of a light cell (volume of 1.1 liters), inlet and outlet flow control baffles, impeller draft tube and various sized black plastic dark compartment fillers. These fillers were constructed to give varying dark time volume. These volumes were 2.65 liters, 3.75 liters, 5.30 liters, 7.75 liters, 9.45 liters, 11.72 liters.

The plan view (Figure 3) shows the relative arrangement of the draft tube, light cell, dark volume, pH meter, electrodes and photovoltaic cell compartment and supports. The photocell supporting mechanism was basically adapted from previous experimental work (77).

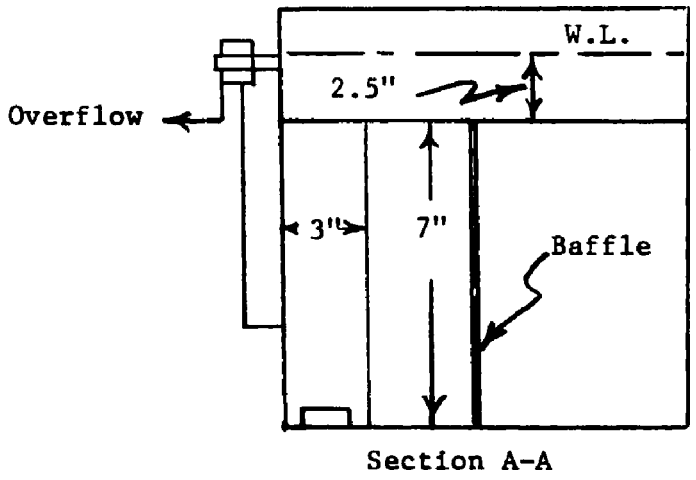
In use, this tank was located in such a way that the impeller was located in the center of the draft tube. The elevation of the tank was regulated so that the impeller blade was located at a constant elevation above the bottom of the tank.

Fig. 3. Schematic diagram of culture apparatus.




No Scale

Fig. 4. Schematic diagram of culture apparatus-- section view.




No Scale

Another basic tank configuration was used in this study. This was an acrylic tank designed primarily to evaluate constant light response. The full front and back area were of clear plastic, with photovoltaic cell supports on the back of the tank. This tank was constructed with a size equal to that of the light cell in the large dark to light tank system. No draft tube or dark volume fillers were used. Mixing was done directly in the light volume with the impeller placed in such a way that it would provide a minimum of interference with the light transmission.

## DARK AND LIGHT VOLUME CONTROL

The dark volume of the major study configuration was designed to be variable. The variation was accomplished by placing appropriate acrylic resin fillers in the space behind and under the light volume cell which provided volume variation from about 3.56 liters to about 11.5 liters. Another extensively used system provided the dark volumes mentioned under the tank discussion.

The volume variation built into the system provided incremental control for variable dark volume. Thus it was possible to obtain dark to light ratios based on the relative volumes. Since the light volume was kept constant at 1.1 liters, the ratios were 2.41, 3.41, 4.81, 7.05, 8.60 and 10.68. These ratios were selected based on indications in the literature that ratios of 5-10 would be desirable.

For some high velocity studies the light cell was partially filled with an acrylic resin filler block. This reduced the volume to 0.56 liters and provided a reduced cross-section area so that velocity would be much higher. The limiting velocity conditions were established by the rpm of

the impeller. At an rpm of about 2500 the culture tended to pump out of the tank and thus the results were not reliable.

## MIXING AND VELOCITY

Two different methods were used in these studies. In the first case the algal culture was circulated in the tank by means of a plastic coated impeller. The driving mechanism was by means of a variable speed electric motor. For any particular study condition, the speed was generally held constant in order to maintain a fixed flow condition, constant mixing and velocity, and most important of all a constant light exposure time. Under such controlled velocity conditions, the mean light detention time was about 0.7 seconds. This was obtained with an rpm of about 1730 and a three-bladed impeller with the angle of the blades set at about 15° to a plane normal to the shaft. Under these conditions the exposure time of 0.7 seconds would appear to be too large for obtaining high efficiency for intermittent light conditions. However, significant effects can be expected in this range under proper conditions as it has been reported that exposure time as high as 30 seconds might be used (142).

For most of the studies the culture was pumped from the dark compartment through the inlet deflectors through the light cell and back into the dark compartment through the outlet baffles. The deflector and baffle systems were designed to accomplish two purposes. The first purpose was to insure that light would be sharply reduced in the transition from light to dark and dark to light. It was also desirable to insure that light would not gain access to the dark compartment. The baffles and deflectors also provided flow control to insure that the flow across the light cell would have very nearly uniform velocity values at all points in the cross-

section. The placement of these thin plastic baffles was developed by careful experimentation because it was thought that good control at these points would more likely provide better light control and better data evaluation.

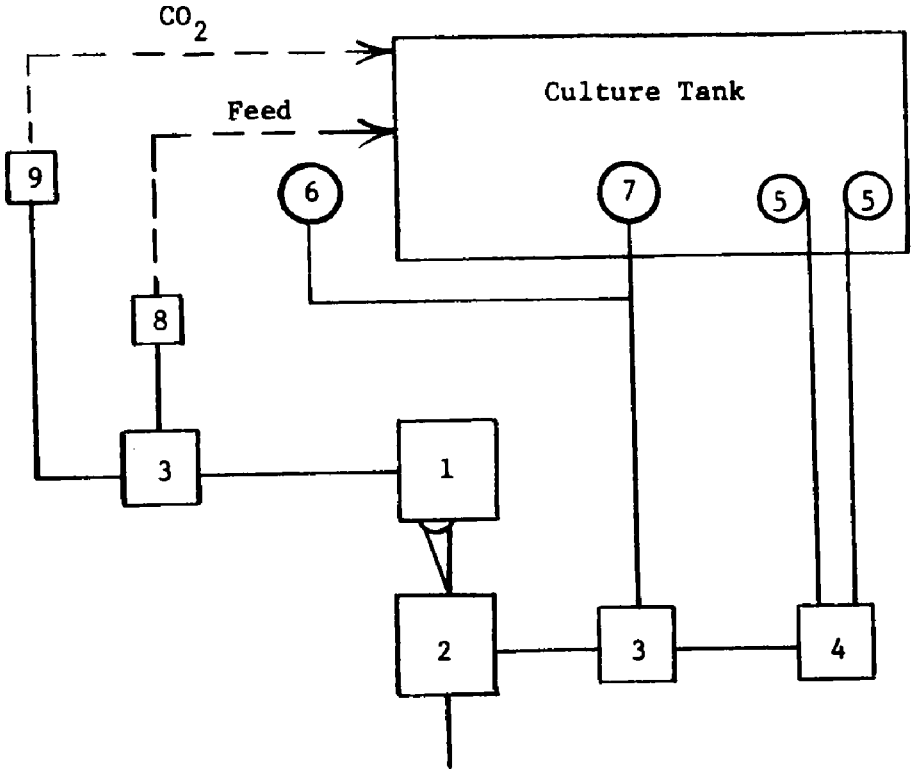
The second mixing arrangement was similar to the one just discussed except that an air motor was used. This method of driving the impeller in the culture tank draft tube was used because it could operate at higher and somewhat lower speeds than the electric motor. With the air motor it was possible to provide mixing up to 3000 rpm. The maximum value for the electric motor was about 1800 rpm.

A special mixing condition was provided for the light tank studies or light cell studies (zero dark time). This mixing was provided by means of a 6-inch stainless steel impeller having a three-bladed impeller about 1 1/2 inch in diameter. This was operated by means of the air motor at about 600 rpm. The value of 600 was selected because any value higher than this tended to produce a very high velocity of mixing and the resulting mixing action was not anywhere near the mixing action obtained at about 1700 rpm with the electric motor.

## CULTURE DENSITY CONTROL

The system used to control the culture density was similar to that used by Witt (150), Gates (76) and Echelberger (77). This system (Figure 5) consisted of two voltaic cells installed in such a way that the current induced by the light action on the cells would be opposite to the current from a reference cell. A balanced condition was set into the system by means of a variable resistance. The balanced condition was, for control purposes, the desired study density. The current reading was measured

Fig. 5. Schematic diagram of culture control system.




1 Photoelectric relay
2 Spotlight galvanometer
3 Reference switching
4 pH meter
5 pH electrodes
6 Photo cell--reference
7 Photo cell--culture
8 Solenoid--feed
9 Solenoid--$CO_2$

every seven minutes, for a period of about five minutes, by means of a one-hour clock-driven cam system. This system was adjusted so that micro switches would be actuated in such a way that one spot-light galvanometer and photo relay system could be used for the light or density control as well as for pH and carbon dioxide control. When the density control micro switches were appropriately activated any unbalance from the photovoltaic cells would be observed on the spot light galvanometer. If a sufficient amount of deflection was obtained in the galvanometer the light would shine on the minature photo cell and this would in turn actuate the media feed solenoid. The media feed solenoid had a time limiting device in the line so that it would only feed for a controlled period of time. This time could be modified to meet specific situations, but in general it was set at 10 seconds. This insured that the solenoid would not continue to feed and produce an overdiluted culture. By limiting the feed time for any particular demand there was time for efficient mixing so that the next reading would be more representative of the true density.

The solenoid control feed produced a dilution effect to reduce the density, but the quantity of liquid in the tank increased. This increase in quantity produced an overflow from the tank through the constant head overflow device. The periodic measurement of this overflow provided the information for rate of growth evaluation.

## CULTURE ROOM

The culture systems for most of these studies were maintained in an insulated, darkened, constant temperature room. The objective of this room was to provide an enclosure which would prevent stray light from entering the culture system and at the same time provide temperature control.

The darkened interior of the room helped insure that any light passing through the culture tank would be absorbed and very little would have any influence on the growth characteristics of the algae in the study system. In addition, all internal lights were turned off except when it was necessary to adjust equipment.

The constant temperature control was provided by two units. One unit consisted of an external milk cooler from which the cooled liquid was pumped through a radiator and the room air was circulated through the radiater by means of a large circulating fan. Additional cooling capacity was provided by a commercial air conditioner which was modified so that it could operate at lower temperatures than normal. This conditioner had a capacity of about 35,000 BTU per hour. The modification of the unit consisted of inserting a relay and thermostatic control in the normal cooling unit control system. The thermostat installed in the room had a sensitivity of 0.1°C and thus it was found that at times the fluctuation of cooling demand would result in blowing the cooling unit fuses. A delay relay was therefore installed in the control system so that the thermostat had to call for cooling for ten seconds before activation of the compressor relay.

Most temperature conditions for the research were maintained at 20°C in the culture tank. The light source was installed outside of the culture room and thus the heat dissipation requirement was that from the culture

tank absorbing radient energy from the lights. Direct heat from the lights and their associated ballasts did not have to be removed by the cooling system. The culture temperature of 20°C could generally be maintained with the room temperature at approximately 13°C. Summer operation usually required a somewhat lower temperature than this and winter operation was usually satisfactory at a temperature of about 14°C.

## CULTURE ORGANISM

Many types of algae have been used to study growth response under various environmental conditions as well as to study nutrient stimulation characteristics. This particular work was done primarily with a blue-green alga, Schizothrix calcicola. This particular alga was used for several reasons. It had been isolated from sewage plant effluent and thus it has potential as far as utilization in organic waste effluent applications. There have also been other growth studies made using the same organism and thus further utilization would tend to maintain a continuity of investigation.

An advantage of this organism has been evidence of desirable characteristics in the culture system. This organism seemed to demonstrate very little clumping and very little coating of the plastic surfaces in the tank. With only moderate mixing the organism did not appear to settle appreciably and thus the culture appeared to remain relatively uniform at all times. There was very little evidence of settling and accumulation in any part of the light or dark cells in any of the culture configurations.

In using this organism, it has been possible to maintain a relatively uniform and dominent culture. Most of the determinations were made with a minimum number of contaminating algal forms. Whenever undesirable

contamination appeared in the study cultures, it was possible to discard the culture and start a new one from stock cultures maintained for this purpose. The stock cultures were maintained on a high nitrate, synthetic medium. By allowing the organism to grow at high pH values, which appeared to be advantageous, it was possible to maintain 99.9% dominance. The organism was able to withstand fairly high pH conditions without too much detriment and evidently with much less detriment to the organism than for many of the other algal contaminating forms and protozoa. When contamination appeared it was frequently possible to eliminate the contamination by allowing the pH to rise to 10.5 or 11.0 and maintain it at this level for some period of time. At a pH of 11.0 it has been observed that the organism starts to show signs of undesirable growth effects and thus it can only be kept at this pH for a short period of time. However, that time increment has been long enough to greatly inhibit if not kill off most of the other organisms in the cultures.

## STOCK CULTURING

Stock cultures of the organism were maintained continuously throughout the study. These cultures were maintained by mixing liter flasks in front of two "Grow-Lux" tubes. The flasks were maintained at a distance of about 10 inches from the two bulbs. This gave a light intensity of about 450 ft.c. This arrangement provided good growth characteristics without excessive heat. However, in warm weather it was often found necessary to place the algae in a cooling tank with cold water running through the tank to help keep the temperature in the flasks at about 20°C. Higher temperatures often appeared to stimulate the growth of contaminating organisms.

These cultures were maintained on a high nitrate media. The pH was checked once a day and adjusted with carbon dioxide to a value of 7.5.

Algae from the stock nitrate media cultures were not used directly in any of the study systems. The standard medium system was prepared by centrifuging the algae from the suspension at 1500 rpm for 10 minutes. The supernatant was then removed from the centrifuged tubes and the accumulated cells were washed with distilled water and centrifuged again to remove the majority of the metabolic bi-products. This also tended to reduce, as much as possible, any effects that the stock culturing growth conditions might have had on the study medium system.

The centrifuged algae obtained from the washing process were used to start study cultures in the mixed ammonia nitrogen and nitrate medium. The washed algae were used as an inoculum in glass jugs and the mixture was aerated for several days before the culture was used for any study purposes. This allowed the organisms to become fully adapted to their new medium before experimental observations were made. This aeration was also carried out at about the same distance and light intensity from the standard bulbs as was done for the stock cultures. The density of the aerated acclimating system was checked each day and density was maintained at about 15% transmittance (425 mu) by wasting some of the culture and diluting with fresh medium. This concentration was maintained because it appeared that it provided the best growth conditions with respect to inhibiting stray algal contamination and still there was very little growth inhibition as the result of high densities.

The pH of this system was checked periodically. However, adjustment was not provided, such as adding carbon dioxide, as the carbon

dioxide from the air appeared to hold the pH at about 8.7 to 8.9. This pH level did not appear to interfere with the growth characteristics of the organism.

## LIGHT ENERGY

The lights used in this research were "Gro-Lux" fluorescent lamps which have been used in other experimental work. These lamps were arranged in a bank of 12 with a space of about 1/8" between the sides of the tubes. This arrangement was made so that it would be possible to maintain as high a light intensity as could be obtained from these tubes.

Light location from the face of the culture was studied to find the most favorable position for the best light intensity and distribution. It was found that a distance of 8 inches from the face of the tubes to the face of the culture tank gave the best results and this placement distance was used throughout the studies.

The back and sides of the holder for the fluorescent tubes was lined with aluminum foil paper to reflect the heat energy and also to help reflect the light from the backside of the tubes and thus increase the amount of light projected onto the face of the culture. This arrangement provided both a high and uniform light intensity to the face of the culture, approximately an average of 3000 ft.c. The variation of intensity was found to be negligible over the surface of the (2.5" x 6.8") light window.

The lights and their associated ballasts contributed a considerable amount of heat when they were operating. In much of the past experimental work these bulbs and their ballasts were included in the constant temperature room and thus all of the generated heat had to be dissipated

by the cooling facilities. In order to provide adequate cooling with the modified cooling equipment (lower cooling capacity) it was decided to install the light source outside of the temperature room. This was done by placing the bulbs at the predetermined distance from the face of the culture with the center bulbs aligned with the center of the culture window. This lighting holder was then surrounded with black plastic from the bottom of the holder to a height of about 9 feet. The space below the lights was enclosed with 1 inch fiberglass filters so that air circulation could be provided, but at the same time floor dust could be kept to a minimum. However, it was found that even with the furnished filters there was a considerable amount of dust which would tend to collect and thus the lights and the culture window had to be wiped clean about every day to insure that the accumulation was not of sufficient quantity to provide a significant degree of light attenuation. The whole system was sealed to the walls of the culture chamber.

The "Gro-Lux" fluorescent lamps used in this work were selected because their light quality was desirable with respect to photosynthesis. The light emission from these bulbs has been reported to be mostly in the visible range which also covers the energy range for the favorable chlorophyll absorption wave length. The peak emission wave lengths were indicated to be about 450 mu and 660 mu. The energy distribution from 400 mu to 700 mu followed the activity level of the chlorophyll synthesis process.

Variable light intensity could be provided by this arrangement. The tubes were installed in pairs with appropriate toggle switches so that two tubes at a time could be turned on or off to modify the existing light system. With new bulbs it was possible to obtain intensities as high as 3500 ft.c. However, most of the work was done with intensities of about 2500

ft.c. because it was found that this was a level of intensity which could be continuously maintained even with gradual tube deterioration. The hope was that the intensity would approach that of sunlight. However, it was found that it was not possible to arrange the lights close enough or to provide any higher intensity. Sunlight intensities have been reported to be in the order of 8000 to 10,000 ft.c. on a bright, clear day. The intensities of 2500 ft.c. for this work have thus been well below sun intensity values.

## MEDIA

The media used in this work were developed from basic media composition reported in the literature. These particular media were developed for the growth of blue-green algae and they had also been used successfully in work done by Gates and Echelberger.

For the purposes of this work the basic media were modified to provide the concentrations of the various components which would be desirable for the objectives in the two distinct situations. One medium was intended to be used as a stock culturing medium. This was provided with higher concentrations of nitrate and phosphate. The study medium was a more general medium with a combination of nitrate and ammonia nitrogen.

The high nitrate and phosphate stock culture medium was found to be useful in maintaining a dominant culture. It appeared that this alga could maintain its dominancy because it could metabolize nitrate more readily than other algae which might tend to contaminate the system. Also, this alga could apparently tolerate higher pH values than the other organisms which might be found in the stock cultures. This characteristic had been indicated by the work done by Gates, in which he indicated a maximum pH

which could be tolerated by this organism. Favorable pH levels were studied in some of Echelberger's work and he indicated that the best pH for growth was considerably higher than some of the other common types of algae.

The medium used for the actual growth studies was a modification to try and provide a solution which would be similar to a sewage plant effluent with a fair degree of alkalinity, high phosphate and moderate nitrate content. The intent was to provide a nutrient concentration which would be satisfactory for growth conditions without any nutrient deficiencies. Tables 1 and 2 show a listing of the concentration and amount of each reagent added to distilled water to make up the particular medium.

## LABORATORY ANALYSIS

Chemical determinations were made daily on all routine continuous culturing systems and as needed for the batch systems. The determinations were for $NH_3$-N, $NO_2$-N, $NO_3$-N, $PO_4$-P (orthophosphate), pH and alkalinity. The procedures used to evaluate the concentrations were according to Standard Methods (164) and generally followed the approach used by Witt, Gates and Echelberger. The procedures, as modified, are indicated in Appendix IV.

The daily chemical analyses were conducted on both the influent and effluent samples. The influent samples were obtained from the discharge nozzle of the feed control device. The effluent samples were obtained by filtering a composite from the overflow through a micro filter. This filtering operation then separated the algae from the substrate. The substrate provided a clear sample for chemical evaluation. The algae collected on

TABLE 1

COMPOSITION OF SYNTHETIC STUDY MEDIUM

| | Reagent (1) | Concentration in Stock Solution--(g/1) (2) | ml of Stock Solution Added per 1 Culture (3) |
|---|---|---|---|
| (A) | $Ca(NO_3)_2 .4H_2O$ | 5.0 | 5.0 |
| (B) | $KNO_3$ | 68.0 | 0.90 |
| (C) | $MgSO_4.7H_2O$ | 90.0 | 5.0 |
| (D) | $K_2HPO_4$ | 50.0 | 1.0 |
| (E) | EDTA-FE | * | 1.0 |
| (F) | Micro-elements | ** | 1.0 |
| (G) | $NH_4Cl$ | 30.6 | 5.0 |
| (H) | $NaHCO_3$ | 55.0 | 5.0 |

*26.1 g EDTA, 268 ml 1.0-N KOH, and 24.9 g $FeSo_4.7H_2O$/1

| **Reagent | Concentration (g/1) |
|---|---|
| $H_3BO_3$ | 2.86 |
| $M_n Cl_2.4H_2O$ | 1.81 |
| $ZnSO_4.7H_2O$ | 9.222 |
| $MoO_3$ (Molybdic acid, 85%) | 0.0177 |
| $CuSO_4 .5H_2O$ | 0.079 |

TABLE 2

COMPOSITION OF SYNTHETIC ALGAL STOCK-CULTURE MEDIUM

| | Reagent (1) | ml of Stock Solution Added per 1 Culture (3) |
|---|---|---|
| (A) | $Ca(NO_3)_2 .4H_2O$ | 10.0 |
| (B) | $KNO_3$ | 10.0 |
| (C) | $MgSO_4.7H_2O$ | 5.0 |
| (D) | $K_2HPO_4$ | 12.0 |
| (E) | EDTA-FE | 1.0 |
| (F) | Micro-elements | 1.0 |
| (G) | $NH_4CL$ | 0.0 |
| (H) | $NaHCO_3$ | 5.0 |

the filter, which had been previously dried and tared, provided a means for determining dry weight concentration of the algal suspension.

## PHYSICAL OBSERVATIONS

Physical observations of the study systems were made daily during any particular study period. The observed values were primarily for light intensity, impeller speed, culture temperature, culture pH, algal count, culture density as percent transmittance and as dry weight of cell, and overflow from the constant density cultures.

### Light Intensity

The intensity of the light on the light cell was routinely measured by making an observation at the center of the cell window. This reading was taken with the illumination target held vertically outside the culture tank and the constant temperature culture room. This appeared to be the most practical way to obtain routine readings. The equipment used in making these readings was a Western Illumination Meter, model 756, equipped with a Western Illumination Target.

It was necessary to consider the light correction with respect to a point observation. It was found that the correction for relative displacement and transmittance through the plexiglas, to give the light intensity on the inside culture face, was about 0.90. This correction factor appeared to vary with respect to the number of bulbs being used and observations were made to consider all possible combinations. The size of the light window was such that there was very little variation over the surface of the clear opening and thus a correction was not necessary for area distribution. A more significant correction was necessary to

provide for evaluation with respect to light restriction in depth of the culture. This was especially true for some special determinations and studies where light area was further reduced.

Impeller Speed

Velocity was a very important determination for these studies. Since velocity could not be measured directly during the actual exposure periods, the rotating speed of the impeller was used as the primary control and observation criteria. This rpm could then be correlated with the various velocities.

The speed of the impeller was obtained by means of a strobotac. This instrument was a General Radio Co. strobotac, type 1531-A, with a range capability of 100 to 5000 rpm.

Most of the time the impeller was driven by an electric motor with a variac control and thus the rpm was very constant. Therefore, observations of impeller rotation were made only once a day, after it was established that more frequent readings were not necessary to obtain a good speed and velocity representation. In the systems where an air motor was used it was found that there was somewhat more fluctuation and observations were made between 10 and 20 times a day, at approximately hourly intervals. The mean rpm was obtained from this information by means of graphic evaluation on normal probability paper.

Culture Temperature

The temperature of the culture was observed once a day by means of a mercury thermometer immersed in the end of the light cell compartment. It was determined that it was not necessary to make readings more frequently than each day because the culture room temperature control

usually held the system in a range of 20°C ± 0.5°C. More frequent readings were made when a new culture system was being started. The greater frequency was necessary as variation of light intensity and/or mixing speed would change the thermostat control setting requirement for maintaining the desired temperature.

Culture pH

The pH of the culture was determined in two ways. Relatively continuous monitoring of the pH was provided by an electric pH meter with the electrodes immersed in the end of the light compartment. Continuous pH readings could be observed on the indicating dial of the meter and for about every 5 minutes out of a 14-minute period the pH was monitored by the carbon dioxide control system. Fluctuations in pH were thus corrected about every 7 minutes, as needed, by adding carbon dioxide and thus reducing the pH to the desired level of 7.5. The functioning of the electric pH meter was also checked by measuring the pH of the samples selected from the system and also the pH of samples from the overflow. Samples were only occasionally taken directly from the culture tank for pH check, when it was suspected that the overflow pH relationship did not appear to correlate well with the indicated value on the continuous culture meter.

In batch culture studies the pH was obtained as needed. In general these observations were made about once a day. An electric pH meter was used to determine the appropriate values.

Culture Density

The evaluation of density of algae in the culture was accomplished by three methods: count, percent transmittance and dry weight of cells. The dry weight indication appeared to be the best indicator of true culture

characteristics. However, the percent transmittance relationship was the primary routine control system for regulating the overall density. It has been shown that throughout the study there was a reproducible and dependable relationship maintained between dry weight density and percent transmittance as shown in Figure 6.

The density evaluation by means of algal count was obtained by taking a well mixed sample from the accumulated overflow sample and counting the number of organisms in a haemacytometer. For the denser cultures, dilution with distilled water was frequently necessary. The intent was to try to maintain a count of about 100 organisms per major field of view in the haemacytometer. Five fields of view were used for the standard count. A new sample was selected from the sample for each field of view, thus giving a statistical distribution of counts for determination of the mean count. In counting this long filamentous alga each filament was counted. The length of the alga tended to be between 10 and 100 microns in length. Table 3 is a tabulation of the correction factors applied for counts obtained for varying dilution factors. Results were expressed as $10^6$/ml. The standard count relationships were obtained using Equation 11.

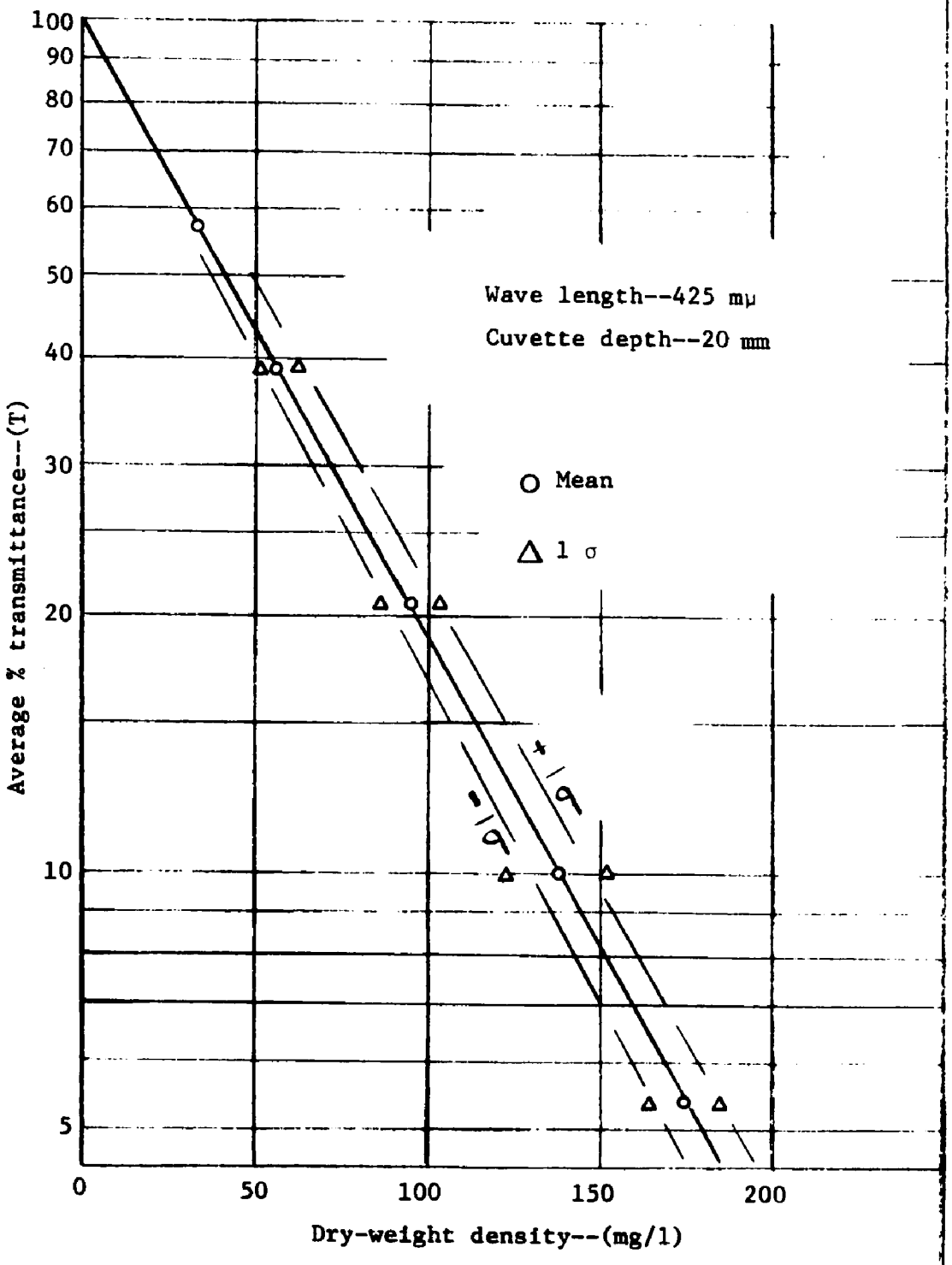
$$\frac{(D.F.) \times 10^4}{\#\ Fields} = \#\ cells/ml \qquad [11]$$

TABLE 3

ALGAL COUNT MULTIPLIER FOR FIVE FIELDS

| D.F. | Multiplier |
|---|---|
| 1 | $0.2 \times 10^4$ |
| 2 | 0.4 |
| 3 | 0.6 |
| 4 | 0.8 |
| 5 | $1 \times 10^4$ |

Fig. 6. Percent transmittance versus dry-weight density.



The percent transmittance method of evaluating density was determined for each sample by taking a well-mixed sample, placing it in a 20 ml depth spectrophotometer tube and comparing its percent transmittance at 425 mu against a distilled water blank.

The dry weight evaluation was obtained similar to the manner used by Gates except that precut grid filters (Type RA 1.2 ± .05 millimicron pore size) were used. This insured the continuous utilization of the correct direction of filtration.

pH Control and Carbon Dioxide Feed

The pH of the study cultures was controlled by means of passing the current from the output of the recording pH meter through the cam operated micro-switch timing device and the spotlight galvanometer and the photo-relay system. This meant that the alternate seven-minute period, alternating with the light sensing time, would measure the unbalance or current output from the meter. This could be provided by the fact that the meter had a zero response at a pH of 7.0. Growth of the culture would raise the pH and thus the increased pH would provide sufficient (at about .05 pH units above the reference level) signal current to deflect the spotlight galvanometer and, by means of the photo relay, actuate the solenoid which controlled the carbon dioxide feed.

The carbon dioxide was obtained as 100% commercial soda fountain compressed gas which was passed through a reducing pressure regulator. The outlet pressure from the regulator to the culture system was maintained at about 11 pounds per square inch. This appeared to give the most desirable regulation.

Carbon dioxide was fed to the culture tank through plastic tubing from the solenoid to a small 1/2 inch diffuser in the dark compartment, just beyond the outlet baffles from the light cells. This point of application was selected because the pH electrodes were placed just above the outlet baffles at the end of the light compartment. Thus this arrangement insured that there was a maximum amount of mixing of the carbon dioxide in the solution before reaching the electrodes. This minimized pH fluctuations and the system maintained a constant pH of 7.5 ± 0.05 throughout most of the studies.

The carbon dioxide served a dual purpose in that it also provided necessary carbon for phctosynthesis in addition to maintaining the desired pH level.

## BATCH CULTURES

The batch culture studies were primarily made for special experimental systems and various conditions of mixing, pH control, media feed and light exposure. The specific characteristics of any study have been discussed with the particular study. In general, most of these studies were conducted in flasks using magnetic stirring bars for agitation. The flasks were exposed to the same light intensities, usually about 400 to 700 ft.c.

The nutrient media used in these studies varied from time to time. The general media used was similar to the normal study medium except that at times all nitrate or all ammonia nitrogen were the only forms of nitrogen. For some studies excess bicarbonate was added to try and maintain a sufficient amount of carbon dioxide without adding a further amount of carbon as gaseous carbon dioxide.

Chemical and physical observations were generally the same as those utilized in the continuous cultural studies. The only additional evaluation was the carbon dioxide determinations for the special carbon dioxide balance studies.

## SPECIFIC GROWTH RATE

This discussion concerns the evaluation of the specific growth rate used in the algal studies. This rate was evaluated as k from the 24-hour overflow data and the culture volume.

For purposes of evaluation the general relationship is shown in Equation 12 (55) and previously discussed in Equations 7 and 8.

$$k = \frac{V}{V_s t} \qquad [12]$$

The terms in Equation 12 are:

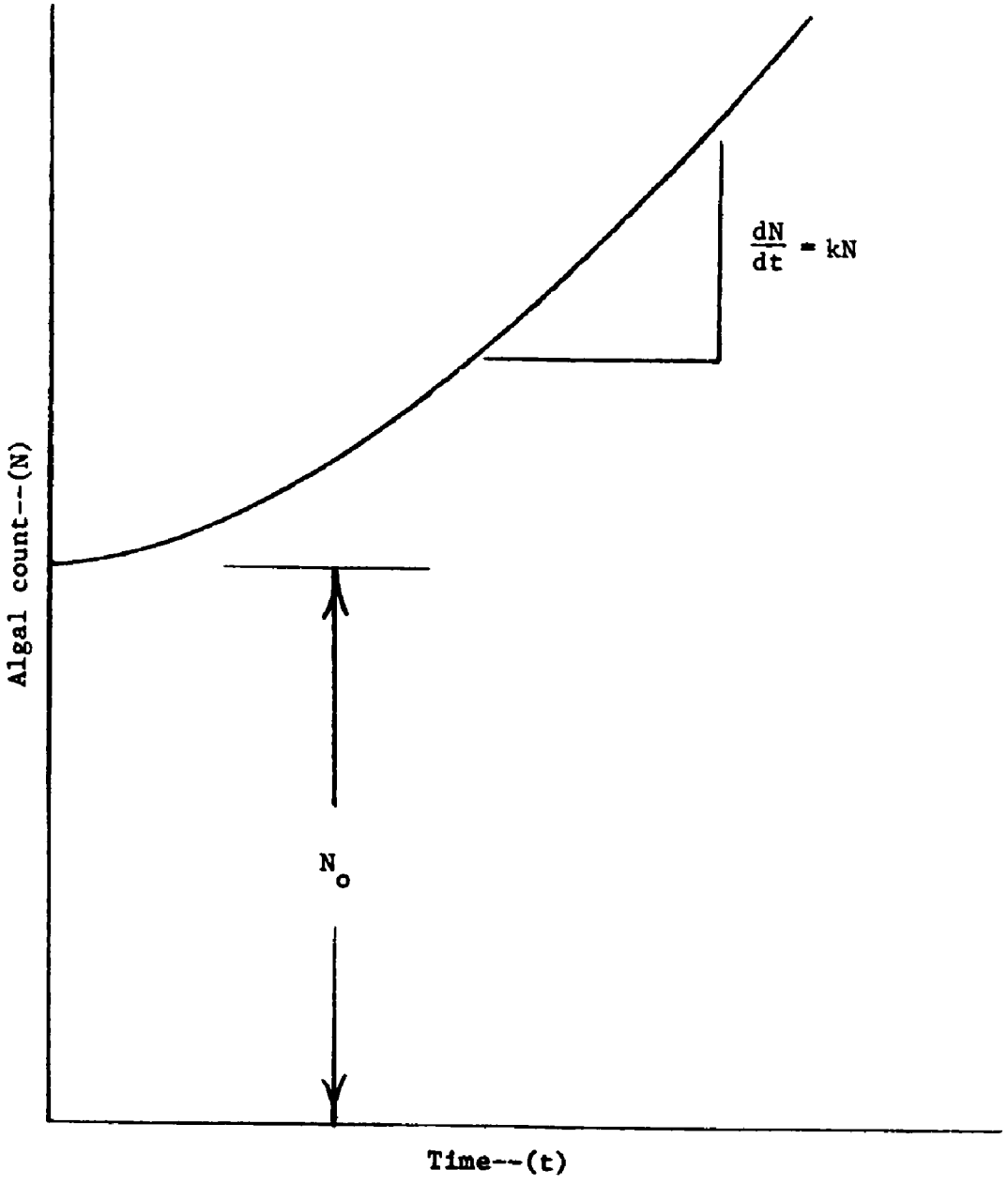
$V$ = overflow (liters)

$V_s$ = culture volume (liters)

$t$ = time (days)

The interpretation used in the culturing conditions for this research was based on representing the growth by a first-order reaction equation. This equation situation could be represented by Figure 7 where we start with some initial number of algae $N_o$ and the number will be increasing with respect to time. Thus $\frac{dN}{N} = kdt$ or at any time t the initial number for the next increment will be equal to $N_t$. Integrating this we obtain the relationship

Fig. 7. Algal growth versus time--theoretical curve



$$\ln N_t = kt + c \qquad [13]$$

and when $t = 0$, $N_t = N_0$. Thus

$$\ln N_t = kt + \ln N_o \qquad [14]$$

or, for purposes of accurate calculation,

$$k = \frac{\ln N_t - \ln N_o}{t} \qquad [15]$$

with t expressed in days.

For the culture conditions used in this research the situation could be expressed as shown in Figure 7 with an increment condition, such as $\Delta t = 15$ minutes, which would be realistic with respect to the actual situation. Initial conditions were when $t = 0$, $N = N_o$ with a concentration of $\frac{N_o}{V_s}$ and then at $t = 15$ minutes (0.010417 days), $N = N_t = N_0 + \Delta N$. This situation may be physically expressed in the culture system by the fact that, in about this period of time, the culture density increased enough ($\Delta N$) to actuate the feed solenoid and thus the culture was diluted and the $\Delta N$ removed to maintain the initial number or amount of organisms $N_o$ and also the concentration $\frac{N_o}{V_s}$. This may be expressed as shown in Equation 16

$$k = \frac{V_o}{V_s} \qquad [16]$$

in terms of $V_o$ (overflow in 1/24 hours) and $V_s$ (culture volume in liters) and thus this value of k should correspond to the value calculated by the first order Equations 8 and 15.

A hypothetical situation of culture, overflow, volume and density would be as follows:

volume of culture = 4.0 liters (constant)

volume of overflow = 1.0 liters per day

culture density of 40 mg/l (approximately 60% T)

Also for purposes of this discussion the increase in N will be drawn off every 15 minutes.

In the culture the amount of algae will be 40 mg/l x (4.0 l) which is equal to 160 mg of algae in the culture system, which represents a specific number of organisms. In the 1.0 l/day of overflow, with a concentration of 40 mg/l there will be 40 mg of algae produced per day. The hourly production of algae will thus be 1.665 mg/hr (or per 0.0417 days) and dividing this by 4 gives a value of 0.4163 mg/15 min (or per 0.010417 days). If we use this information the specific growth rate may be calculated as follows:

$$k = \frac{\ln N_t - \ln N_o}{0.010417} = \frac{\ln 160.416 - \ln 160.000}{0.010417}$$

$$= \frac{5.07776 - 5.07517}{0.010417} = \frac{0.00259}{0.010417} = 0.2485 \ (\text{days}^{-1})$$

Overflow basis:

$$k = \frac{V_o}{V_s} = \frac{1.0}{4.0} = 0.25 \ (\text{days}^{-1})$$

Thus, this shows that the log calculated value of 0.2485 and the volume calculated value of 0.25 agree on a daily basis and this numerical value is equal to the theoretical growth constant.

The increment of time of feed would affect the calculation of the constant, and some values have been calculated to estimate the amount of this effect. The results of such calculations are shown in Table 4. This table shows that fairly long periods between feedings could occur without

seriously affecting the calculation of the specific growth rate. Even with three hours of growth between feeds, or dilution, the variation in the growth rate would be less than 2%.

TABLE 4

COMPARISON OF CALCULATED GROWTH RATE WITH VARIATIONS OF FEED TIME

| Time Between Feed (hours) | k (algal wt basis) | k (overflow volume basis) | Percent Variation | Culture Density (mg/l) |
|---|---|---|---|---|
| 0.25 | 0.249 | 0.250 | 0.4 | 40 |
| 1.0 | 0.248 | 0.250 | 0.8 | 40 |
| 2.0 | 0.247 | 0.250 | 1.2 | 40 |
| 3.0 | 0.246 | 0.250 | 1.6 | 40 |
| 0.25 | 0.1249 | 0.125 | 0.08 | 160 |
| 3.0 | 0.1240 | 0.125 | 0.8 | 160 |

With a constant density and constant feed culture system the growth rate may also be calculated by means of volume and algal count, assuming that the algae maintain a uniform size and shape.

The volume calculated specific growth rate is an accurate representation of the rate as compared with the theoretical exponential growth rate calculated on the basis of weight, count or volume. This condition is true so long as there is steady-state growth, and thus a uniform overflow condition. This steady-state overflow can and has been shown to exist for this research as shown in Figure 42.

# CHAPTER III

## EXPERIMENTAL RESULTS AND DISCUSSION

### GROWTH STUDIES

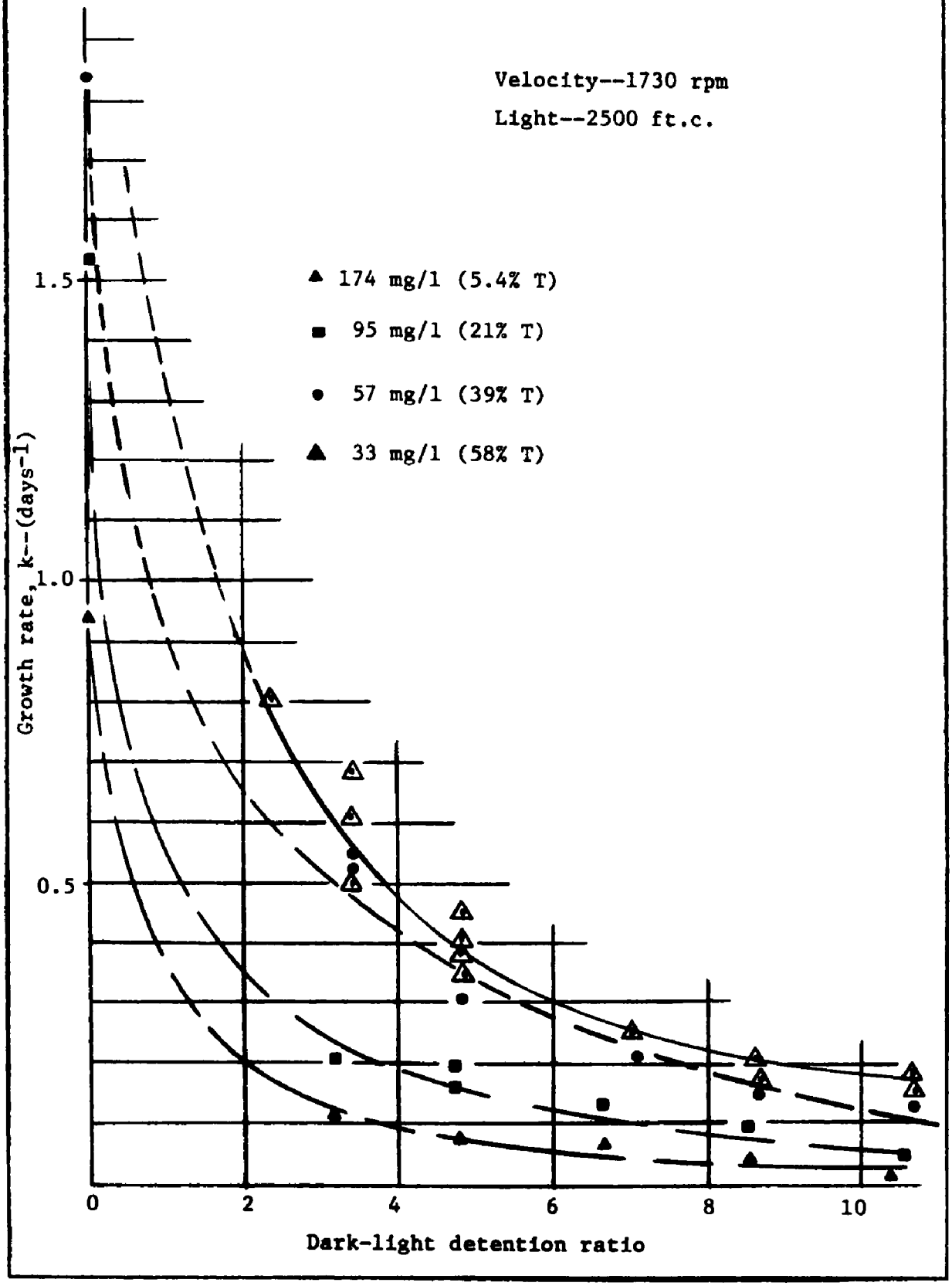
#### Dark and Light Ratio

The growth rates for 5.4%, 21%, 39% and 58% transmittance algal study cultures have been plotted with respect to the dark-light detention ratio configurations. These results are shown in Figure 8 in which the growth rate values (k) are very low at dark-light detention ratio values of about 10, increasing to larger values at dark-light detention ratios of about 3. Pronounced increases in growth rate are shown at zero dark-light detention ratios (continuous light conditions).

The curvilinear representation in Figure 8 was fitted to the data on the basis of best fit, and the apparent zero integration growth rate curve shown in Figure 37. Therefore, these curved response curves could be expected for any culture density, in the range of dark-light detention ratios of 10 to 0, as this condition, shown by Figure 37, represents a situation whereby more and more effective light becomes available for any constant velocity condition.

Each density curve, from 5% - 60% transmittance, shows an increase in growth rate for any particular dark-light detention ratio. This increase is the result of increasing effective light as a result of the reduction in density and thus the reduction in shading effect throughout the culture depth. From this information it was decided that the most favorable light conditions for further studies would be the 60% transmittance cultures

Fig. 8. Growth rate versus dark-light detention ratio.



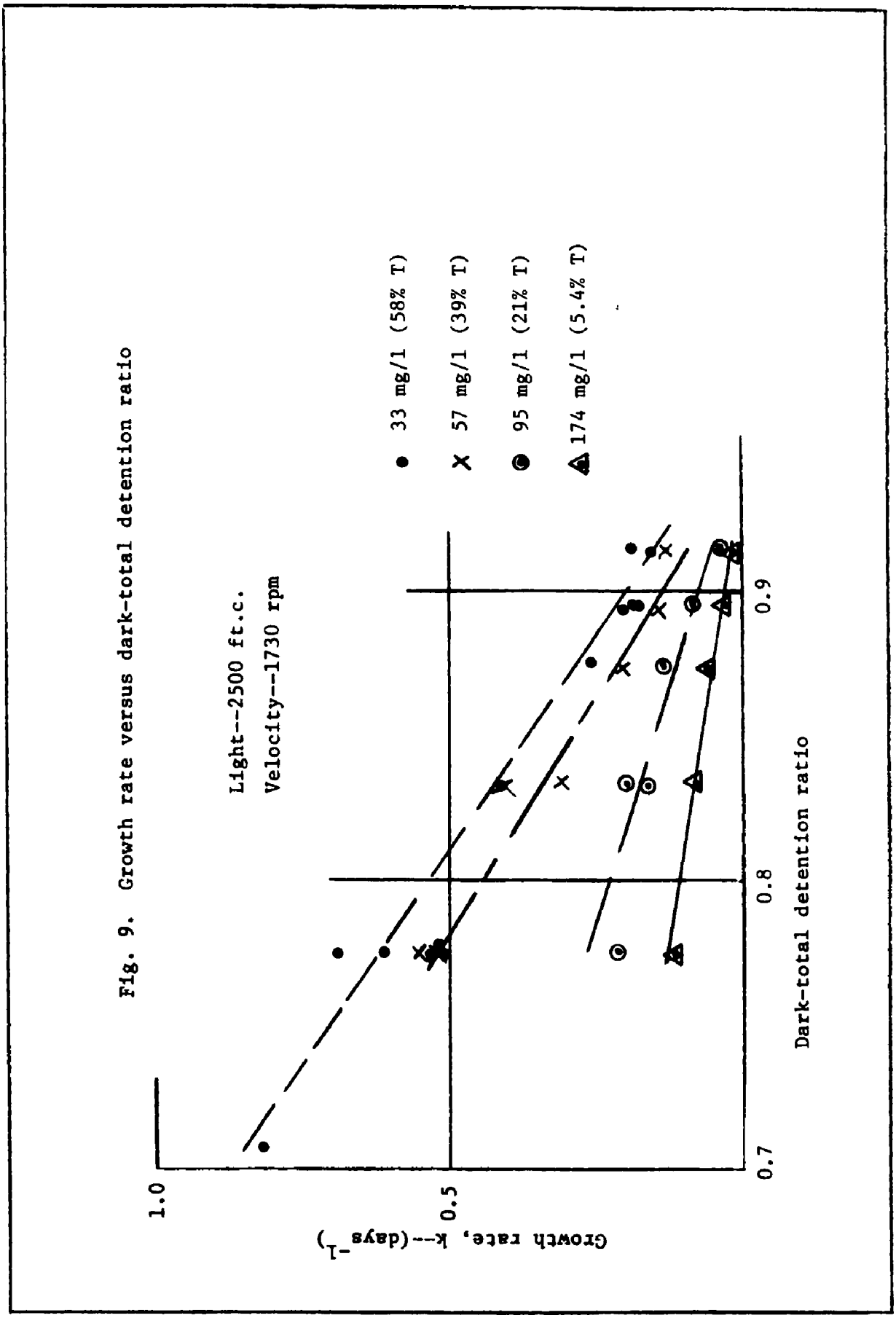
because this would provide the most light intensity for effective velocity evaluation and possible integration evaluation.

It is apparent from these studies that the dark time is advantageous in the lighter culture densities, such as the 39% and 58% transmittance cultures. This advantage is primarily the result of the high light intensities. In the lower percent transmittance values the culture density greatly reduced the mean light intensity.

The growth characteristics observed in the lighter density culture systems could be expected because it has been reported (71,72) that integration studies, using variable dark time values, have usually been conducted at intensities in the order of ten times the maximum intensity for maximum growth rate. The intensities for such reported studies have also been for relatively shallow cultures and thus each cell would normally receive about the same amount of high intensity light. The mass culture results, shown by these studies, would thus show some variation because the organisms would receive varying light intensity as a result of the distance from the source and from shading effects.

The growth rate with respect to controlled dark and light conditions may be further investigated by comparing the resulting rate of growth with respect to the ratio of dark time to total time, as shown in Figure 9. The zero integration growth rate versus dark-total detention ratio is indicated in Figure 38, and this shows that the zero integration growth limit plots as a straight line under these conditions.

In Figure 9 the growth rate values have been plotted with respect to dark-total detention ratio for the 5.4%, 21%, 39% and 58% transmittance cultures. For the 5.4% and 21% transmittance cultures the relationship


Fig. 9. Growth rate versus dark-total detention ratio
Light--2500 ft.c.
Velocity--1730 rpm
33 mg/1 (58% T)
57 mg/1 (39% T)
95 mg/1 (21% T)
174 mg/1 (5.4% T)
Growth rate, k--(days$^{-1}$)
1.0
0.5
0.7
0.8
0.9
Dark-total detention ratio

has been plotted as a straight line from a ratio of about 0.9 to 0.7 (dark-light ratio of about 2.5 to 10).

Figures 8 and 9 show favorable growth characteristics in the dark to light ratio range of 2.5 to 3.5 (dark to total ratio of about 0.72 to 0.79). This effect served as the basis for selecting the configuration giving a dark to light ratio of about 4.7 and for using a density of 58% transmittance for most of the subsequent studies.

Velocity Effects

In the studies shown for Figures 8 and 9 light intensity and velocity of mixing were kept constant throughout. Therefore, to evaluate growth rate response more complete velocity studies were made utilizing the density and dark to light information which indicated that in most cases a dark to light ratio of 4.7 (Configuration II) and a culture density of 58% transmittance would be the most desirable conditions.

The routine observation relating velocity was obtained by recording the rpm of the mixing device and thus it was necessary to study the velocity response for the various configurations if meaningful velocity values were to be utilized in the evaluation.

Figures 10 and 11 represent the results of such velocity studies and show the relatively consistent response of the various configurations. These results indicate that for most conditions the mixing characteristics were relatively the same for a constant rpm so that results could be reasonably compared if introduced in the analysis. For some purposes the rpm value of about 1500 would have been desirable because the velocity with respect to rpm was just about equal for all situations. This was, however, too low a velocity in most cases for desirable mixing conditions.

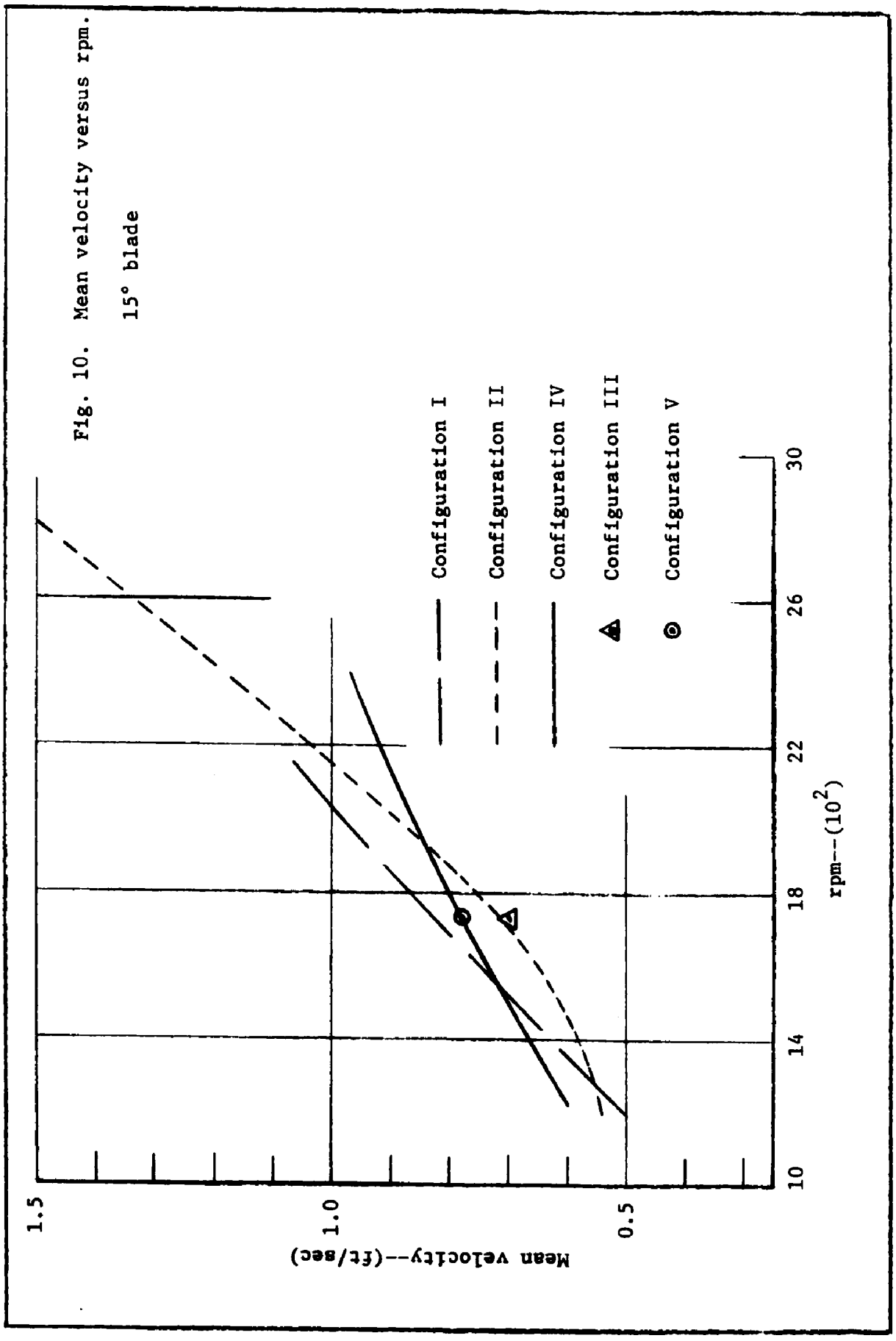



Fig. 10. Mean velocity versus rpm.

15° blade

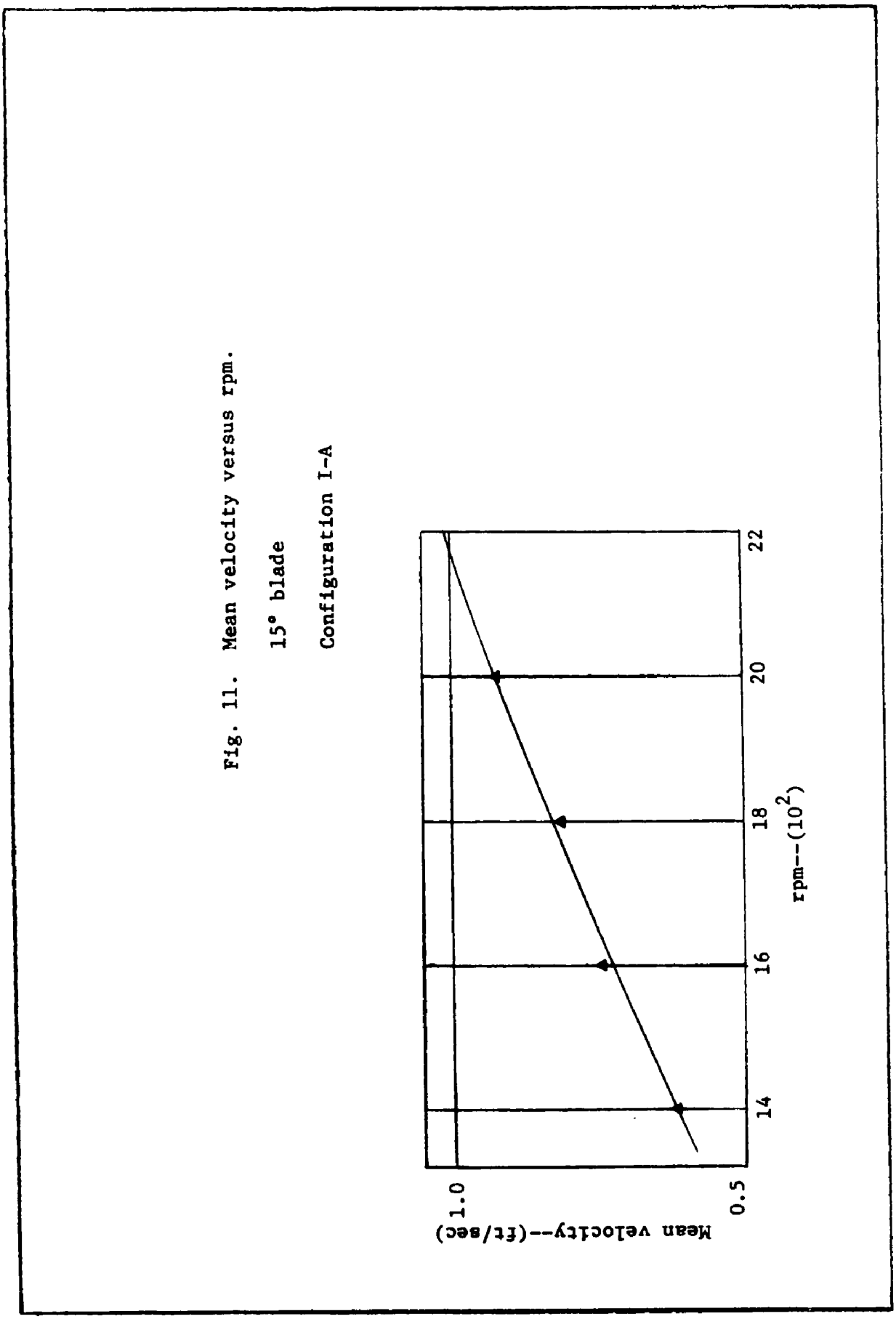



Fig. 11. Mean velocity versus rpm.

15° blade

Configuration I-A

The higher mixing rate conditions tended to produce a sufficient amount of divergence in velocity response so that the velocity had to be related for each configuration and rpm condition.

The growth rate versus velocity effects are shown in Figure 12 for the various velocity conditions studied. These curves show that there is a pronounced improvement in growth rate with respect to increased velocity. Velocity values, for these configurations, of about 0.6 ft/sec or less gave relatively constant growth rates. Values of velocity of 0.8 ft/sec and greater provided the most pronounced improvement for constant light and density conditions.

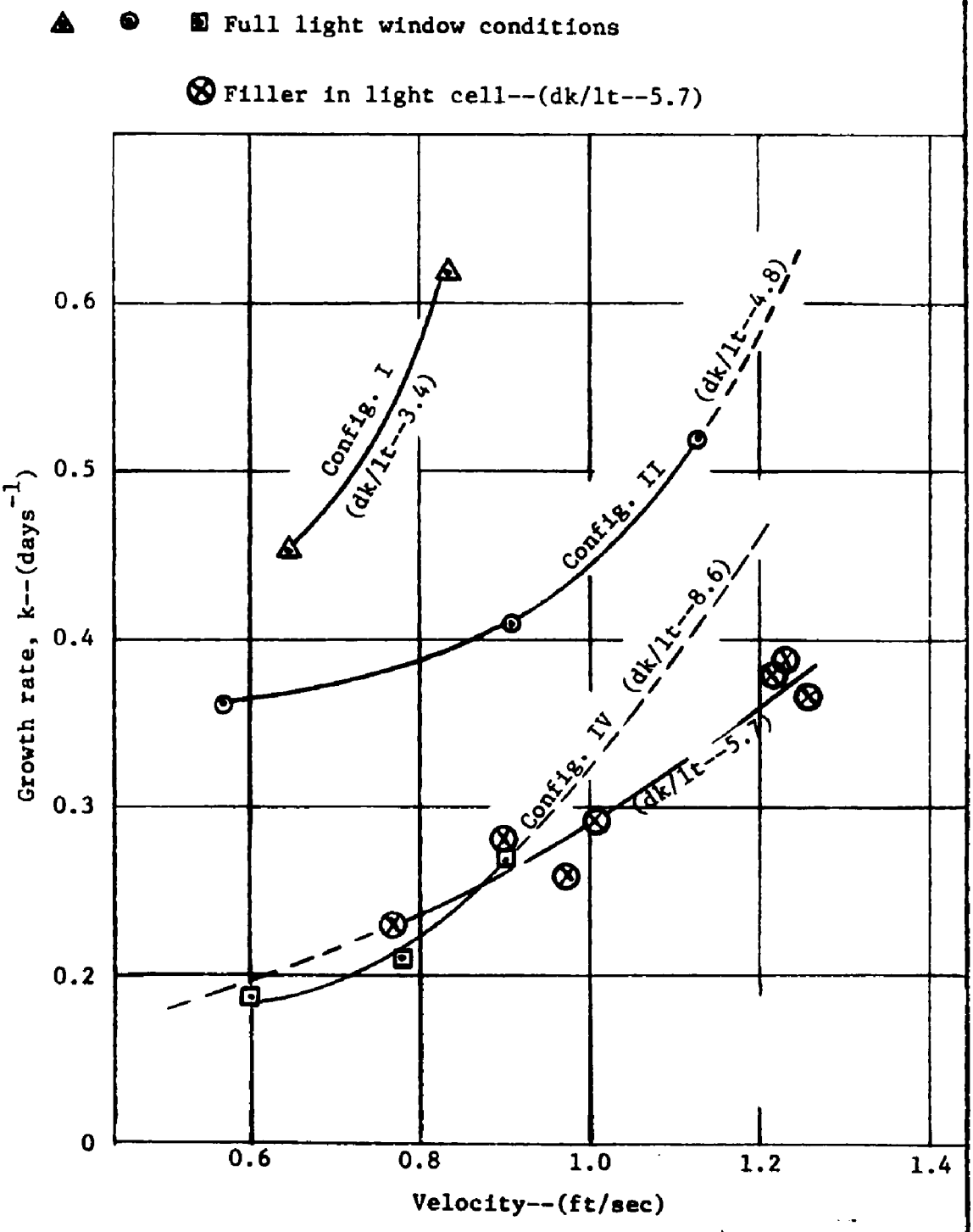
The increase in growth rate for the various configurations, for any particular velocity condition, is the result of increased effective light as the dark-light detention ratio was decreased with decreasing configuration designation. This is consistent with the results observed in Figures 8 and 9 where growth rate increased with decreasing configuration designation, velocity constant.

The tendency for the curves to become somewhat steeper for the higher light intensity conditions (lower dark-light detention ratio values) would be expected because higher velocity is more effective in stimulating growth rate when the light intensity is higher than when the intensity is low.

The light and velocity relationships were emphasized by the results of a special study, shown in Figure 12, as the dark-light detention ratio curve of 5.7. This special study was made to try to obtain some higher velocity conditions using a modification of Configuration I in the same light and culture density conditions. This configuration normally had a dark to light ratio of 3.41. For this particular study the ratio was

Fig. 12. Growth rate versus velocity--
several dark-light ratios

Light--2500 ft.c., 58% T



modified by placing a filler block in the light cell which reduced the cell volume to about a half of its original volume. This arrangement resulted in a dark to light ratio of 5.7, a total volume of 3.76 liters, and a light window area of 0.005 square meters. This new arrangement provided for continuous operation at velocities in the range of 1.1 to 1.25 ft/sec.

The results for this study show the same general trend of increasing growth rate with respect to velocity, up to about 1.3 ft/sec. However, this growth rate curve is considerably lower in value for the dark-light detention ratio of 5.7 as compared to Configuration I (dark-light detention ratio 3.41) as well as Configuration II (dark-light detention ratio 4.81) and Configuration IV (dark-light detention ratio 8.6). The first impression would be that the results do not agree. However, there is an additional factor which has to be considered, and that is that the filler block in the light cell influenced the total amount of light available to the algae in the culture.

This effect was studied and a comparison was made of light availability from the front of the culture to the back of the culture for Configuration I, no light filler block, and Configuration I, with the light filler block, as shown in Figures 13 and 14. These values were obtained for all possible combinations of lights but the most important ones are shown in the figures. Figure 13 shows the variation when the filler block was not present. In this situation the higher light intensities tend to agree with the expected square of the distance variation, but with a considerable decrease from the expected relationship in the last five centimeters of depth. At low intensities the light distribution agrees almost exactly with that expected by considering the distance effect.

Fig. 13. Light intensity versus depth for several light configurations--no cell filler

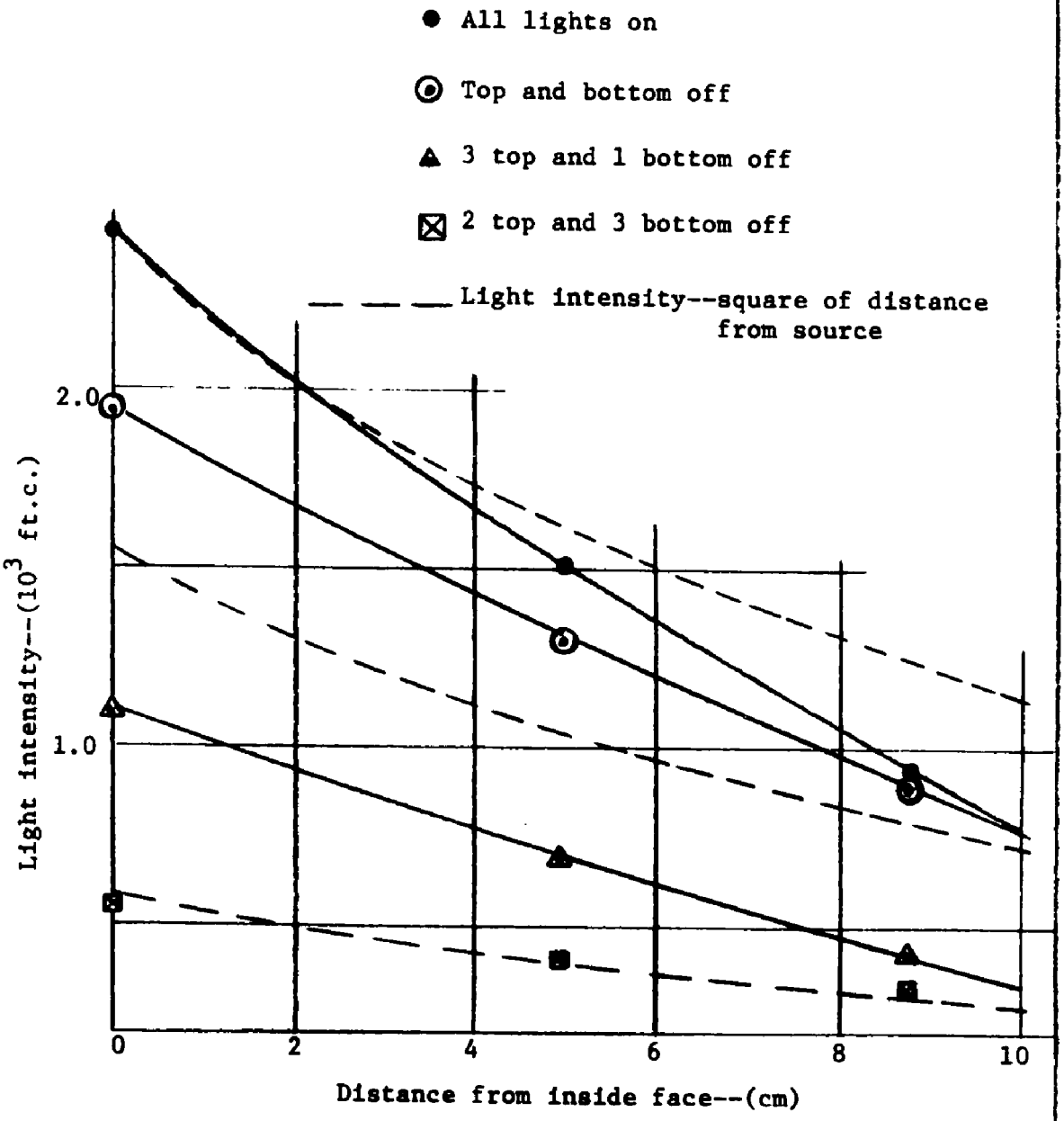
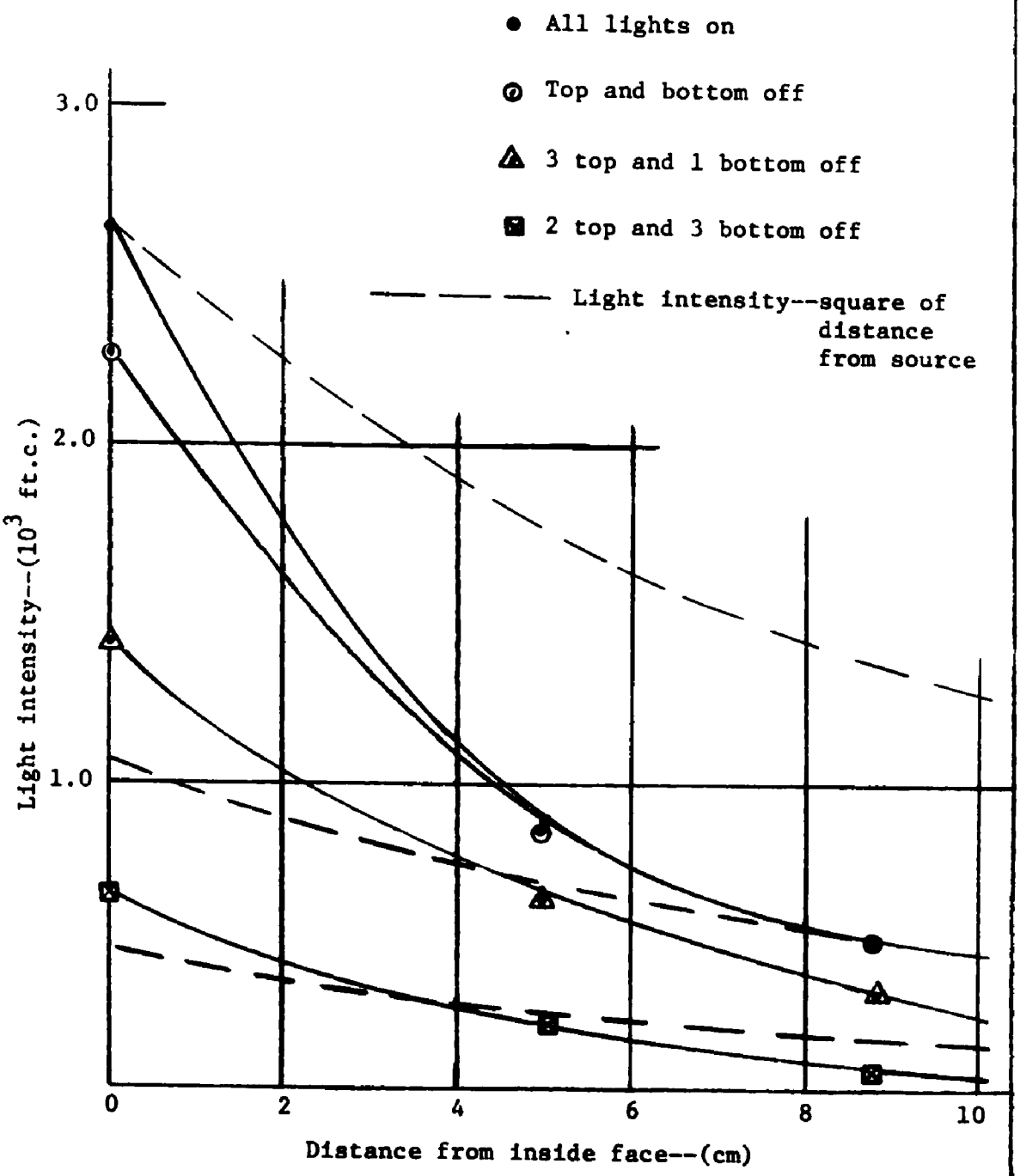


Fig. 14. Light intensity versus depth for several light configurations--with light cell filler.



The comparison of the light distribution, front to back, was also made for Configuration I with the light cell filler block. The results of this are shown in Figure 14, for the arrangement of conditions for the special study, show a considerable reduction of light from the inside front face of the light cell to the back of the cell. At the higher initial intensities the light deviated considerably from the expected intensity predicted by consideration of light intensity with respect to the square of the distance from the source. For lower light intensities the relationships more nearly approach the expected variation with respect to the square of the distance through the full depth of the light volume.

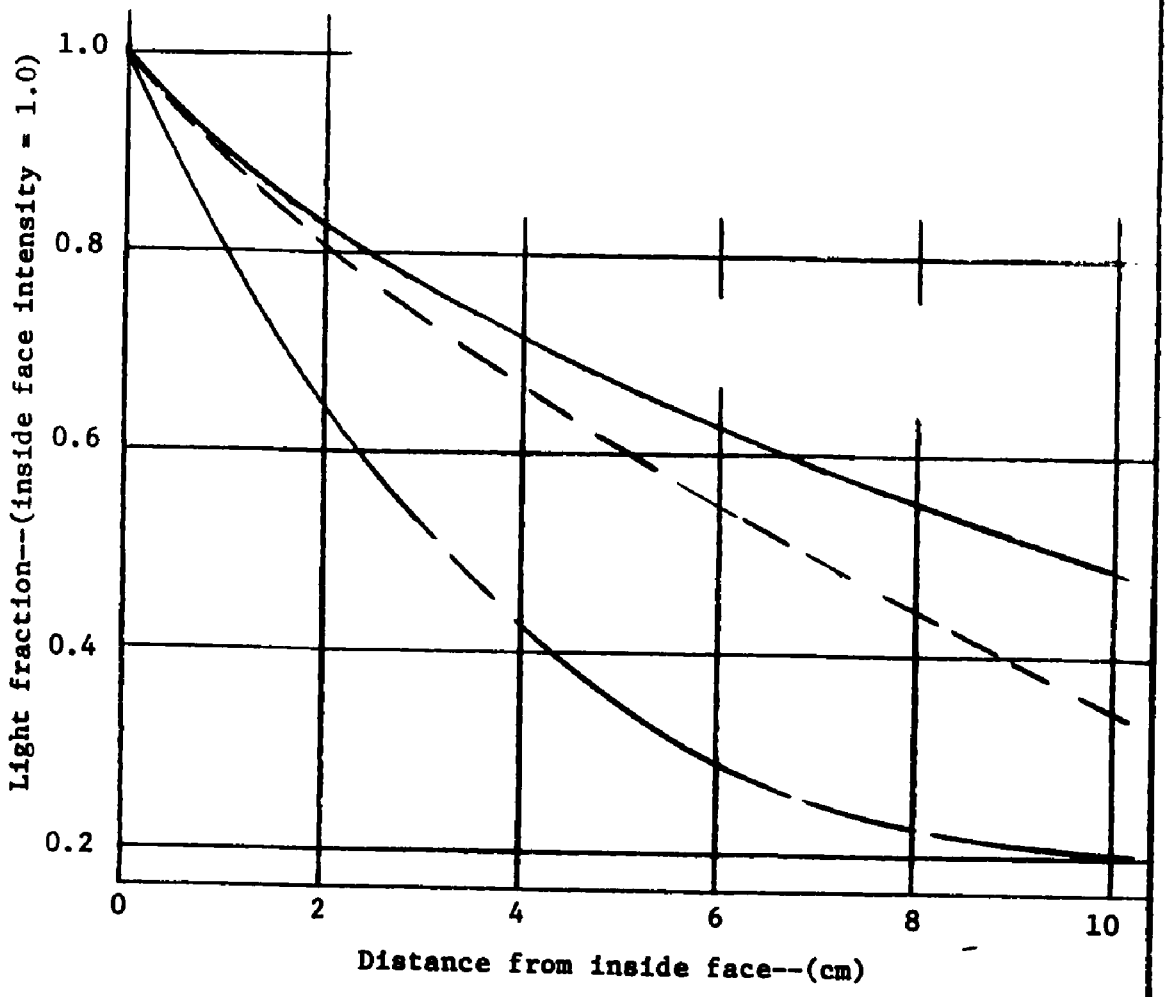
Figure 15 compares the configuration and light intensities for the condition with and without the light cell filler. These values have been plotted as a fraction of the light intensity, at the inside face of the light cell. This curve again shows that for the full light cell there was only moderate variation from the expected distance-intensity relationship. In the case of the filler block in the light cell there was a considerable variation from the square of the distance curve for the condition of all lights on. An analysis of the mean light intensity of the light cell, front to back, for the two indicated conditions shows that with the cell filler block there was a 33% reduction in light as compared with the full light cell condition. This reduction in total light availability would explain the reduced growth rate for the curve obtained from this special study shown in Figure 12. Adjustment of this curve upward by 33% in the growth rate, correcting for the total light reduction, would place the curve in the expected position for the dark-light detention ratio. Such an adjustment could be justified on the basis of Figure 16 which shows the variation of growth rate with respect to mean light intensity for

Fig. 15. Fractional light intensity versus distance from inside face.

———— Intensity with square of distance

— — — — No light cell filler--all lights on. Mean light factor--0.63

———— — — — Light cell filler--all lights on. Mean light factor--0.42



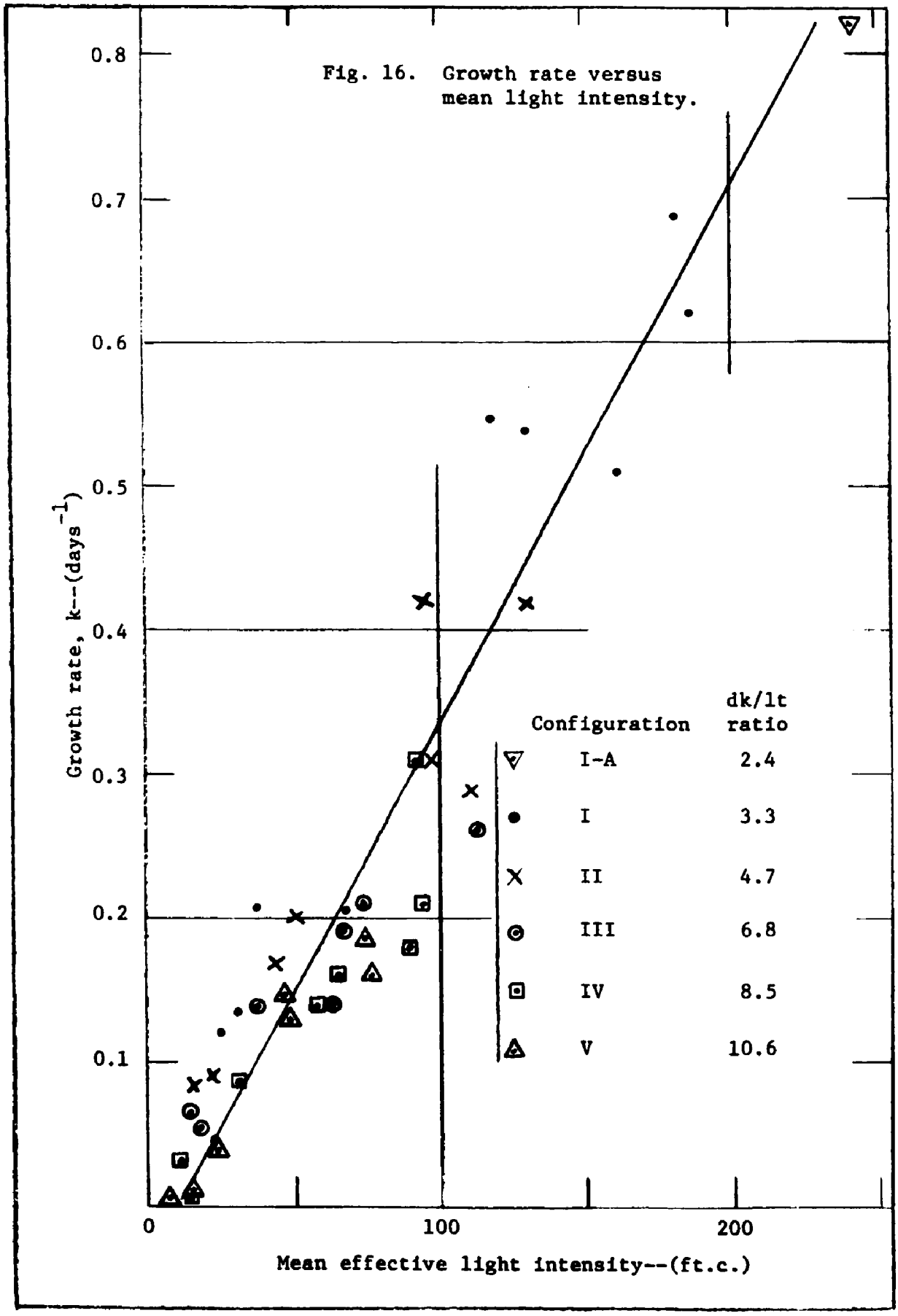



Fig. 16. Growth rate versus mean light intensity.

many ranges of light to dark ratio. This shows that as the total mean light intensity increases in these particular culture configurations there is a proportional increase in growth rate.

The special growth study (dark-light ratio 5.7) could not be carried to a velocity value higher than about 1.3 ft/sec because when mixing was increased above 2300 rpm the mixing or pumping process tended to produce relatively constant velocity conditions. The velocity conditions for each situation were evaluated using an electrode-salt solution method of checking the velocity as well as by means of a calibrated drag gauge.

Another reason it was not possible to increase the velocity above 1.3 ft/sec was because the culture tended to splash out of the tank during the runs at rpm values which might have produced higher velocities. The higher rpm only produced an increase in turbulence without increasing the velocity. Thus, this value of 1.3 ft/sec appears to be the upper limit of the mixing rpm for this particular configuration.

Yield Relationships

The yield of an algal culture (grams per square meter per day) is an indication of the efficiency of light utilization. In order to consider this possibility for the growth studies the yield has been plotted versus dark-light detention ratios for the various density cultures (5.4%, 21%, 39% and 58% transmittance).

The culture density showing the most pronounced growth rate change with respect to dark to light ratio was the lightest density culture, 34 mg/l (58% transmittance). The yield relationships for this density are shown in Figure 17 and for the yield versus dark-total detention ratio in

Figure 18. In Figure 17 the yield shows a similar response to increasing light as the dark-light detention ratio becomes smaller, optimum at values of about 3 to 4. This is consistent with the growth rate characteristics shown in Figure 8 where the growth rate in this range was only exceeded by the growth rate in continuous light. In the yield curve the yield value at zero dark to light ratio (continuous light) would appear to be anamalous. However, if we examine the growth rate curve we find that the maximum expected rate would be 2.1, if ideal conditions were realized in continuous light. Thus it would be apparent that the yield curve would have to show a downward trend from the maximum value of about 10 grams per square meter per day shown in Figures 17 and 18. This would have to occur because if the value of k = 2.1 is used in calculating the yield at the dark-total detention ratio or dark-light detention ratio of zero, the yield will be about 7.2 grams/square meter/day. Since the growth rate was somewhat less than 2.1 under these conditions the yield was even less pronounced.

For the 39% transmittance culture the yield analysis produced a similar relationship to the 58% values, as is shown in Figure 19. There was a slight increase in the effectiveness of the light or efficiency of light utilization as the yield was about 12.5 grams/square meter/day in the favorable dark-light detention ratio of 3-4 with a similar decrease occurring in the constant light condition. This general relationship could be expected, as Figure 8 indicates growth rate characteristics for the 39% culture as being similar to the 58% culture, having almost the same rate values, but being a somewhat denser culture, which would account for the slightly higher yield.

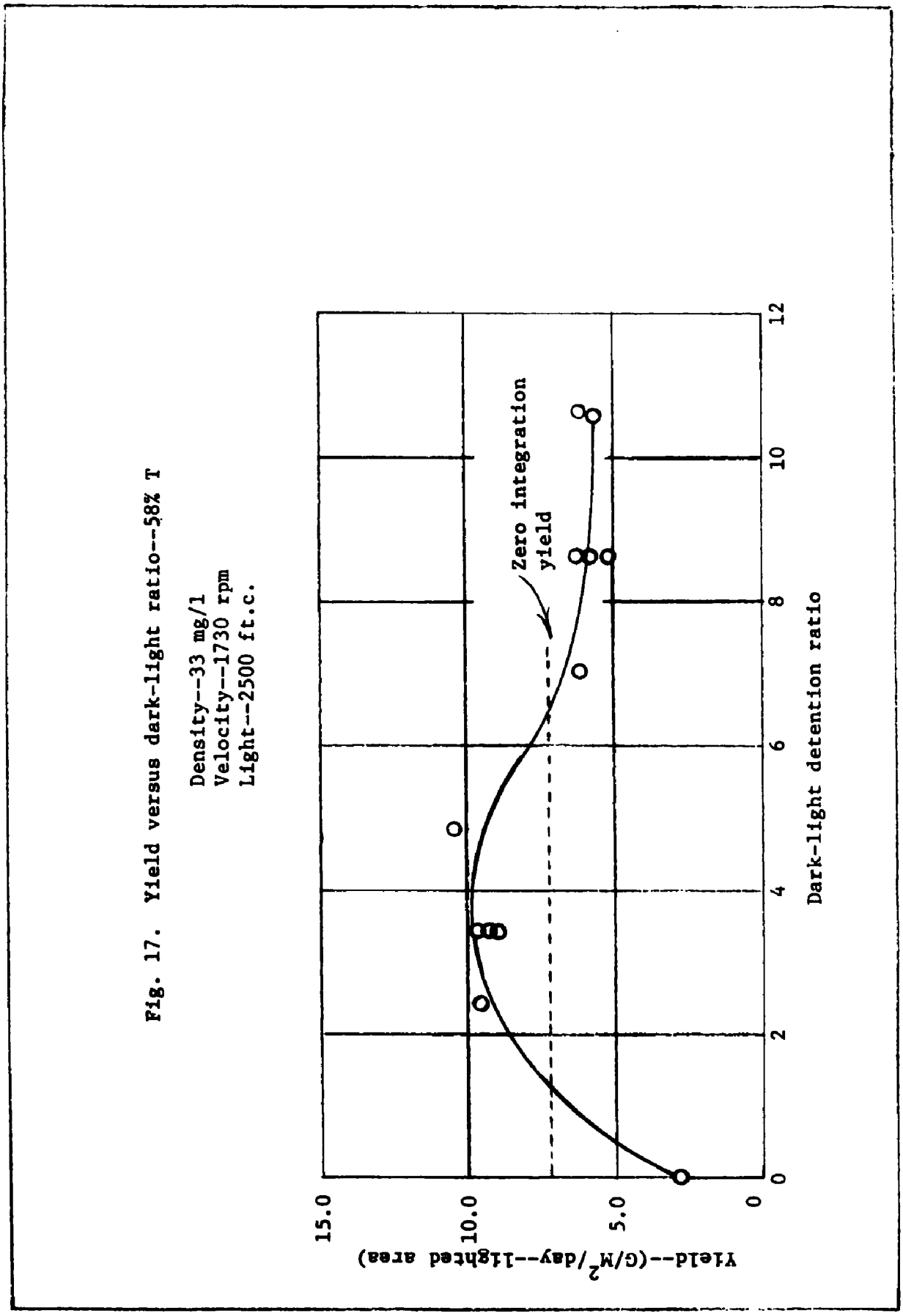
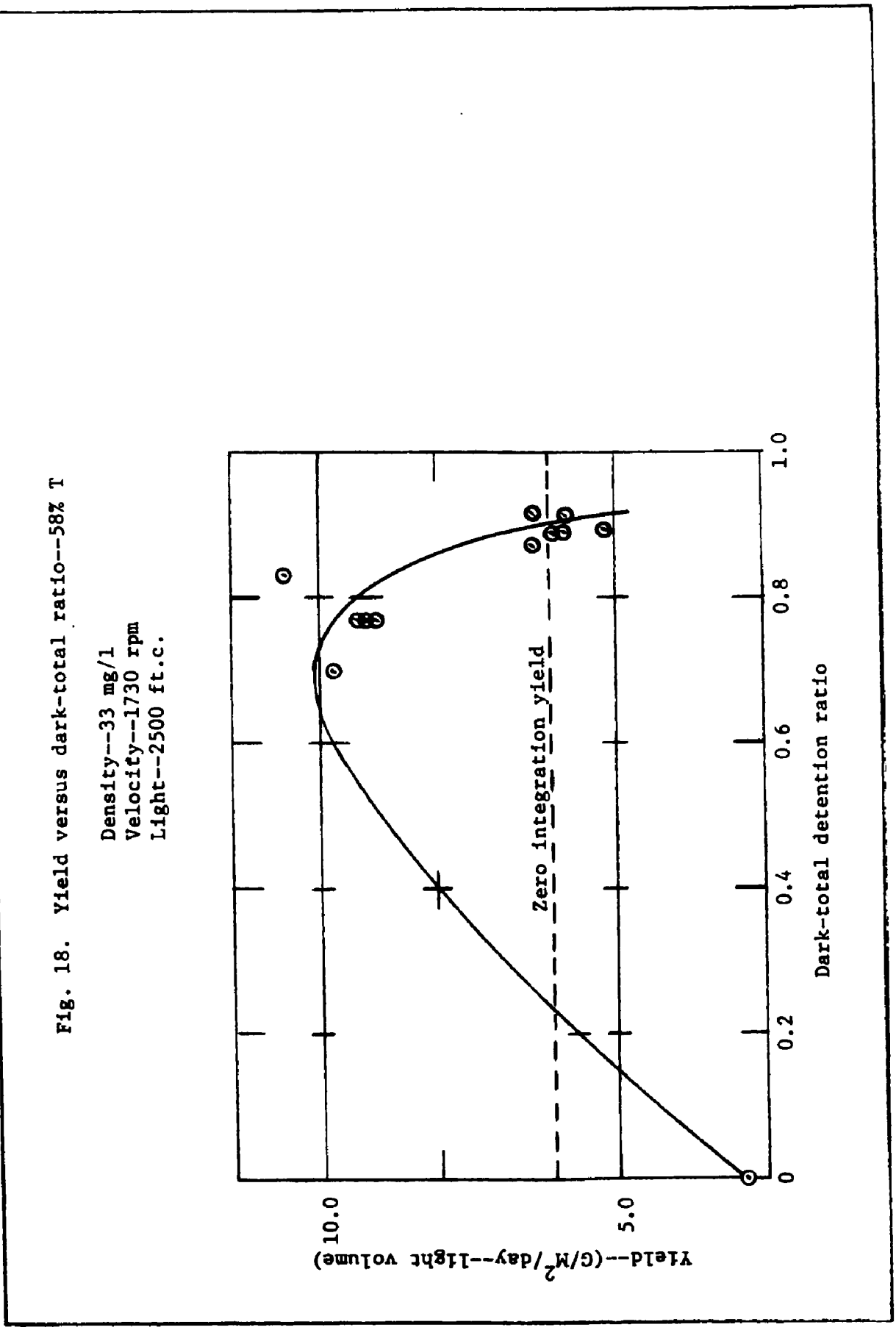



Fig. 17. Yield versus dark-light ratio--58% T

Density--33 mg/l
Velocity--1730 rpm
Light--2500 ft.c.


Yield versus dark-total ratio--58% T
Density--33 mg/l
Velocity--1730 rpm
Light--2500 ft.c.
Yield--(G/M²/day--light volume)
10.0
5.0
0
0.2
0.4
0.6
0.8
1.0
Dark-total detention ratio
Zero integration yield

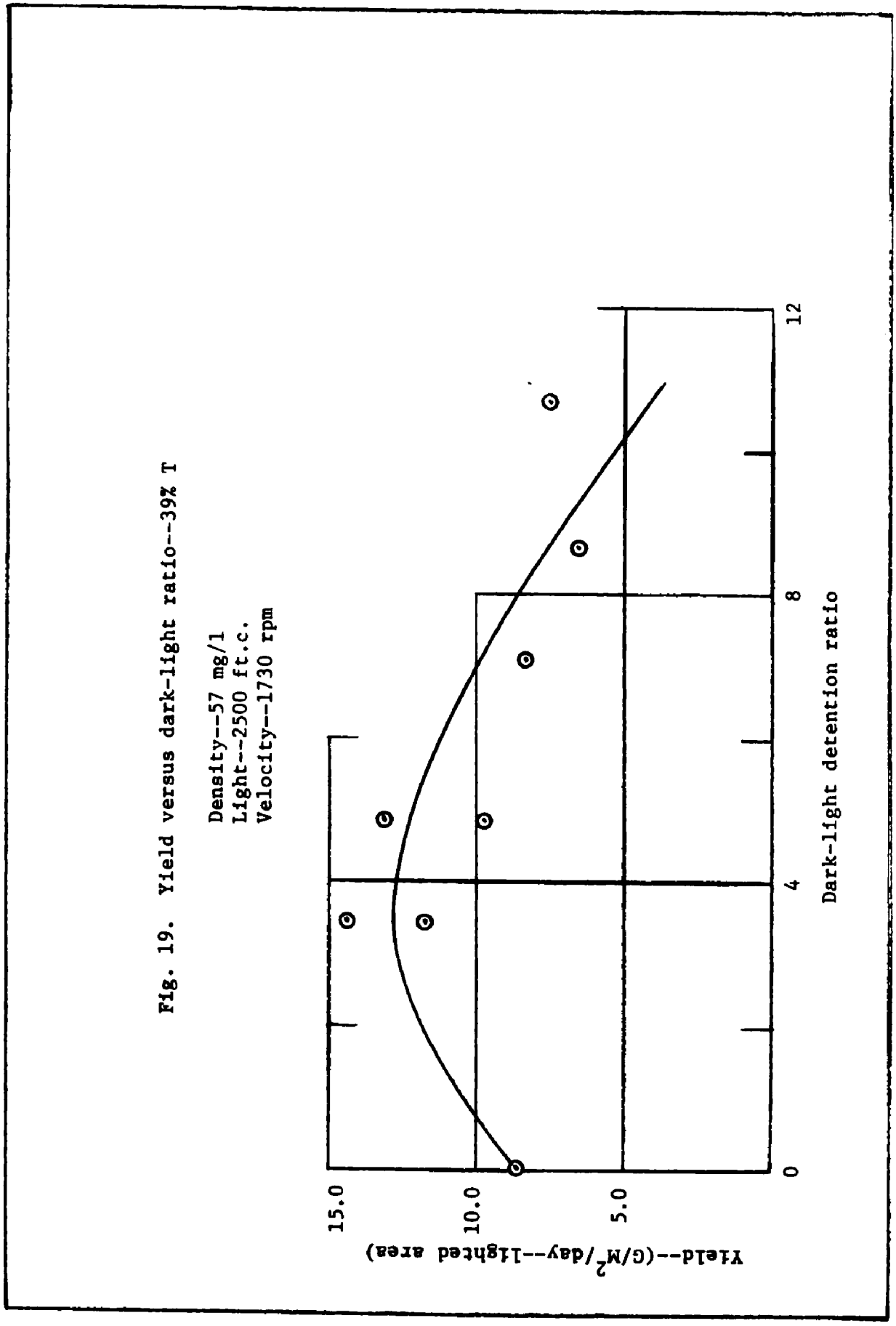



Fig. 19. Yield versus dark-light ratio--39% T

Density--57 mg/l
Light--2500 ft.c.
Velocity--1730 rpm

The yield relationships for the 21% and 5.4% transmittance studies give somewhat different results from the 39% and 58% transmittance curves. This could be expected because of the lower growth rate response shown in Figure 8. This would indicate again that the response tends to be a linear light response in these density ranges, being able to utilize more and more light as it becomes available. The pronounced increase in yield under continuous light conditions (zero dark-light detention ratio) is not indicative of a particularly effective yield increase over the values in the 39% and 58% range. To completely evaluate this, light integrating levels of yield need to be considered and are discussed more completely in the section on light integration.

The apparent discrepancies in yield at zero dark to light ratio in Figures 20 and 21 are primarily the result of a different density response, which is discussed and analyzed more completely in the section on continuous light culturing conditions.

To further evaluate the effectiveness of light utilization the velocity results were analyzed to provide a relationship of yield versus velocity for Configurations II and IV, used as the representative configurations studied for velocity effects. Figure 22 shows the response for this condition of increasing velocity with increasing yield, all of the conditions remaining constant. Thus effective utilization of light is increased as velocity increases with pronounced effectiveness taking place in the range of 0.8 to 0.9 ft/sec. This shows that yield is improved with increasing velocity with only moderate influence from configuration. This would be expected as it agrees with the results shown in Figures 8 and 17.

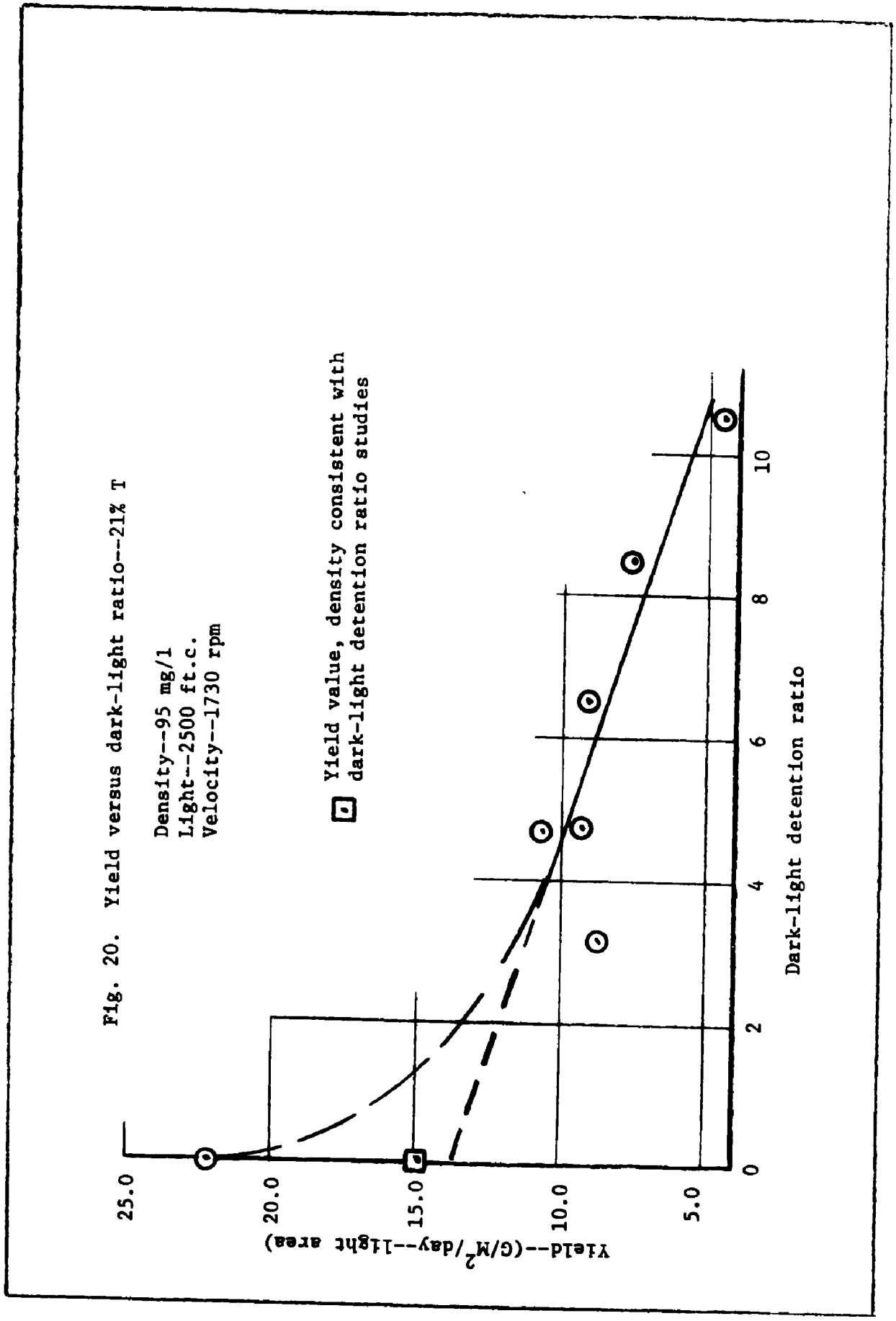
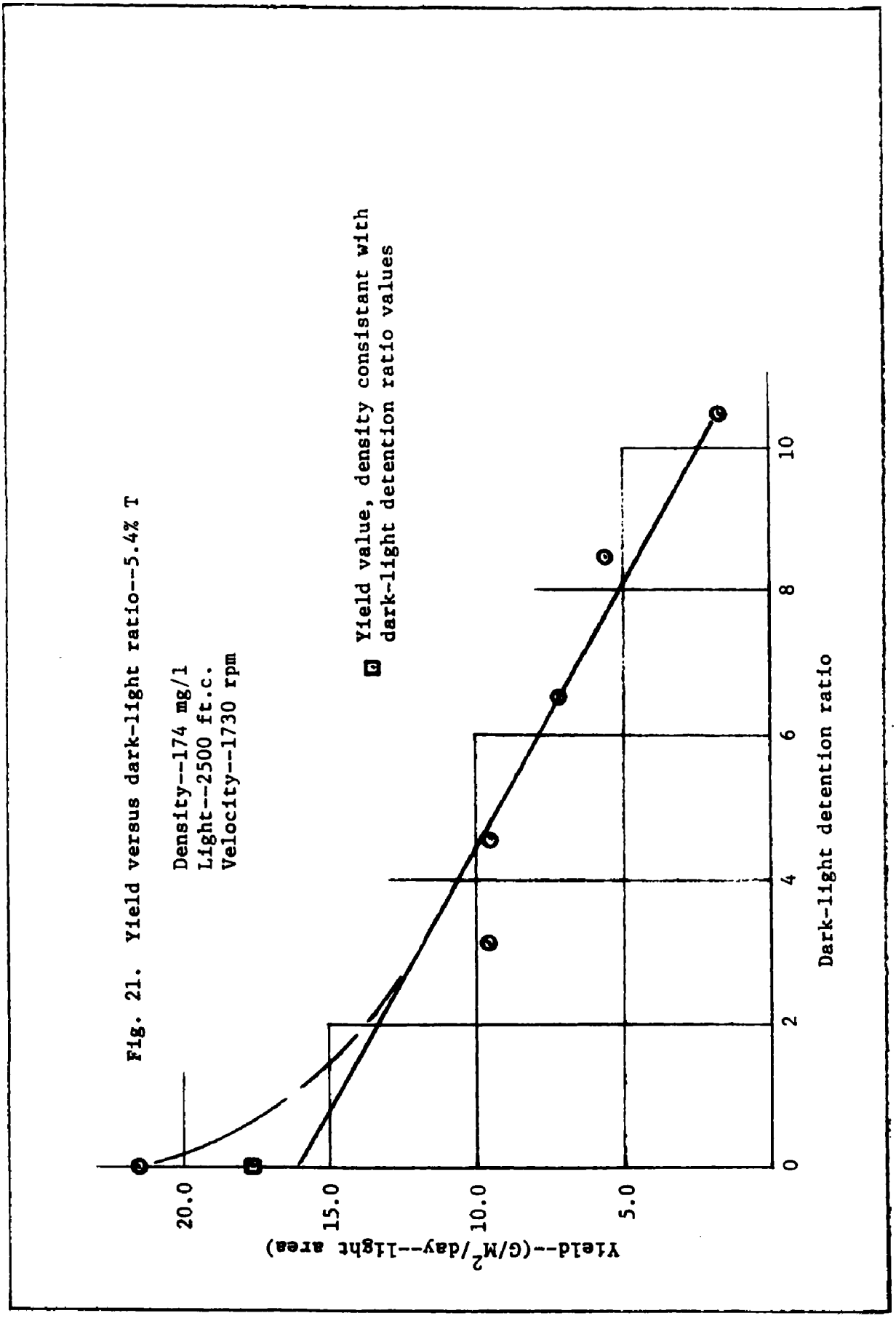



Fig. 20. Yield versus dark-light ratio--21% T


Fig. 21. Yield versus dark-light ratio--5.4% T
Density--174 mg/l
Light--2500 ft.c.
Velocity--1730 rpm
Yield value, density consistant with dark-light detention ratio values
Yield--(G/M²/day--light area)
20.0
15.0
10.0
5.0
0
2
4
6
8
10
Dark-light detention ratio

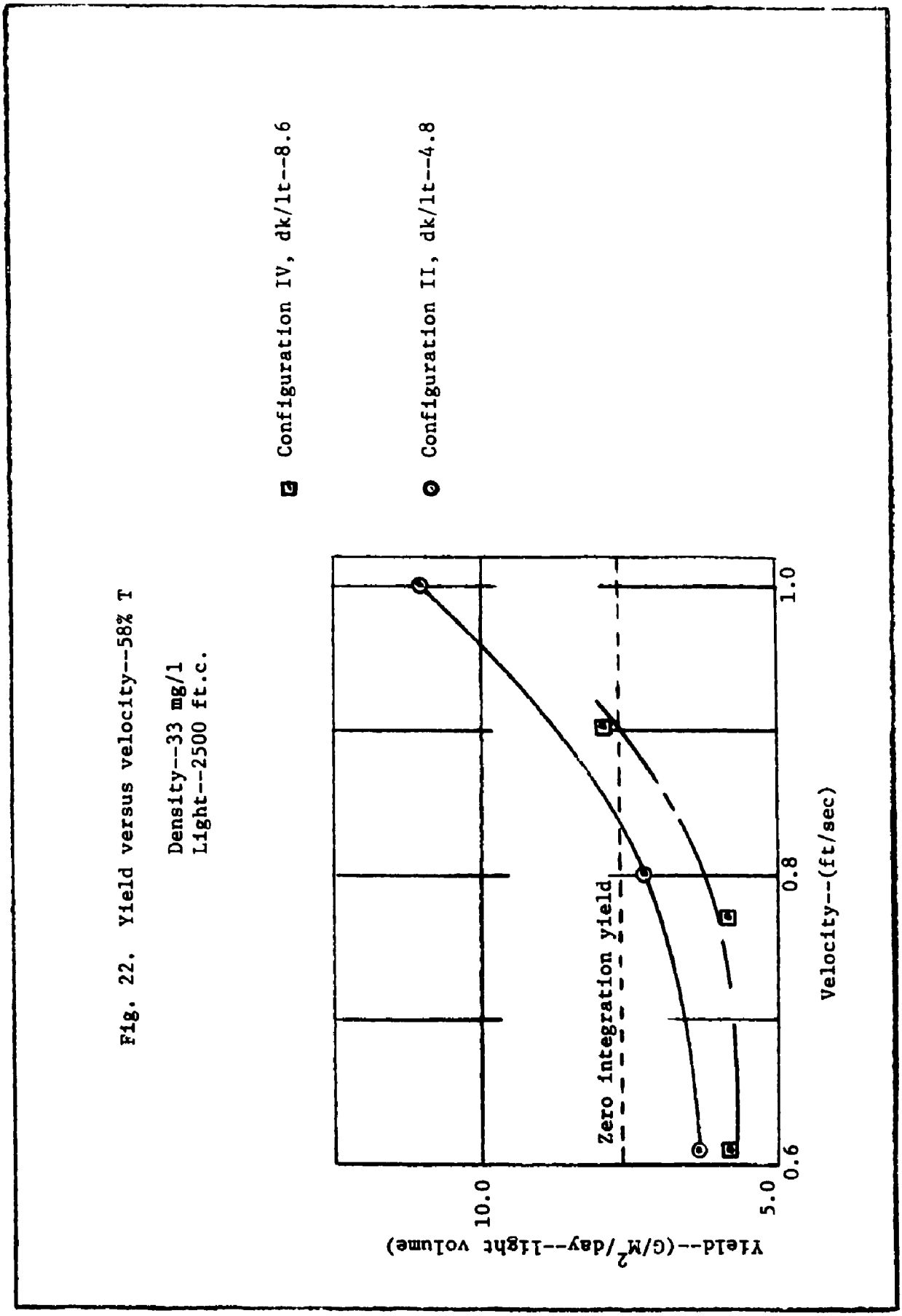



Fig. 22. Yield versus velocity--58% T

Density--33 mg/l
Light--2500 ft.c.

Growth in Continuous Light

In Figure 8 and the yield relationships the values for zero dark time, continuous light conditions, have been included along with the various dark to light ratio results. These growth rates have varied from about five times the growth rate obtained at a dark-light detention ratio of about three, to about two times as large as that for dark-light detention ratio of about three, for the range of 174 mg/l density (5.4% T) to 34 mg/l density (58% T) respectively. All of these values are below the k value of 2.1 assumed as the maximum probable growth rate expected. If optimum conditions were obtained it might be expected that the value of 2.1 would approach this value or possibly equal it as this is considered to be the upper value for Chlorella which is considered to be a very rapid growing green alga. The Schzothrix calcicola would not be expected to produce a value of k greater than 2.1 under these temperature conditions because the growth rate characteristics of Chlorella and many blue-green algae are not much different (68) and also this particular organism has been indicated to have thermophilic characteristics (52). For analysis purposes a k value of 2.1 is felt to be a very severe growth rate value to use in integration comparison. The actual values would be expected to be somewhat lower because it would be very difficult to obtain an ideal condition under mass culture situations.

It appears that the values are reasonable representations because in most cases the growth rate was obtained by increasing the light intensity from low values to high values and the final growth rate for 2500 ft.c. was relatively constant for each density. If the actual maximum zero dark-light detention ratio growth rates were somewhat less than

2.1 this would make very little difference in the significance of the results as this would only appear to make the evaluation more significant.

The results of the zero dark time with respect to growth rate conditions are shown in Figure 23. This shows that for this arrangement of mixing, light and culture depth, the favorable density for growth appeared to be in the range of 80 - 130 mg/l (36% - 20% T), decreasing in growth rate with denser or lighter algal concentrations. This appears to be the result of effective light concentration in that up to about 130 mg/l, from the denser culture conditions, the algal culture could utilize the higher light intensities. Beyond 80 mg/l, toward lighter densities, the organisms tended to suffer from excessive light and thus showed some inhibition.

The growth rate curves, Figures 8 and 9, as well as the yield relationships, indicate that there is some agreement between the two systems, dark-light configurations and constant light conditions but there are also some anomolies. Figure 24 shows the relationship of the two distinct systems with respect to mean effective light intensity. Curve (A) is derived from the dark-light configuration results in Figure 16 which was developed for all growth studies. Curve (B) is related to this in terms of mean effective light intensity for the constant light conditions. This was corrected on the basis of percent transmittance density response shown in Figure 25. This shows reasonable agreement since the systems were rather distinctly different.

The higher growth rates in (B), for the given mean effective light conditions, may be the result of the physical differences or the result of analytical differences. The physical differences could be

Fig. 23. Growth rate versus culture density.

Light--2500 ft.c.
No dark time

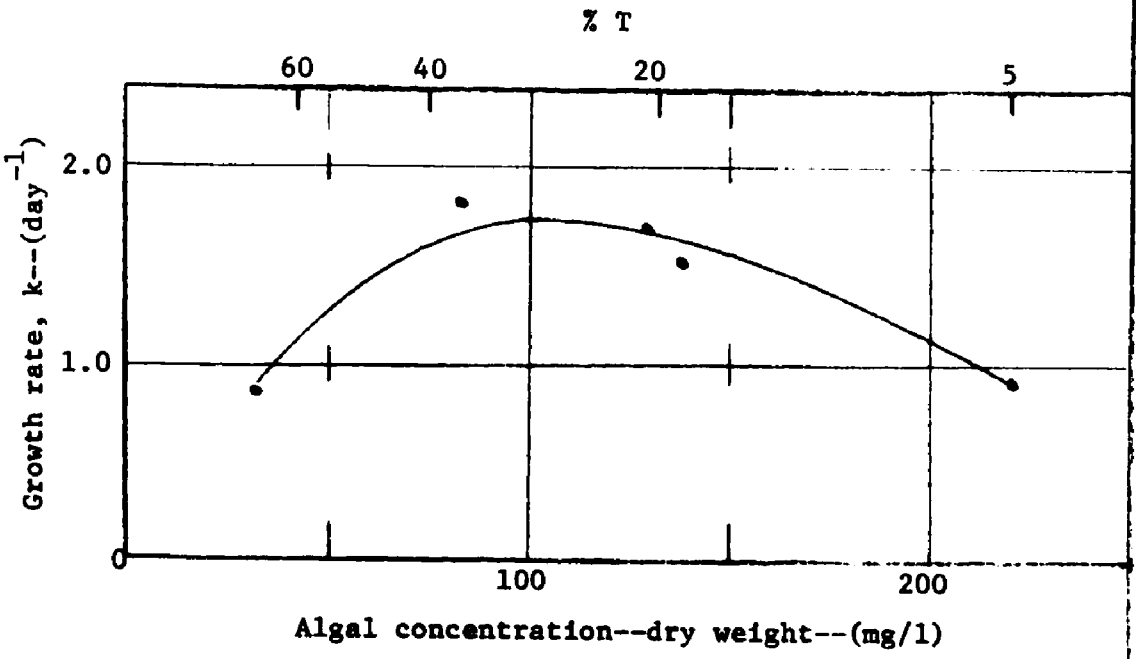


Fig. 24. Growth rate versus mean light intensity.

A -- Dark light configurations
Velocity--1730 rpm

B -- Constant light conditions

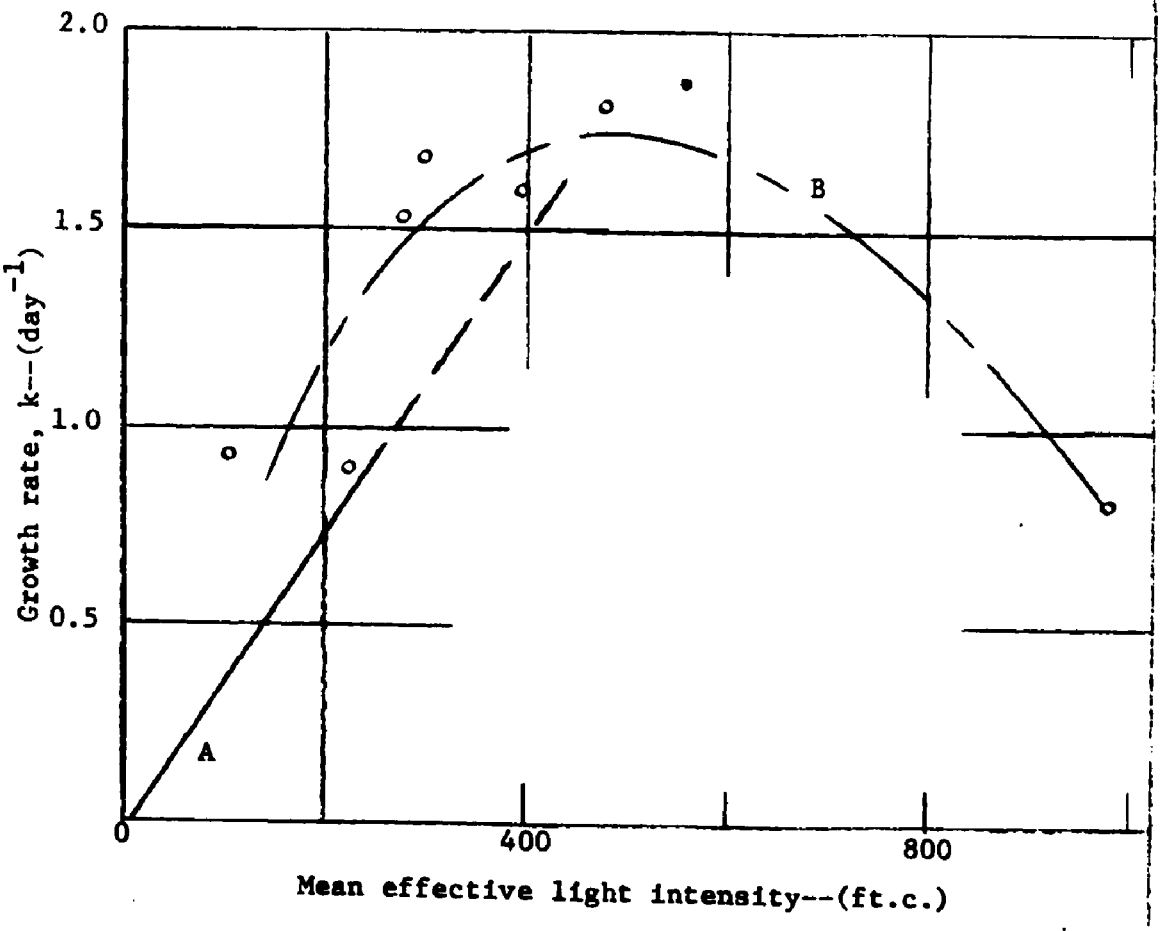
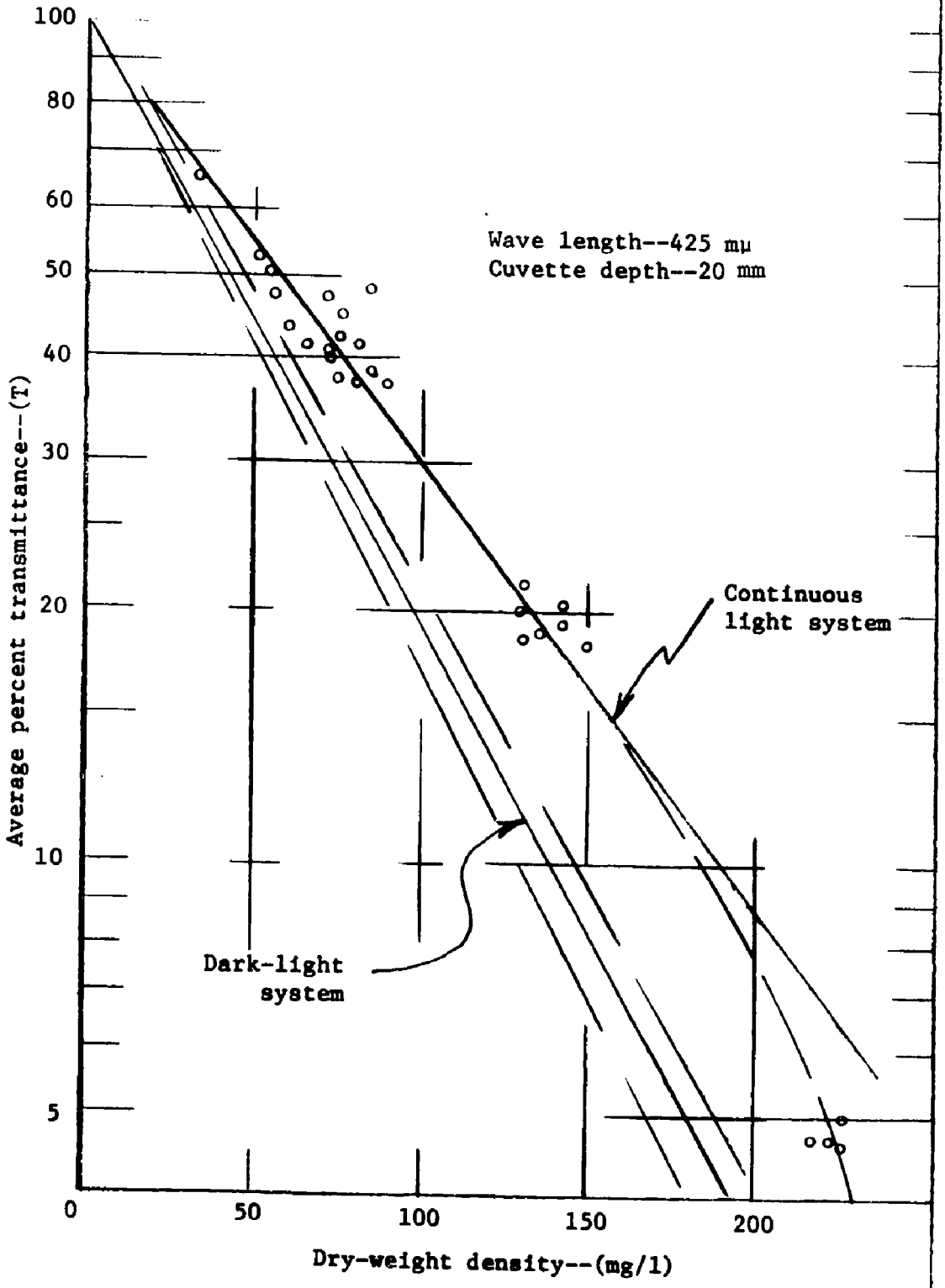


Fig. 25. Percent transmittance versus dry weight for two culturing systems--continuous light cultures and dark-light detention ratio cultures.



accounted for on the basis that the mixing conditions would possibly produce somewhat higher growth rates. However, this would appear to be a minimal effect. Of more concern would be the variation in extinction coefficient and thus the mutual shading variation. These variations would tend to contribute to a higher actual mean effective intensity because of the observed lighter cell characteristics and the percent transmittance-density response shown in Figure 25.

In Figure 24 the constant light results, (B), indicates a pronounced detrimental effect on growth at a mean effective light intensity of around 1000 ft.c. This tends to confirm the relationship observed in Figure 23 where growth rate reduction was observed in the lighter density cultures, which would produce higher light intensity conditions.

It would appear that an intensity of 1000 ft.c. would not normally be detrimental, but some intensities in this range have reportedly caused toxic effects (42). However, the mean effective intensity would probably be somewhat higher because of the changed algal cellular characteristics just mentioned. Also, of more importance, would be the light inhibiting effect as the result of rather prolonged light exposure in the range of 2300 to 2600 ft.c., which existed near the front face of the culture. The mean effective light intensity would no longer be effective in describing the growth characteristics under such light inhibiting conditions. Cells exposed to these higher light intensities would tend to take longer and longer to recover, in the absence of pronounced dark time periods, and in the constant light cultures there would be no real dark time. This could be expected because the dark-light conditions indicate there is a real advantage to such a system under high light intensity

situations.

In obtaining the data for Figure 23, and related to the dark-light studies in Figure 24, there were observations which help substantiate the contention that light inhibition was part of the problem. There was progressive difficulty in obtaining satisfactory culture growth as culture density was decreased. The 220 mg/l (5% T) culture was started without any great difficulty. It grew satisfactorily, and it did not have any tendency to die out, bleach, or become overgrown with other organisms. The 20% transmittance culture did show some tendency to be sluggish when starting from full light intensity; however, growth could be developed quite satisfactorily by starting at low intensity and acclimating the cells to the higher intensity of 2500 ft.c. The lower density cultures, 75 mg/l and 32 mg/l (40% T) and (65% T), cultures had to be started at very low intensities and carefully worked up to the full light values. In the situations where this was not done there was very little realization of algal growth. This physically confirms the growth rate and yield curves which have indicated a definite advantage to the dark-light ratio, especially in the range of 3-5, as being advantageous for the culturing of algae in the higher light intensities. Higher light intensity must be expressed as mean effective intensity so that it takes into account all the variables for density, depth and other relationships.

Some other differences were also noticed in addition to the acclimation requirements. The color of the cells for the lighter densities tended to be much lighter than normally encountered under dark-light detention ratio conditions, with almost a white appearance to the mass culture in the 60% transmittance system.

In order to evaluate this difference the percent transmittance versus dry weight relationships were compared with the results obtained from the dark-light detention time studies, as shown in Figure 25. The cells grown under full light intensity appeared to have or require a greater mass of cells for a given percent transmittance value. This would tend to indicate a bleaching or pigmentation reduction characteristic which corresponds with the previously mentioned visual observation. This deviation from the dark-light ratio studies was very pronounced in the low density cultures and it appears that the dense cultures, such as the high percent transmittance range, tend to show a return to the spectral transmittance-density relationships shown in Figure 6. Therefore, the high light intensity shows a pronounced effect on the algae under conditions of zero dark time.

Evaluation of Effective Light

In order to evaluate the significance of light intensity it is necessary to consider the mean effective light under the conditions of dark to light ratio as well as the functions related to density, including mutual shading and probability of exposure, and distance from the source of light. The direct measurement of light intensity at the face of the culture is not indicative of the true lighting effects unless the conditions are all maintained constant. In these studies the conditions were significantly different.

Figure 16 shows the relationship of growth rate with respect to the mean effective light intensity in ft.c. For conditions of constant impeller rpm, density and light intensity, various dark-light detention ratios have been included in these values.

This figure is of considerable interest because it is typical of the expected characteristics for light dependent growth conditions as the curve of best fit for this data appears to be a straight line with a zero growth rate value of about 10 ft.c. This zero value would appear to be a mean effective compensation level. Also it is of significance to note that if the curve is projected to the maximum expected growth rate of k - 2.1, the light intensity at this point is a value of about 600 ft.c. This agrees well with the values reported in the literature as being the order of the upper range of saturating light intensity for shallow and low density cultures. This curve would be expected to produce the maximum mean effective light saturation condition since the impeller speed of 1730 rpm tended to produce a relatively constant growth rate which did not change much when the rpm was reduced below this value. Intensities in the order of 400 ft.c. have also been reported as being indicative of saturating intensity and such a value could be approached by the conditions shown in Figure 16 by increasing the velocity of the culturing conditions as this would steepen the positive slope.

This steepening effect, which is deduced from the velocity studies, can be more effectively evaluated by considering Figure 26 in which the growth rate is expressed with respect to unit effective light versus the velocity in the light exposure portion of the system. These data are for the condition of 58% T culture density as this is the range where the most effective light intensity could be obtained. From these data it appears that up to a velocity of 0.8 ft/sec, for the general culturing system, the growth rate tends to be relatively constant. Pronounced improvement in growth rate is obtained when the velocity is increased to about 1.0 ft/sec or better. If this is compared with Figure 16 it is apparent that

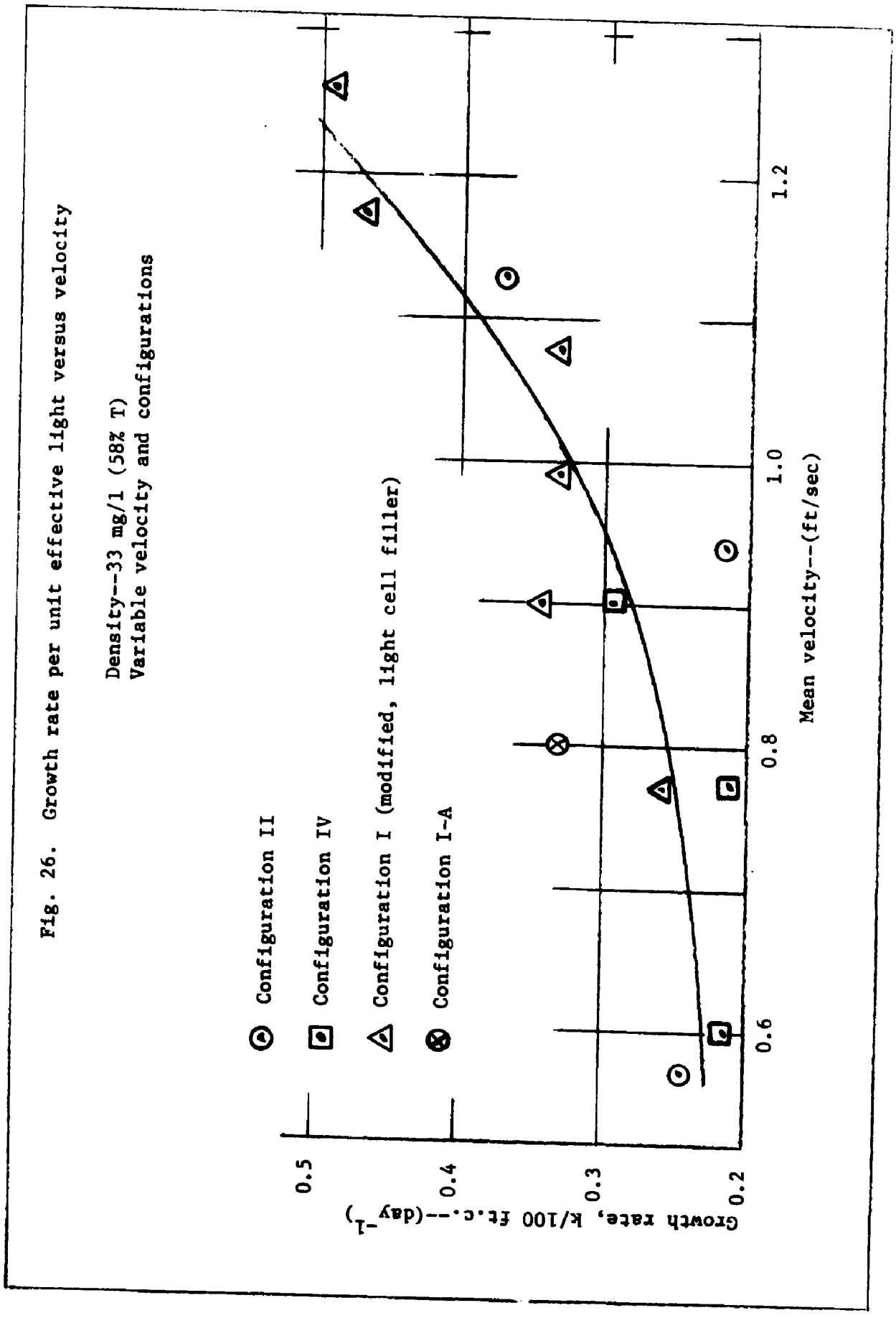



Fig. 26. Growth rate per unit effective light versus velocity

Density--33 mg/l (58% T)
Variable velocity and configurations

the slope of that curve would become steeper as the result of increased velocity. Figure 26 also indicates that there is a relatively constant efficiency up to a velocity of 0.8 ft/sec with increasing light utilization efficiency increasing as the velocity increases beyond this point. This conclusion is obtained from the fact that the value of growth rate per 100 ft.c. goes from about 0.25 to about 0.47 in the range of 0.8 to 1.2 ft/sec (velocity in the light cell).

The significance of this effect can be more completely related to flash time by considering the time of exposure in the light cell as velocity is varied. Figure 27 shows the unit effective growth rate versus light exposure time in which it appears that for this overall configuration there is little improvement in growth characteristics until the exposure time reaches a value of about 0.7 sec. From 0.7 sec to lower flash time values the effectiveness of the light increases, with a resulting increase in efficiency, which is the relationship expected in controlled dark-light time systems (72). This would mean that in applied situations it would be desirable to have exposure times of half a second or less if 10 cm depth culture condition systems (148) are utilized and efficient light utilization is to be obtained. This is of particular interest in considerations of light integration.

The velocity curves shown in Figure 12 show very good agreement if they are corrected for velocity and lighting conditions. These relationships are further amplified in Figures 26 and 27 by relating growth rate on a unit light basis to the velocity effects. A pronounced increase in production is evident at a velocity of about 0.8 ft/sec for the various conditions. These adjustments also show that the vertical displacement (in Figure 12) is primarily a result of effective light availability

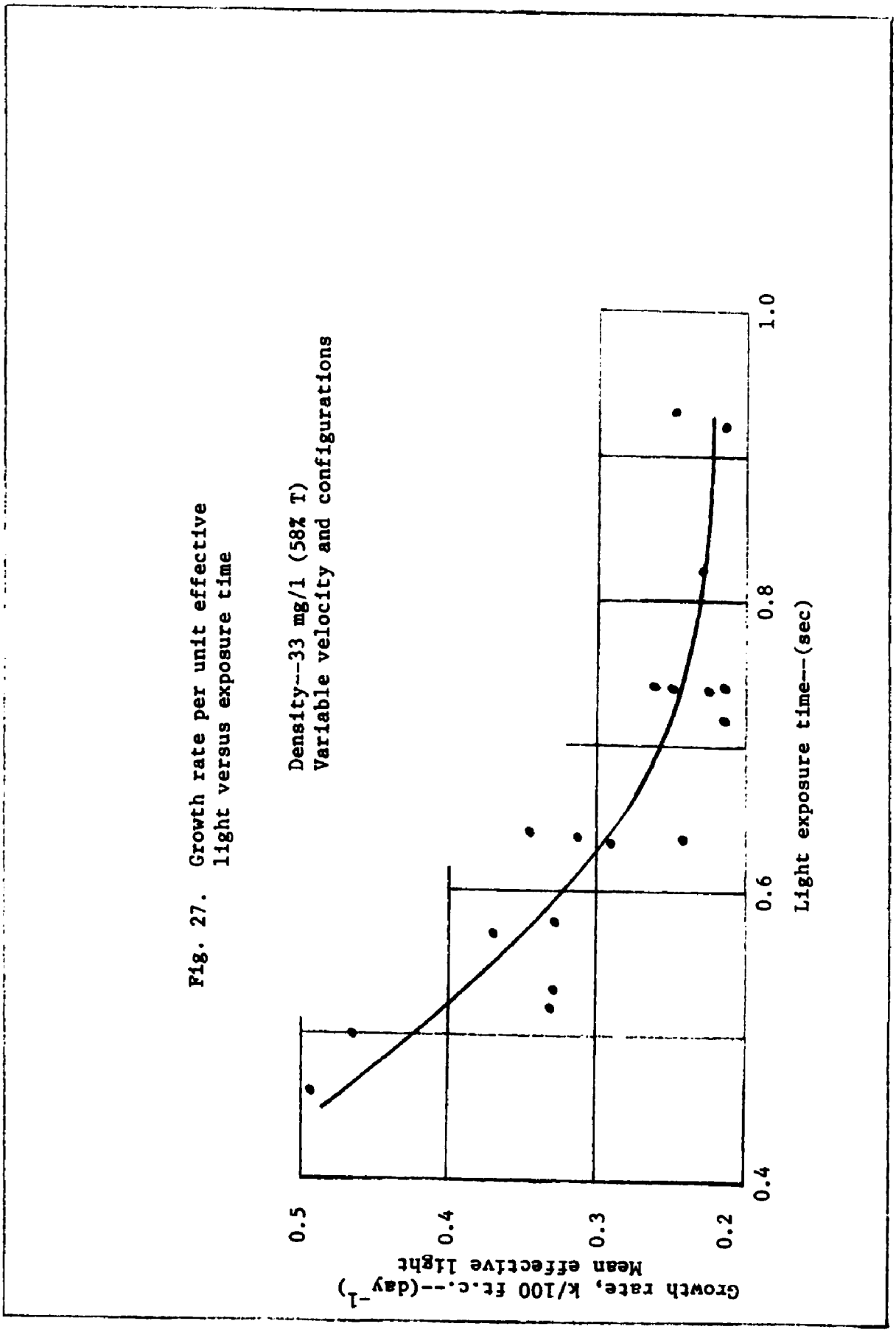



Fig. 27. Growth rate per unit effective light versus exposure time

as the results tend to give a consistent curve on a unit light basis. This appears to be relatively consistent for the various configurations, such as the dark to light ratio of 5.7 shown in Figure 12 as well as at the upper part of the curve for the range of dark to light ratio (Configuration I) of 3.41.

The variations around the curves in Figures 26 and 27 can be explained on the basis that it was found that in the low dark to light ratio configurations there was a tendency for the velocity to be somewhat uneven across the cross-section of the light cell. The mean velocity would therefore not give the same growth results as it has been shown that a velocity of about 0.8 ft/sec produced a pronounced increase in growth characteristics. Some portions of the light cell tended to have relatively high velocities and this would contribute to a higher growth rate than would be expected if the point velocities and the mean velocity were very nearly the same.

## LIGHT EVALUATION

The evaluation of light in mass algal cultures poses several problems because there is considerable variation of light intensity from one algal cell to another as a result of physical culture differences and depth. The depth factor involves the effect of reduction of light intensity from the source varying as the square of the distance and the effect of mutual shading. The culture depth evaluation involves both the square law and any other limiting physical features. Mutual shading involves probability distribution of particles in a mixed culture, transmittance through a single algal cell, and the extinction coefficient.

Depth

For these studies the fluorescent light source was located at a distance of 22.4 centimeters (8.8 inches) from the inside face of the culture tank, and thus the distance from the light source to the back of the tank was 32.7 centimeters (12.8 inches). Figure 13 shows the light intensity variation which would be expected with respect to physical position from the face of the culture to the back of the culture, as expressed by light intensity variation with the square of the dis-distance. This would mean that if there was only one algal cell moving through the light cell it could be exposed to various light intensities just because of its physical location. With a value of 3000 ft.c. at the culture face a single cell would be exposed to only 2150 ft.c. at a distance 4 centimeters from the face, 1625 ft.c. at a distance 8 centimeters from the face, and only 1410 ft.c. at a distance of 10.3 centimeters from the face.

This example of light variation assumes that there are no differences with respect to possible light configuration and cell configuration. Table 5 gives some examples of variation of intensity for various light configurations and culture face intensities with corresponding correction factors. Observations were also made in the cell itself for light intensity variation with respect to depth for both conditions of full cell volume and for conditions of study where the volume was reduced to about one-half. Tables 6 and 7 show the data obtained from such an evaluation.

TABLE 5

LIGHT TRANSMISSION CORRECTION*

| Light Configuration | Outside (ft.c.) | Inside (ft.c.) | Coefficient |
|---|---|---|---|
| All on | 2800 | 2450 | .88 |
| Top off | 2600 | 2200 | .85 |
| Top and Bottom off | 2225 | 1950 | .88 |
| 2 Top and 1 Bottom off | 1925 | 1550 | .81 |
| 3 Top and 1 Bottom off | 1350 | 1100 | .82 |
| 2 Top and 2 Bottom off | 1450 | 1250 | .86 |
| 3 Top and 2 Bottom off | 775 | 630 | .82 |
| 2 Top and 3 Bottom off | 660 | 580 | .88 |

*For inside culture face values from outside readings, through light cell window.

TABLE 6

EFFECT OF DEPTH ON LIGHT INTENSITY IN
LIGHT CELL--MEASURED IN AIR--
(NO CELL FILLER)

| Light Configuration | 0 cm. | 5 cm. | 8.8 cm. |
|---|---|---|---|
| All on | 2450 | 1500 | 950 |
| Top off | 2200 | 1400 | 950 |
| Top off and Bottom off | 1950 | 1300 | 900 |
| 2 Top and 1 Bottom off | 1550 | 1100 | 750 |
| 3 Top and 1 Bottom off | 1100 | 700 | 410 |
| 2 Top and 2 Bottom off | 1250 | 900 | 650 |
| 3 Top and 2 Bottom off | 630 | 450 | 320 |
| 2 Top and 3 Bottom off | 580 | 420 | 320 |

TABLE 7

EFFECT OF DEPTH ON LIGHT INTENSITY IN
LIGHT CELL--MEASURED IN AIR--
(WITH LIGHT CELL FILLER)

| Light Configuration | 0 cm. | 5 cm. | 8.8 cm. |
|---|---|---|---|
| All on | 2650 | 920 | 560 |
| Top off | 2500 | 920 | 525 |
| Top and Bottom off | 2280 | 900 | 540 |
| 2 Top and 1 Bottom off | 1990 | 885 | 540 |
| 3 Top and 1 Bottom off | 1400 | 685 | 390 |
| 2 Top and 2 Bottom off | 1400 | 710 | 465 |
| 3 Top and 2 Bottom off | 810 | 495 | 335 |
| 2 Top and 3 Bottom off | 680 | 290 | 160 |

Figure 13 shows some of the relationships of the data for various light configurations and for the full light cell volume. This shows that with all the lights on there is some variation from the square law distribution across the depth of the culture. As some of the lights are turned off, such as in the case of two top and one bottom bank of lights being off, the variation closely approximates that which would be anticipated by the square of the distance effect. In the case of the last two curves, three top and two bottom off or two top and three bottom lights off, the observed curve and the square relationship are almost identical. The variation of this light can be shown more graphically in Figure 28 where the face intensity is taken as the unit value for all variations and initial intensities. This shows that the relative light variation approximates the square law for most of the source light combinations.

In the velocity studies, using a reduced light cell volume, the data show more pronounced variation of intensity with respect to depth or distance. The data for the observations made in the light cell filler configuration are shown in Table 7 and the plotted results are shown in Figure 14. Thus Figure 14 shows that there is a considerable amount of variation between the light distribution with the filler and the square law variation. This shows that the filler tended to produce a pronounced light restriction effect. However, with only two center bulbs on, the variation still tended to have a steeper negative slope than would be expected for a normal distance function. This is shown quite graphically in Figure 15 where the variation tends to be more pronounced across the culture cell depth, more than would be expected from the square of the distance reference curve. The difference from this reference is more pronounced with the filler present than in the case without the filler.

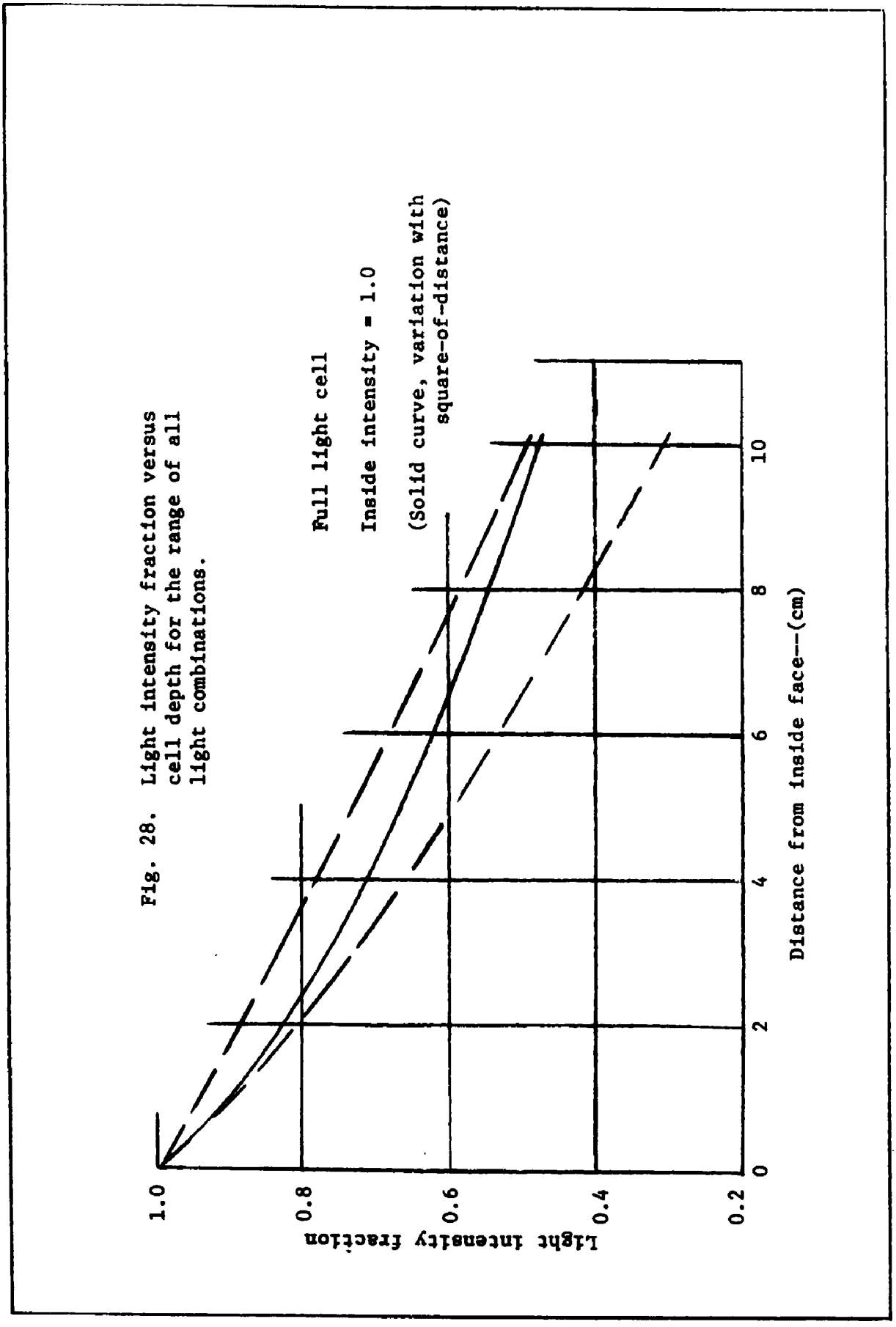



Fig. 28. Light intensity fraction versus cell depth for the range of all light combinations.

Figure 15 also shows that with all lights on, for the two light cell configurations, there is a pronounced difference in mean light value between the light in the full cell configuration as compared to the light in the reduced cell volume configuration. By integrating the relative light intensity area under the two curves and obtaining a mean value it can be shown that without the filler the mean light intensity would be about 63% of the face intensity, and with the filler it would be about 42% of the face intensity. This indicates that with the filler there is about a 33% reduction in available light as compared with the situation without the filler.

This shows that in considering the mean effective light intensity exposure the depth of the culture must be taken into account as well as the light source configuration. This correction factor has been included in each value for light evaluation.

Unit Algal Cell Light Transmission

The unit algal cell light transmission ($\Gamma$) is the amount of light transmitted by an equivalent solid layer of algal cells, one alga thick. This is an important evaluation in light consideration as this gives information with respect to the light intensity which will be received by one alga behind another alga.

For this study the value for light transmission through one cell was arrived at by microscopic examination of samples of the culture and by measuring the algal configuration and relating this to count and density data. A typical count for this evaluation is shown in Table 8. This information was analyzed statistically on normal probability paper and the mean length was obtained as 47 microns ($47 \times 10^{-4}$cm) with a mean

TABLE 8

COUNT AND LENGTH OF ALGAL CELLS FROM
A SAMPLE OF A CULTURE OF
SCHIZOTHRIX CALCICOLA

60% T

| Number | Length (μ) | Number | Length (μ) | Number | Length (μ) |
|---|---|---|---|---|---|
| 1 | 5 | 18 | 35 | 35 | 55 |
| 2 | 10 | 19 | 40 | 36 | 60 |
| 3 | 10 | 20 | 40 | 37 | 60 |
| 4 | 15 | 21 | 45 | 38 | 60 |
| 5 | 15 | 22 | 45 | 39 | 65 |
| 6 | 15 | 23 | 50 | 40 | 75 |
| 7 | 15 | 24 | 50 | 41 | 75 |
| 8 | 15 | 25 | 50 | 42 | 75 |
| 9 | 20 | 26 | 50 | 43 | 80 |
| 10 | 20 | 27 | 50 | 44 | 80 |
| 11 | 25 | 28 | 50 | 45 | 80 |
| 12 | 25 | 29 | 50 | 46 | 90 |
| 13 | 30 | 30 | 50 | 47 | 90 |
| 14 | 30 | 31 | 50 | 48 | 100 |
| 15 | 30 | 32 | 50 | 49 | 100 |
| 16 | 30 | 33 | 50 | | |
| 17 | 35 | 34 | 55 | | |

diameter of 3 microns ($3 \times 10^{-4}$cm). In the 20% to 60% transmittance culture density samples, the average count per unit of weight was $2.5 \times 10^{6}$ (counts/ml/100mg/l). If we say that the mean volume per count is $V_m$ then: $V_m = 47 \times 10^{-4}(3 \times 10^{-4})^2$; $V_m = 4.23 \times 10^{-10}$ with $V_m$ having units of $cm^3$/count. This is calculated on the basis of a square end area with a cubic volume. The reciprical of this value will then give the number of counts in a cubic centimeter of solid algae which for this case would be $2.36 \times 10^{9}$ counts/ml and thus if we divide this $2.36 \times 10^{9}$ by $2.5 \times 10^{6}$ (counts/ml/100mg/l) this would indicate that there would be a density of about $9.45 \times 10^{2}$ times as much cellular material in a unit volume of solid algae as was used in the study culture. For the purpose of this example calculation, the unit dry weight was 100 mg/l and thus the solid layer of algal cells would represent a concentration ($\rho$) of $9.45 \times 10^{4}$ mg/l. Using this value in the relationship of Equation 17

$$\Gamma = e^{-E\rho b} \qquad [17]$$

where E is the extinction coefficient (1/mg-cm), $(6 \times 10^{3})$

$\rho$ is the density (mg/l), $(9.45 \times 10^{-4})$

b is the light penetration distance or thickness of absorber (cm), $(3 \times 10^{-4})$

Substituting, we have $\Gamma = e^{-6x10^{3}\ (e \times 10^{-4})(9.45 \times 10^{-4})}$. This gives a value of $\Gamma = 0.84$. An alternate method of calculation for this value of $\Gamma$ would be to consider the volume on the basis of the circular cross-sectional area which gives a mean volume per count of $3.32 \times 10^{-10}$ cc/count. Using this in the same manner as discussed before, the value of $\Gamma$ becomes

0.803, a variation of about 4-1/2%. The true situation could probably be more nearly represented as being about half way between the two extremes or by using the average value of $\Gamma$ calculated by the two methods. This would give a value of $\Gamma$ as 0.82. It would not be justifiable to change the calculations to this mean value as other percent variations are much greater.

Extinction Coefficient

The extinction coefficient may be evaluated from the fundamental absorption equation (62)

$$\frac{I_b}{I_o} = e^{-Ebc} \qquad [18]$$

where $I_b$ is the light intensity after penetrating a distance b (cm) and $I_o$ is the initial intensity before penetration of an absorber; c is algal concentration and E is the extinction coefficient (1/mg - cm). If we say that $\tau$ is the average fractional transmittance over the range of wave length being considered, the relationship may be expressed as Equation 19

$$\frac{I_b}{I_o} = \tau \qquad [19]$$

where $\rho$ is the culture density (mg/1). Equation 19 may then be written as shown in Equation 20.

$$\tau = e^{-\rho Eb} \qquad [20]$$

Using this form of the equation it is then possible to evaluate the extinction coefficient (E).

The term $\tau$ has been determined for this study by measuring the percent transmittance of an algal suspension of known density. The range for these studies was selected as 400 to 700 mμ with observations made at 10 mμ increments. This range of wave length was selected because the light source emits most of its energy in this range, and thus the algae would receive only a small fraction below 400 mμ or above 700 mμ and this would not be a very effective fraction.

The data obtained from several studies of percent transmittance at various wave lengths are shown in Table 9 and the results are plotted in Figure 29. The results of the plotted percent transmittance values for change in wave length were integrated to give the total area under the curve from which the average fractional transmittance ($\tau$) was calculated for the various densities.

Using these values of $\tau$, b = 2.0 cm (the depth of the spectrophotometer light cell), $\rho$ = the various culture densities (mg/l). An example of this relationship can be shown by the 40% transmittance culture where $\tau$ = 0.51, b = 2.0 cm and $\rho$ = 55 mg/l. Substitution gives Equation 21

$$0.51 = e^{-Ex110} \qquad [21]$$

which is obtained from Equation 20. If we solve this for E we obtain a value of $E = 6.13 \times 10^{-3}$ (1/mg - cm).

The extinction coefficients shown in Table 10 show agreement for these density values. These values then define the extinction coefficient constant for these density ranges and the 2.0 cm depth of the spectophotometer cell used to evaluate the extinction coefficient for this particular alga.

TABLE 9

PERCENT TRANSMITTANCE OF ALGAL CULTURES OF SCHIZOTHRIX CALCICOLA FOR VARIOUS WAVE LENGTHS OF LIGHT

(400-700 mμ-2.0 cm curvette)
Density (mg/l)

| λ (mμ) | 55 % T | 62 % T | 137 % T | 177 % T |
|---|---|---|---|---|
| 400 | 41.5 | 38.7 | 12.0 | 6.5 |
| 410 | 41.0 | | 11.0 | 6.0 |
| 420 | 40.5 | | 10.5 | 5.5 |
| 425 | | 36.0 | | |
| 430 | 40.0 | | 9.5 | 5.0 |
| 440 | 40.0 | | 9.0 | 4.5 |
| 450 | 41.0 | 34.5 | 10.0 | 5.0 |
| 460 | 42.0 | | 11.0 | 7.0 |
| 470 | 45.0 | | 12.5 | 8.0 |
| 475 | | 40.0 | | |
| 480 | 49.0 | | 15.0 | 10.0 |
| 490 | 51.0 | | 16.0 | 11.0 |
| 500 | 53.0 | 44.5 | 18.0 | 12.5 |
| 510 | 53.0 | | 20.0 | 14.0 |
| 515 | 55.5 | | | |
| 520 | 58.0 | | 22.0 | 16.0 |
| 525 | | 52.2 | | |
| 530 | 58.0 | | 25.0 | 17.5 |
| 540 | 59.5 | | 25.5 | 18.0 |
| 550 | 59.0 | 54.0 | 25.0 | 17.5 |
| 560 | 57.5 | 56.5 | 24.0 | 16.0 |
| 570 | 56.0 | | 23.0 | 15.0 |
| 575 | | 52.5 | | |
| 580 | 54.5 | 21.0 | 13.5 | |
| 590 | 53.5 | | 20.0 | 12.0 |

Table 9, continued

(400-700 mμ)
Density (mg/l)

| λ (mμ) | 55 % T | | 62 % T | 137 % T | 177 % T |
|---|---|---|---|---|---|
| 600 | 51.5 | | 46.0 | 19.0 | 11.0 |
| 610 | 50.0 | | | 15.0 | 9.0 |
| 620 | 46.5 | | | 14.0 | 8.0 |
| 625 | | | 41.5 | | |
| 630 | 46.5 | | | 13.5 | 8.0 |
| 640 | 47.0 | | | 14.0 | 8.0 |
| 650 | 48.0 | purple | 43.2 | 14.5 | 9.0 |
| 650 | 50.0 | red | 45.0 | 15.5 | 9.5 |
| 660 | 51.5 | | | 16.0 | 10.0 |
| 670 | 54.0 | | | 17.0 | 10.0 |
| 675 | | | 46.0 | | |
| 680 | 54.0 | | | 18.0 | 11.5 |
| 685 | | | | | 13.0 |
| 690 | 58.0 | | | 21.5 | 15.0 |
| 695 | 62.0 | | 56.0 | 28.5 | 21.0 |
| 700 | 62.0 | | 56.0 | 28.5 | 21.0 |

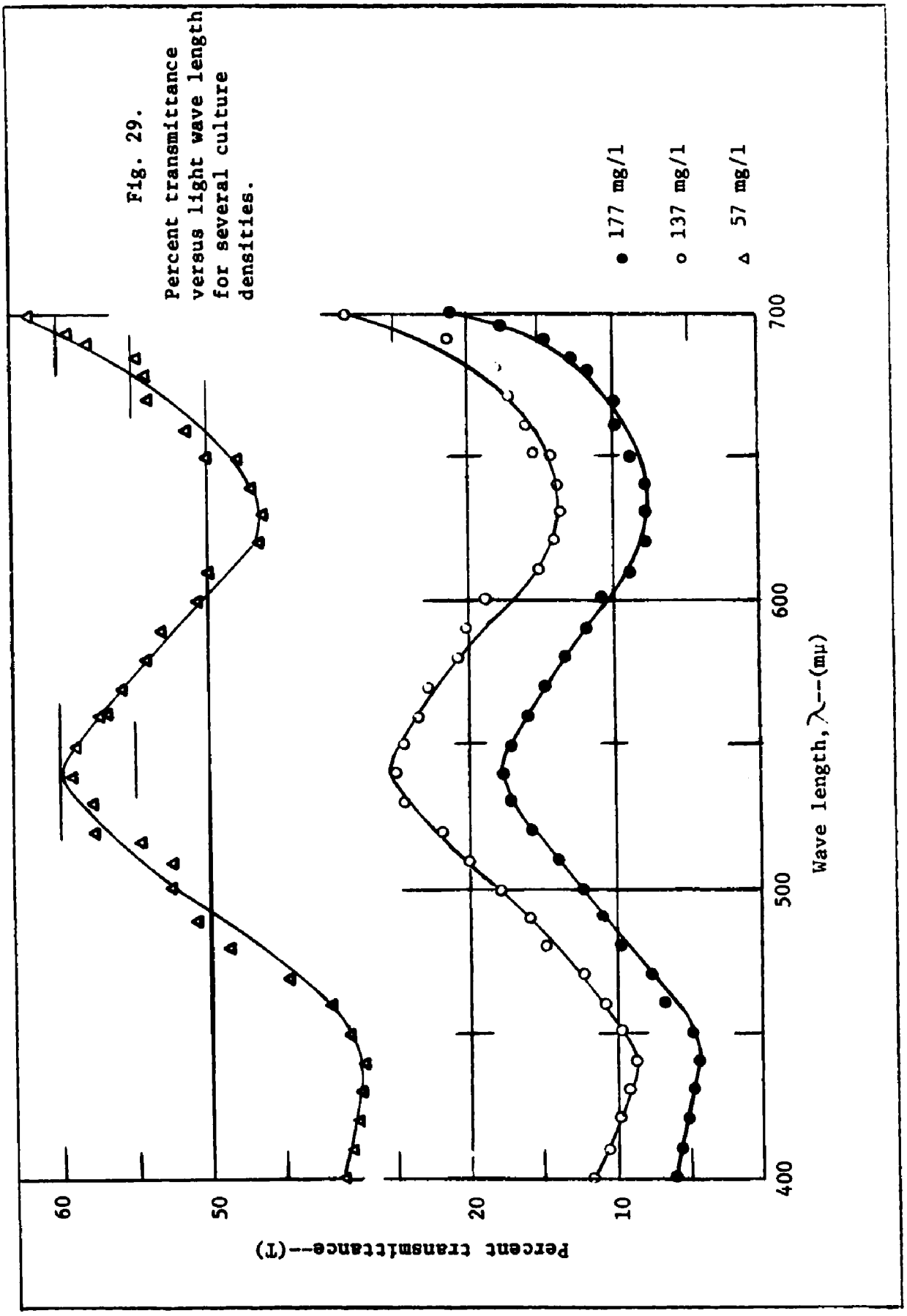



Fig. 29.
Percent transmittance versus light wave length for several culture densities.

TABLE 10

EXTINCTION COEFFICIENTS

| % T | (mg/1) | E(1/mg - cm) |
|---|---|---|
| 40 | 55 | $6.13 \times 10^{-3}$ |
| 57 | 33 | $6.21 \times 10^{-3}$ |
| 5 | 180 | $6.15 \times 10^{-3}$ |

Probable Mean Light Intensity

In a mixed culture of more than una-algal thickness, such as in mass culture, there will be shading of algae by others and this must be taken into account when considering the total mean light intensity.

In a mixed culture there will be random position of algal cells and thus the probability expression would more completely define the amount of light that an algal cell might receive. This probability may be described in terms of a Poisson Distribution Analysis after the manner discussed by Tamiya, et al. (99).

This may be related (Equation 22) on the basis of 0 to m number of shading cells where $r_n$ is the fraction of total cells shaded n times.

$$r_n = \frac{1}{x}(1 - e^{-x}) \sum_{0}^{m} \frac{x^i}{i_!} \qquad [22]$$

In this relationship x is a function of the concentration and size of the algae, as well as depth of the culture, Equation 24. The term i is the number of shading organisms. The evaluation of average light intensity for a shaded culture can be accomplished by summing all of the

portions of the probable light intensity fractions. If we designate $\bar{\tau}$ as the average light intensity fraction it may be related to $\Gamma$ by the summation expression shown in Equation 23.

$$\bar{\tau} = \Gamma^0 r_0 + \Gamma^1 r_1 + - - - \Gamma^m r_n \qquad [23]$$

$\Gamma$ is the fractional transmittance or the fractional amount of light transmitted by one algal cell of thickness. Expansion calculations of this relationship show that n should not be much larger than 5 to 10 as the additional results become relatively insignificant.

Table 11 shows some typical calculations of $\bar{\tau}$ for the conditions for which the extinction coefficient and unit algal transmittance were calculated. These values were developed on the basis of a value of $\Gamma = 0.84$.

In the first column of calculations shown for $\bar{\tau}$ in Table 11 the fractional light intensity was determined on the basis of a cubic configuration of the algae. However, the microscopic analysis shows that the algae have a mean length of about 47μ and a diameter of about 3μ. This would mean that the cubic configuration involved in the probability analysis must be modified in some way and this has been done in the second column of Table 11 by incorporating a shape factor ($\gamma$) in the value for x, Equation 24.

$$x = cd^2b\gamma \qquad [24]$$

The magnitude of $\gamma$ was based on the ratio of length to diameter which for this particular algal system L = 47μ and d = 3μ, thus $\gamma = \frac{L}{d} = \frac{47}{3}$ or approximately 15. In the expression for x, c is the number of algal units per milliliter for the particular culture density, d is the unit size of the

TABLE 11

PROBABLE AVERAGE LIGHT INTENSITY FRACTION

| % T | Density Dry Wt. (mg/1) | $\gamma$ | $\bar{\tau}$ | $\gamma$ | $\bar{\tau}$ |
|---|---|---|---|---|---|
| 57.5 | 33 | 1 | 0.94 | 5 | 0.75 |
| 57.5 | 33 | - | - | 15 | 0.50 |
| 39.0 | 57 | 1 | 0.91 | 5 | 0.61 |
| 39.0 | 57 | - | - | 15 | 0.32 |
| 20.8 | 95 | 1 | 0.84 | 5 | 0.54 |
| 20.8 | 95 | - | - | 15 | 0.18 |
| 5.4 | 173 | 1 | 0.65 | 5 | 0.23 |
| 5.4 | 173 | - | - | 15 | 0.073 |

algae (cm) and b is the culture depth parallel to the light path (cm).

The results of these calculations and the comparison between zero shape factor and the shape factor of 5 and 15 are shown in Figure 30. It would appear that the most representative value would be for the shape factor of 15 but this would be the upper limit. The shape factor 10 to 12 could probably be considered as a more justifiable mean value because the effect of shape would probably not have a direct linear relationship to the slenderness ratio. Figure 31 shows the effect of the variation of the shape factor ($\gamma$) around the values of 12 to 15.

These figures indicate that mutual shading has a very pronounced effect on mean probable light intensity for an algal organism up to a density of about 60 mg/1. Beyond this density (to higher values) there is less density variation effect, especially with long filamentous algae.

Fig. 30. Average light intensity versus dry-weight density.

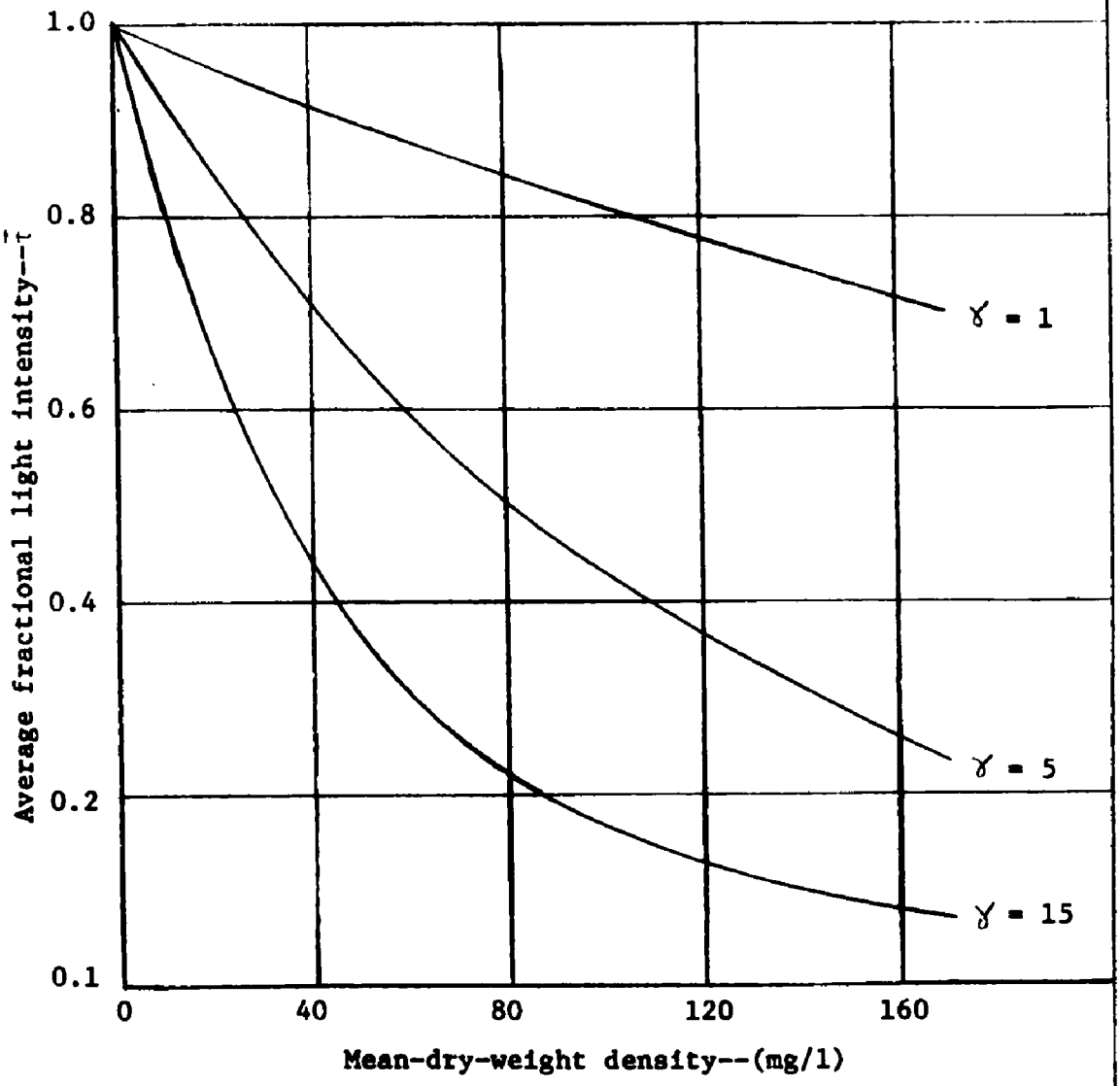
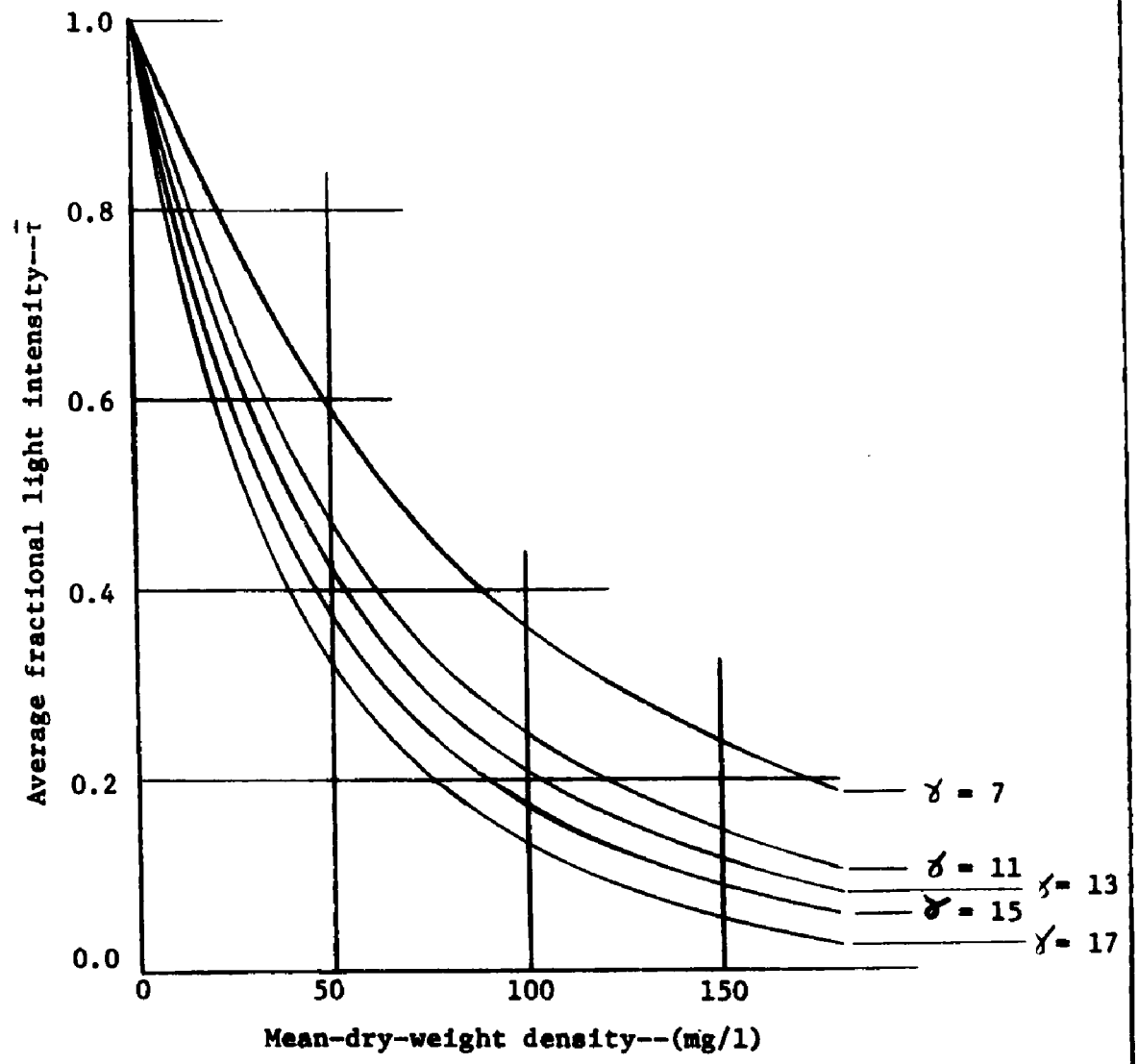


Fig. 31. Average light intensity versus dry-weight-density for several shape factors--($\gamma$)

(Shape factor based on mean count of $2.5 \times 10^6$/ml/100mg/l dry weight)



Mean Effective Light

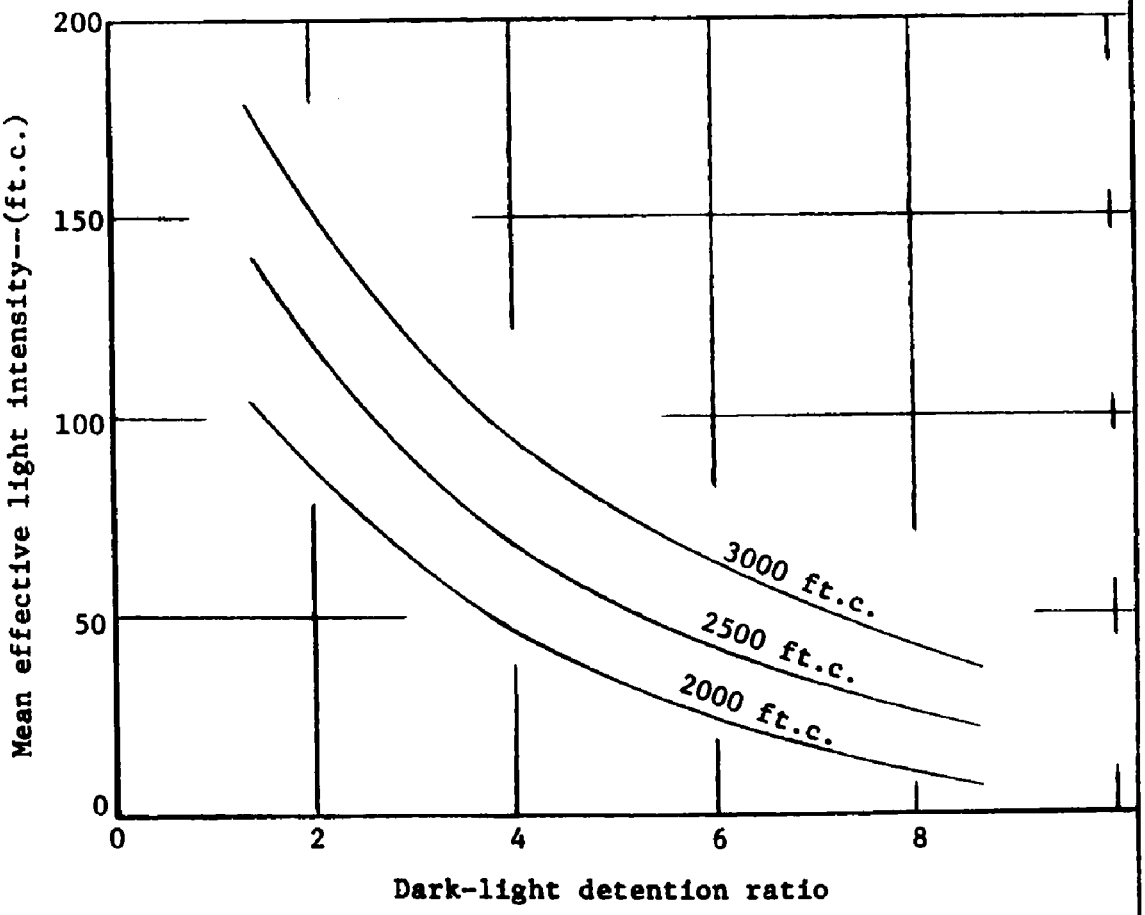
The light intensities used for most of the studies were in the range of outside light readings of 1000 ft.c. to 3500 ft.c. These intensities were obtained by either varying the light source configuration or as the result of bulb aging. In most cases a combination of the two factors was utilized to maintain relatively constant light conditions for particular studies. Table 5 shows the tabulation of necessary correction factors for the various configurations of the light source in order to obtain the culture-face light intensity. These factors are applicable for both the normal full light cell studies and also for the special velocity studies in which a light cell filler was utilized.

The table shows that in many instances a special or different correction factor is needed to correct for the inside light intensity depending upon the position of the fluorescent tubes in the on-position. In general, the configuration has been one with all the bulbs on. Intensities have been obtained by having the top bulb off, the top and bottom bulbs off, and in a few instances, two top and two bottom sets of bulbs off with a few of the other configurations used in very rare situations.

Figure 32 shows the plot of light values at the culture-face with respect to the mean effective light intensity based on corrections for culture depth, the probable light value, the average light intensity for the particular culture tank configurations, and the indicated culture density. This set of typical curves have been developed for the light configuration for all lights on. Slightly modified conditions would

Fig. 32. Mean effective intensity versus dark-light ratio for several light intensities.

Density--30 mg/l



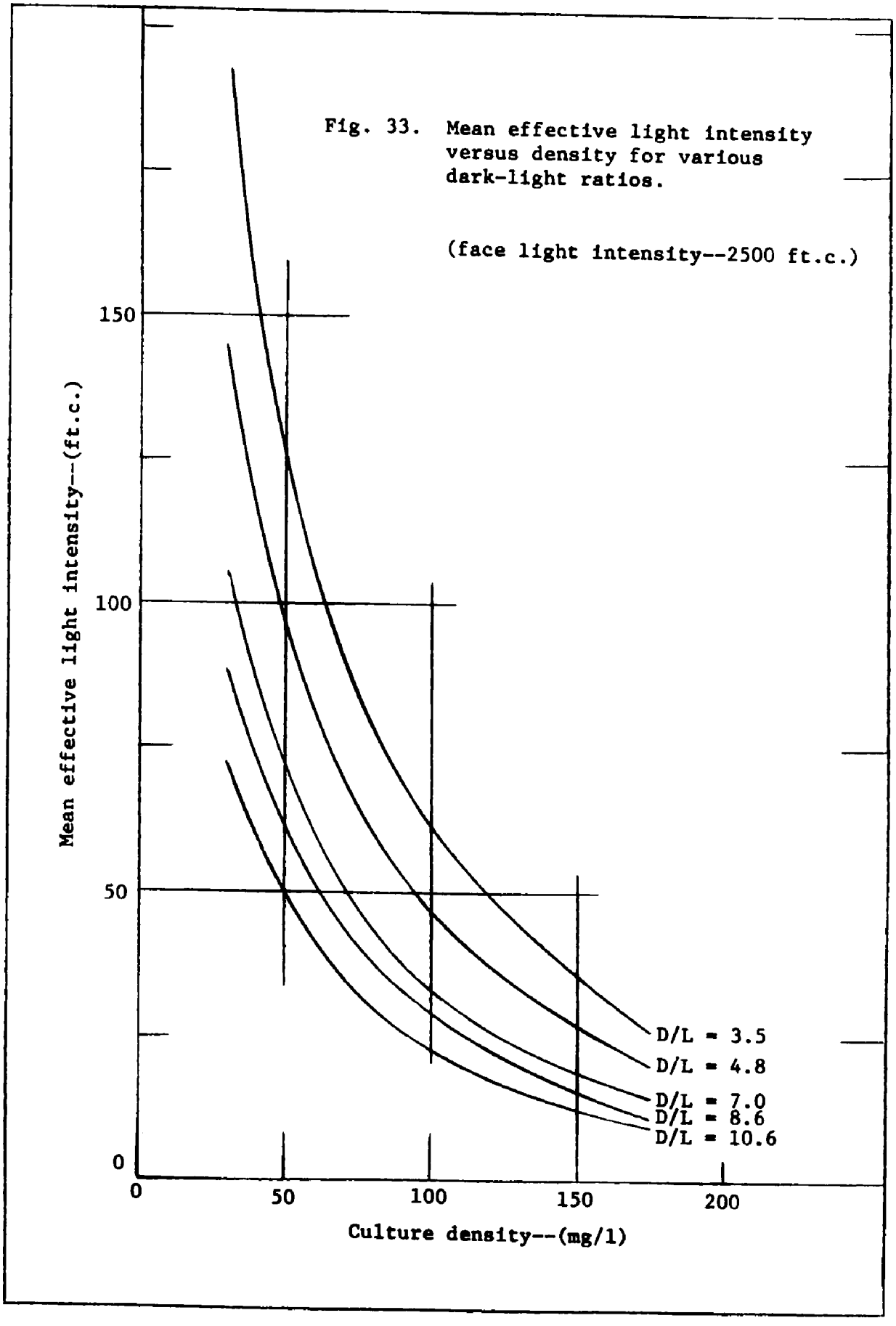
exist for the other configurations and this effect would be taken into account in individual calculations for a particular applied project.

Thus, Figure 32 shows that there is a definite relationship, at any particular density, between mean effective light intensity and the dark-light ratio. The mean light intensity increases when light source is increased and in direct proportion, as expected; but also the dark-light ratio has pronounced influence on mean available light. This combined effect may be related for all configurations in Figure 35. This means that the mean effective light about doubles in going from a dark-light detention ratio of 10.7 to 4.8 and increases by 2.6 times in going to a dark-light ratio of 3.4.

Figures 33, 34 and 35 show the variations of mean effective light intensity with respect to culture density, configuration, and relative configuration effect for a constant light-source intensity. Figure 33 shows that the effective light intensity increases sharply at culture concentrations of 75 mg/1 (29% T) or less. This figure (Figure 33) also shows a more pronounced trend in increased effective light in the lower dark-light detention ratios, thus showing that culture density plays a primary roll in controlling light intensity effect in mass cultures. This relation is more clearly shown in Figures 34 and 35. These figures indicate that the most effective and significant light effect areas of study are in the culture density range of 75 mg/1 or less and dark-light detention ratios of six or less. This confirms and substantiates the characteristics shown in the growth results (Figure 8) and yield results (Figure 17). Thus, it appears that in mass cultures the dark-light detention ratio must be somewhat lower than indicated from shallow, low density conditions of dark-light ratio values of 5 - 10.

Fig. 33. Mean effective light intensity versus density for various dark-light ratios.

(face light intensity--2500 ft.c.)



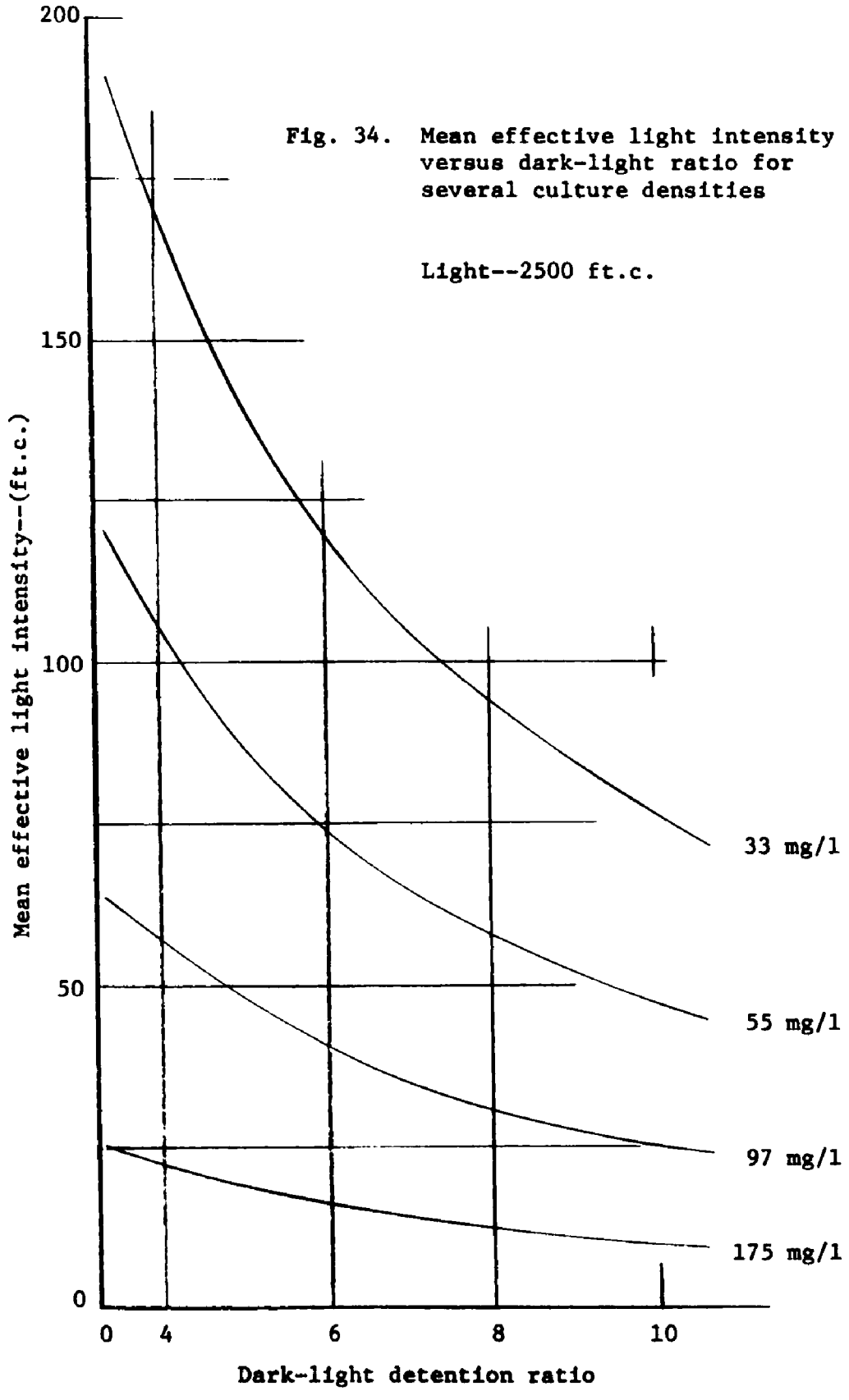
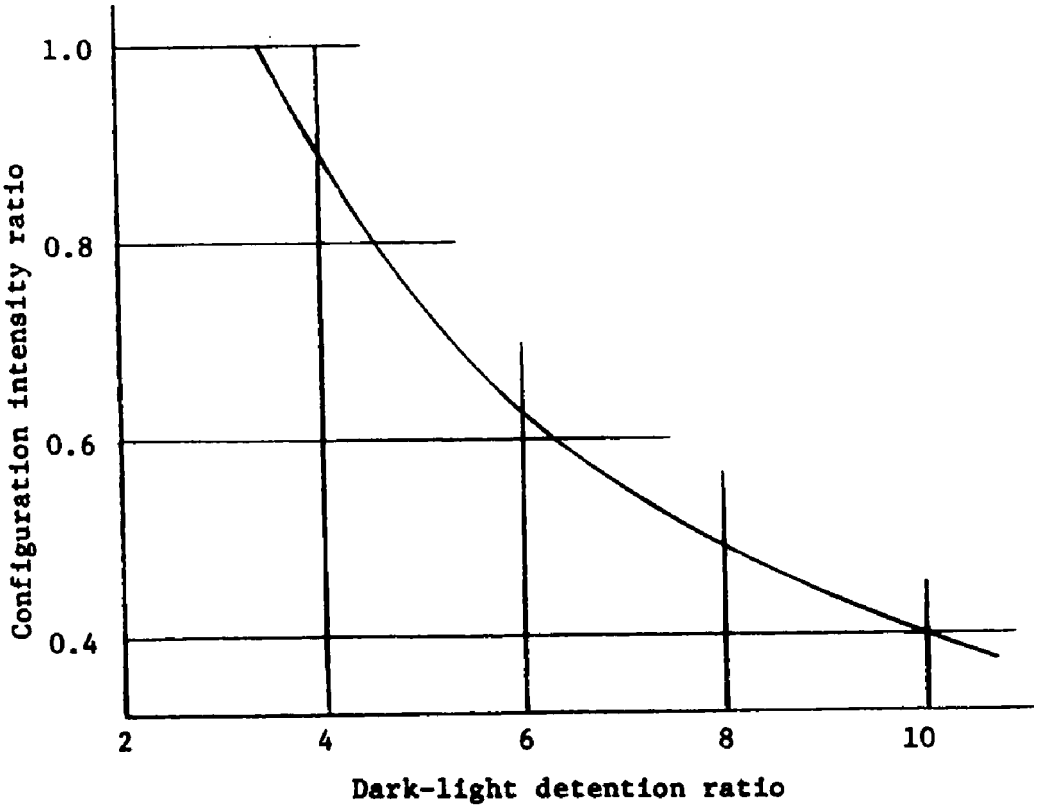



Fig. 34. Mean effective light intensity versus dark-light ratio for several culture densities

Light--2500 ft.c.

Fig. 35. Mean effective light intensity ratio versus dark-light ratio for all configurations--related to Configuration I

All culture densities
Light--2500 ft.c.



## LIGHT INTEGRATION

Light integrating growth is the efficient utilization in the dark of light energy received during a period of high light intensity and short duration. This effective utilization will produce a growth rate, or yield, over the total time interval, greater than expected from the maximum rate of growth during the light exposure time. Full integration is obtained if the growth rate in intermittent light is equal to the maximum possible growth rate for the average of the intermittent light intensity. This average intensity is equivalent to the mean intensity in continuous light.

This condition of intermittent light utilization has been discussed extensively by Phillips and Myers (71) in which they indicate that for Chlorella the compensation point would be about 24 ft.c. The light exposure used in their work was about 10 times the continuous light saturation level.

### Zero Integration

The integrated intensity of light, according to Phillips and Myers (71), is a function of the light intensity times the fraction--light time to total cycle time. Thus, full integration could be expressed as a condition where maximum growth rate could be obtained under flashing light conditions and this rate of growth would be equal to that obtainable under optimum continuous light conditions.

The mathematical relationship of light intensity-duration for integration analysis can be expressed as the relation of an area under a high light intensity time curve being just equal to the area of the continuous light intensity-time curve over the total time interval (71,72), before cells receive another burst of light. Mathematically this could be

expressed as Equation 25 (see Figure 36).

$$\int_0^{t_f} f(I_f)dt = \int_0^{t_T} f(I_c)dt = \int_0^{t_T} (I_M)dt \qquad [25]$$

where $t_f$ = light time

$t_d$ = dark time

$t_T$ = total time interval

$t_T = t_f + t_d$

$I_f$ = intensity of intermittent light

$I_c$ = intensity of continuous light

$I_M$ = integrated intensity

Zero integration growth ($k_o$) can be expressed as a condition of the maximum growth rate [k(max.)] expected in continuous light times the light time fraction (71), Equation 26.

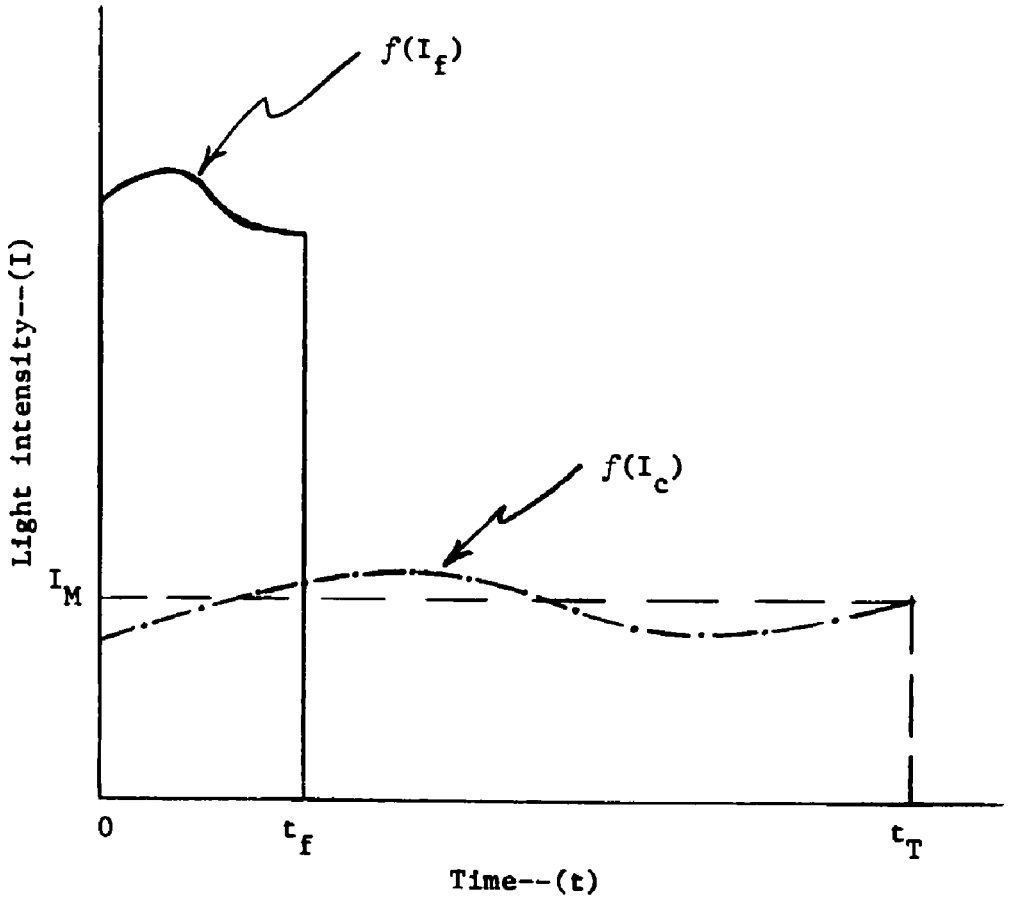
$$k_o = 2.1 \left[ \frac{t_f}{t_f + t_d} \right] \qquad [26]$$

where $t_f$ = flash time (function of light vol)

$t_d$ = dark time (function of dark vol)

Table 12 shows values for $k_o$, calculated by Equation 26, for the system configurations used in these studies. These results mean that a growth rate less than the configuration value would show poorer light utilization than could be expected in an equivalent uniform continuous light situation. A value just equal to the magnitude of $k_o$ would indicate that growth was taking place only equal to the maximum possible during the light period; thus, the light period would appear to be the effective growth period. A value greater than $k_o$ would indicate growth rates over

Fig. 36. Functional relationship of mean effective light intensity with respect to time.



the total time greater than would be expected in the light exposure time, if the calculated growth rate is distributed over total time ($t_T$). In other words, complete integration would show a plot of k versus integrated light intensity which would be the same as a plot of k versus continuous light intensity, for an algal culture of one algal thickness.

TABLE 12

ZERO INTEGRATION

| Configuration | Dark Vol. (1) | Light Vol. (1) | $\frac{t_f}{t_f + t_d}$ | $k_o$ |
|---|---|---|---|---|
| I-A | 2.65 | 1.10 | 0.292 | 0.613 |
| I | 3.75 | 1.10 | 0.227 | 0.477 |
| II | 5.30 | 1.10 | 0.172 | 0.362 |
| III | 7.75 | 1.10 | 0.124 | 0.260 |
| IV | 9.45 | 1.10 | 0.104 | 0.218 |
| V | 11.72 | 1.10 | 0.086 | 0.181 |
| I With Light Filler | 3.2 | 0.56 | 0.149 | 0.313 |

Thus growth rate values obtained in the area between zero integration and complete integration, for any constant light intensity, are a function of flash time, or, in the case of mass culturing, culture velocity. A similar expression for integration has been reported by Kok (72) and expressed in a similar manner. The major difference in his expressions was the utilization of a fractional value of integrated

light intensity as the average intensity over time, divided by the incident intensity, thus reducing it to a unity value.

Light Integrating Growth

The data for this work have been analyzed with respect to growth characteristics under various light intensities, velocity, dark to light ratio and density conditions. An important area which has not been examined extensively has been the possibilities of light energy integration and growth under these various conditions.

In order to develop an evaluation of possible indications of integrating growth the data were plotted as shown in Figures 37 and 38. These figures were plotted for several conditions of culture density, light intensity approximately 2500 ft.c., and a velocity of approximately 0.77 ft/sec in the light cell. The effective light intensity was varied primarily by variation of the dark time.

These figures were plotted for considerations of k versus two possible expressions of the dark-light relationship. The data are compared with a probable light integration condition, solid lines in Figures 37 and 38. These lines were obtained from the relationship shown in Table 12 (71). Integrating growth would be for values above the solid lines and nonintegrating growth would be below the line.

Figures 37 and 38 show that there appears to be evidence that integrated growth might be demonstrated under the conditions of higher available light energy. At a dark-light ratio of three (Figure 37) k is 0.55 for a culture density of 57 mg/l and 0.64 for a density of 33 mg/l. At this dark-light ratio integration growth rate (k) is 0.53 and thus these growth values indicate possible light integration. This evidence of

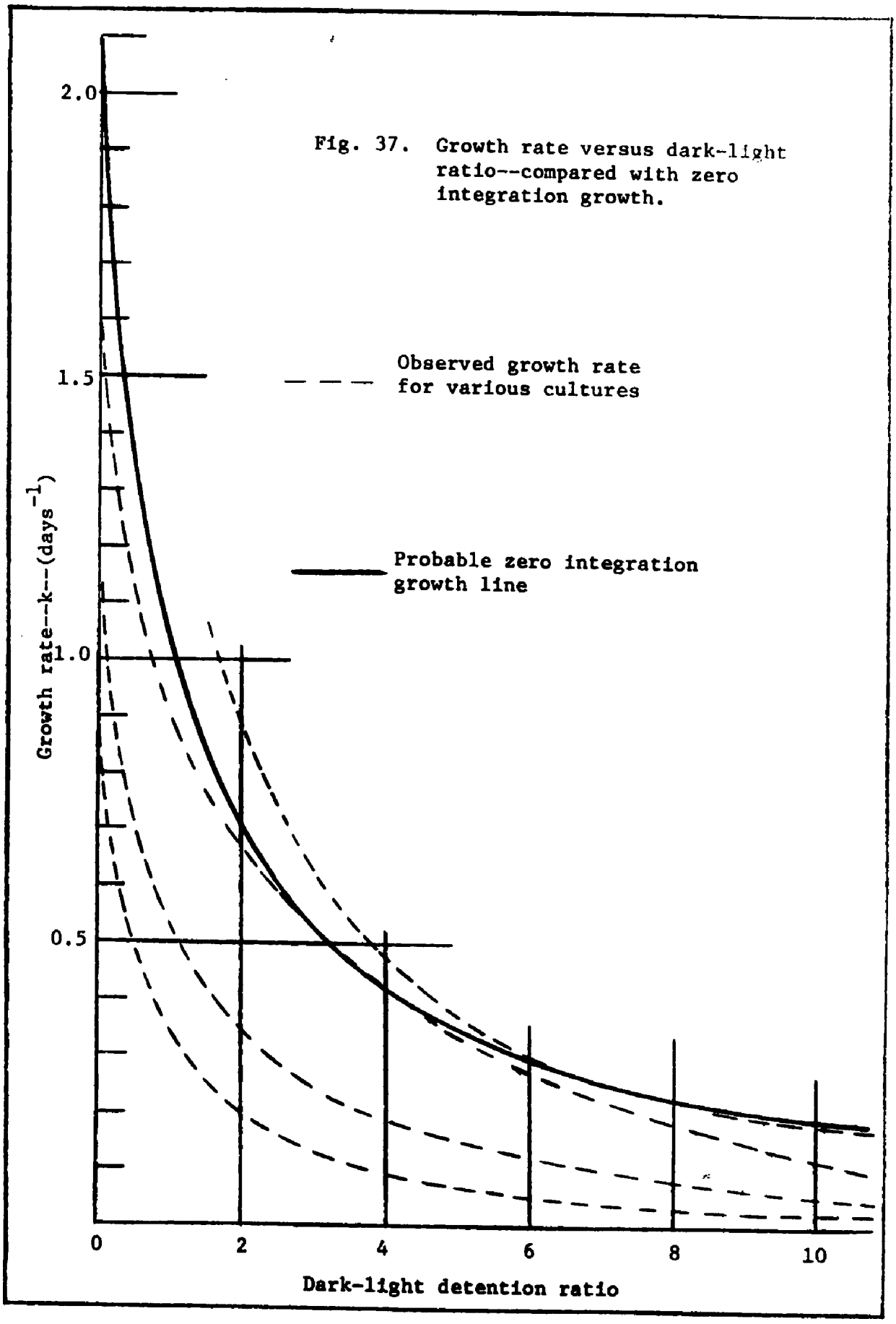
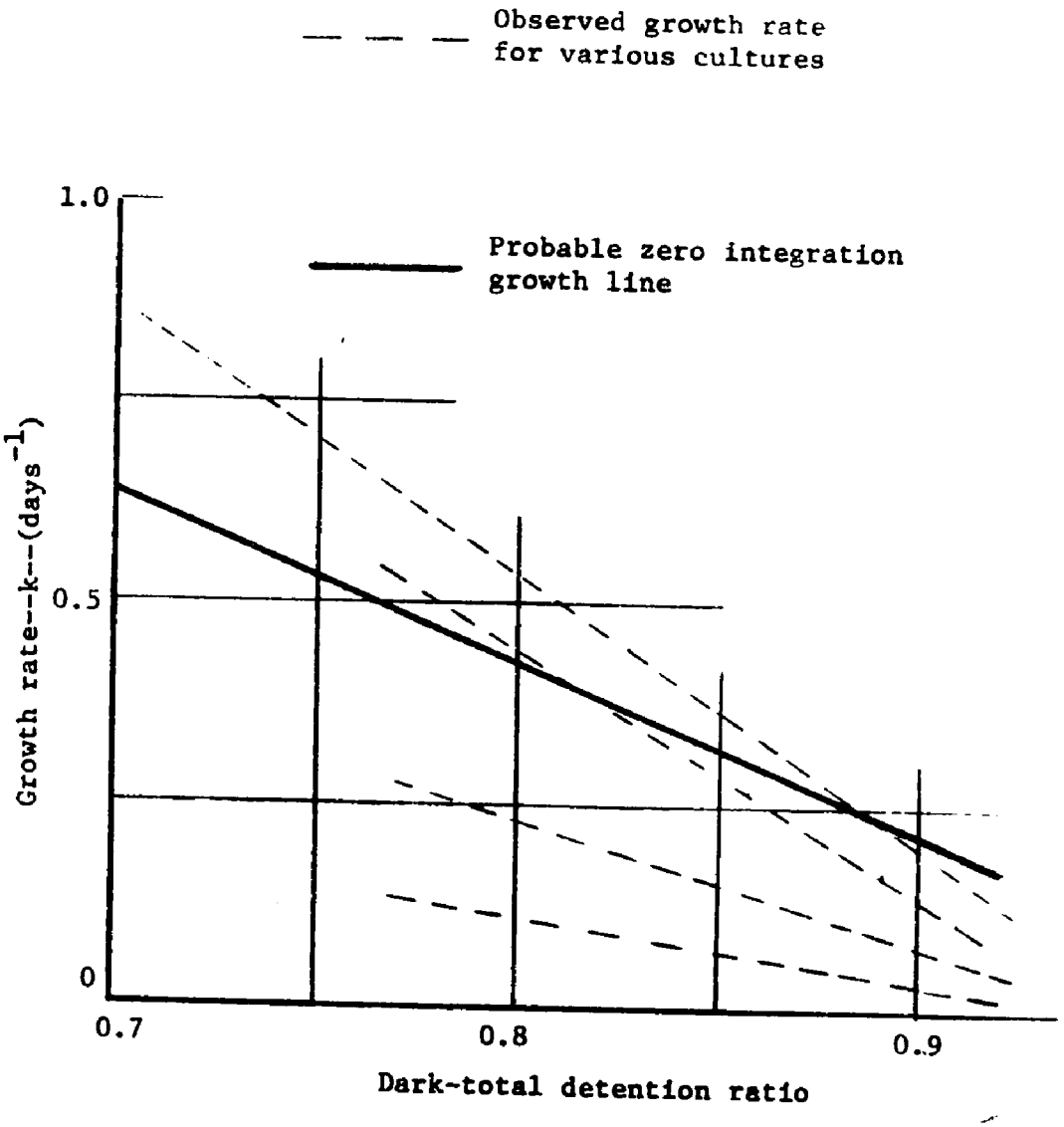



Fig. 37. Growth rate versus dark-light ratio--compared with zero integration growth.

Fig. 38. Growth rate versus dark-total ratio--compared with zero integration growth.



growth response is even more pronounced in Figure 38, growth rate versus dark-total ratio. This comparison indicates that for the 57 mg/l culture density integrating growth could be expected in the range of dark-total detention ratios of about 0.5 to 0.83. This density shows very marginal integration potential but it does show the effect of increased light availability. In the 33 mg/l culture, density shows more pronounced integrating growth possibilities in the dark-total detention ratio range of 0.5 to 0.8. It is significant to note that this evidence of integration was obtained at the average culture study condition, 2500 ft.c. and a mixing speed of 1730 rpm. Thus, for this part of the analysis, the higher light energy conditions were obtained by reducing the period of time the cells were in the dark. This produced a growth rate higher than would be expected if growth took place at the maximum possible rate only during the light period.

If light integration effects are really indicated by the values shown in the respective figures then it should be possible to show improved integration possibilities by increasing the velocity for any given configuration. Figure 39 shows the variation of growth rate with respect to the mean configuration light fraction for a culture density condition of 33 mg/l, light approximately 2500 ft.c., and for several configurations. The zero integration growth line is drawn through these values. At low velocities it can be seen that the growth rate is below the integrating growth value. Increasing the velocity produces results which show an increase in growth rate with the highest values being well above the zero integration line. The figures for the highest and lowest points for each configuration indicate the light exposure time in $10^{-2}$ seconds. For these conditions the light exposure times of

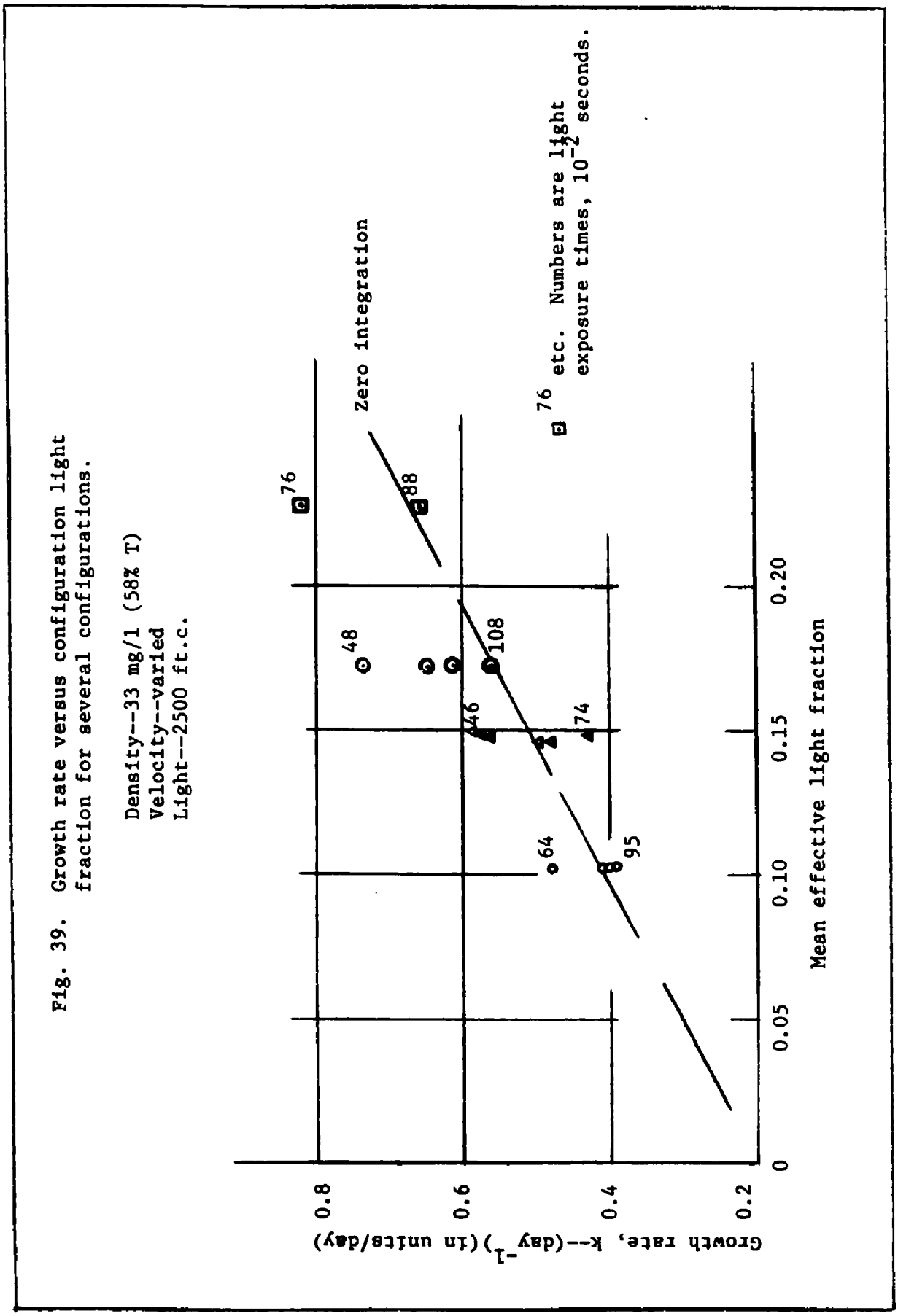



Fig. 39. Growth rate versus configuration light fraction for several configurations.

Density--33 mg/l (58% T)
Velocity--varied
Light--2500 ft.c.

0.7 - 1.0 seconds tend to be in the range where less than integrating growth rate is produced. In all instances increasing the velocity, thus, decreasing the light exposure time showed a transition from marginal to pronounced possibilities of integration. An exposure time of less than 0.6 seconds tended to produce such growth.

These border-line velocity and exposure time situations may be defined more completely in Figures 26 and 27. These figures show that there is marginal integration in these systems and at a velocity of about 0.77 ft/sec, or for an exposure time of 0.7 or less, there would be marked increase in the integration potential with increased velocity as the growth rate increases markedly with respect to unit effective light.

In considering the effects of velocity, it may be noticed in Figure 39 that integrating growth tends to occur at the longer light exposure times in the shorter dark time configurations. Thus, it appears that the velocity increase in the dark-light ratio of about 3 - 4 was more effective than in the ratio of about 10. This substantiates the indication of integration being marginally reached in the ratio range of 2 to 6 shown in Figure 37.

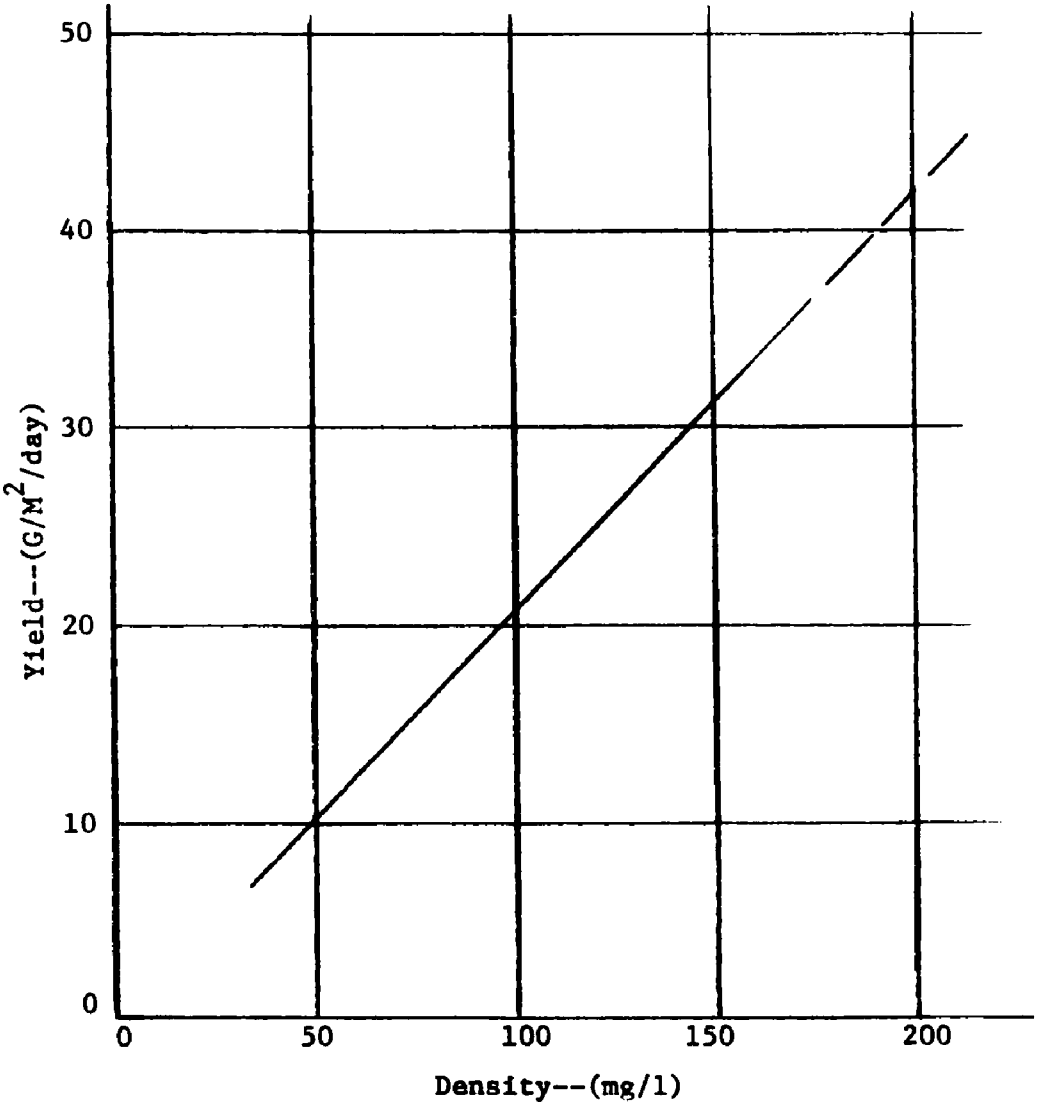
In considering light integration we need to evaluate the effect on yield because if there is integration there should be a corresponding increase in yield which would be greater than the zero integration yield. This increase is shown for the highest light intensity cultures, under constant velocity conditions, in Figures 17 and 18. It should be noted again that the most advantageous range is shown to be in the range of dark-light detention ratio of 2 to 6. The other yield relationships, Figures 19, 20 and 21, indicate similar yield situations. In order to evaluate more completely the zero integration yield level,

the yield condition has been plotted in Figure 40 for varying density conditions. This shows that at a culture density of 57 mg/l (Figure 19) zero integration yield would be obtained at 12 gm/sq.m/da which for these conditions would be obtained in a range of dark-light detention ratio of 2-5. For a higher density such as 95 mg/l (Figure 20) integrating yield would be 20 gm/sq.m/da and thus no integrating yield was produced under these conditions, and for the 174 mg/l density cultures (Figure 21) the yield relationships were well below integration yield of 36 gm/sq.m/da.

These figures show that under constant velocity conditions some apparent integration was evident in the lower density cultures (higher light intensities), and thus if velocity is increased integration should be obtained from any marginal configuration. This is evident in Figure 22 where the effect of velocity and configuration, as compared with the zero integration yield, indicate that velocities of less than 0.8 to 0.9 ft/sec produce marginal integration yield. Increasing the velocity above this level increases the yield above the zero integration point showing the quantitative effects of the advantages of high velocity.

From these studies it would appear that the best conditions for obtaining light integrating or apparent integrating growth in mass cultures would be to have a dark to light ratio of 3-6, a minimum mean effective light intensity of at least 100 ft.c. and a maximum light time of 0.7 to 0.8 seconds. This would appear to indicate that for this depth of culture and general system configuration, the mean effective light intensity can be varied by changing the intensity of the source, by changing configuration or by varying the density of the culture. A change in culture depth would primarily change the effective mean light intensity.

Fig. 40. Yield versus density for zero integration.



## CONSTANT FEED

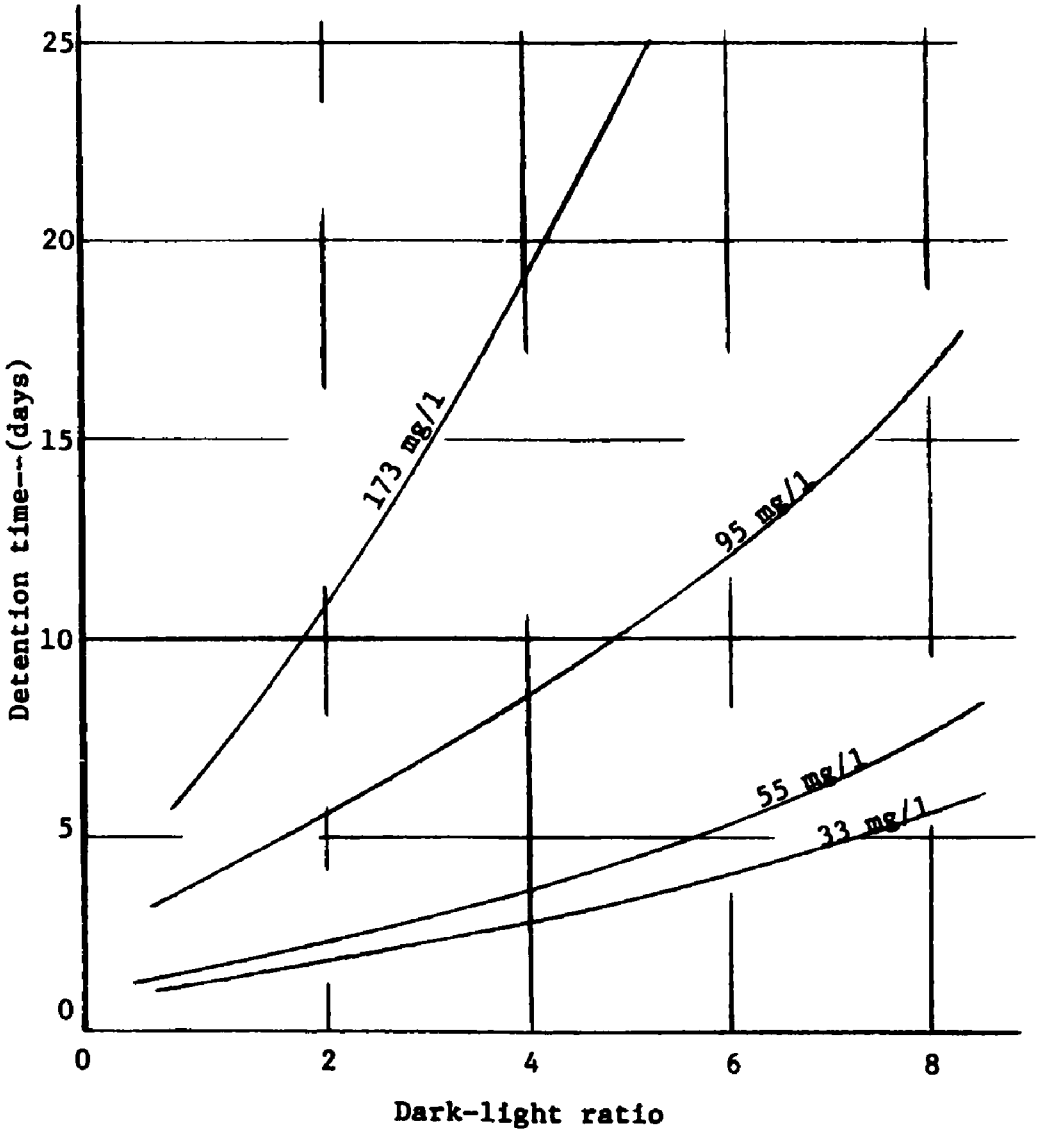
Throughout the growth response studies the results of the algal systems have been based on an effluent overflow controlled by density sensing equipment. This provided a means for evaluating results and at the same time maintain a constant density. Under an applied condition this type of control would probably not be the situation, but instead the flow would tend to control the resulting conditions. The assumption has been that regulation of the density by overflow from the influent feed would be comparable to the effluent discharge control effect from a constant feed system.

The relationship of the feed and overflow characteristics to actual application is of significance because detention volume and flow rates, which will establish detention time, are frequently the controlling relationships in any operating system. The data from this experimental work has been translated into a representation as shown in Figure 41 as detention time versus dark-light ratio. These curves show that the detention time (days) is a reciprical function of the growth rate (see Equation 27).

$$D.T. = \frac{1}{k} \qquad [27]$$

This figure shows that if a given density is to be maintained in a controlled dark-light system the detention time provided must be increased as the dark-light ratio increases or conversely if a detention time is established the culture density will tend to decrease as the dark-light ratio increases. This reduction would be the result of mean effective light reduction and since, under constant velocity and light

Fig. 41. Detention time as a function of dark-light ratio.



intensity conditions the culture is light dependent, it is expected that there would be a decrease in the growth rate as a result of longer dark time. This effect is similar to the conditions which may be observed in cultures under continuous illumination, especially when the average illumination intensity is modified by shading effects as a result of density or depth of culture.

From a design point of view the information in Figure 41 would then be of considerable concern in determining size relationships, total and dark-light values, for artificial or mechanical culturing systems. This information would be useful in conditions where it is desirable to grow algae for production pruposes or in systems where nutrient removal is the objective.

In order to evaluate the validity of these curves a system was developed to provide continuous feed to the culture at a rate which would be expected to maintain a density in the range of one of these curves. The most effective density study was 60% T and thus this density was selected for study purposes.

The system selected for comparison consisted of Configuration II (dark to light ratio of 4.81), with external light intensity of about 3000 ft.c., mixing speed of 1730 rpm. This was selected as a relatively average and representative arrangement for many of the 33 mg/l (approximately 60% transmittance) cultures.

The culturing system was set up in the usual manner with all the appropriate controls for pH and temperature but the density sensing equipment was eliminated. The influent feed was regulated by a 10 milliliter buret with a rubber tube placed over the open end and connected to the medium supply tank. A pinch clamp was also placed on the tubing to

help control the rate of flow through the buret. The pinch clamp provided coarse adjustment and the buret the fine adjustment. This buret was adjusted to give a flow of about 2600 milliliters per day.

The results of this evaluation, Figure 42, show very good agreement with the cumulative overflow with respect to time as the constant feed results are almost superimposed on the curve for cumulative overflow for a similar density controlled rum. From this it can be shown that the growth rate was identical under the two control methods. This then showed the validity of the relationships expressed in Figure 41.

In order to evaluate further the justification that there would be similar conditions the tabulation of lighting, speed, percent transmittance, density and nutrient conditions are tabulated in Table 13 which shows similar relationships for each condition. It is significant that the percent transmittance is about 59% in the continuous feed culture (Table 13), dry weight equilibrium 30 mg/l with a growth rate of 0.43, in units per day. For the density controlled culture comparison the comparable percent transmittance was 56%, dry weight density 34 mg/l and similar nutrient removal characteristics. The variations noted are well within the observed variations for most of the studied conditions. This shows that the overflow and growth rate information can be related quantitatively to continuous-flow systems.

Fig. 42. Overflow versus time.

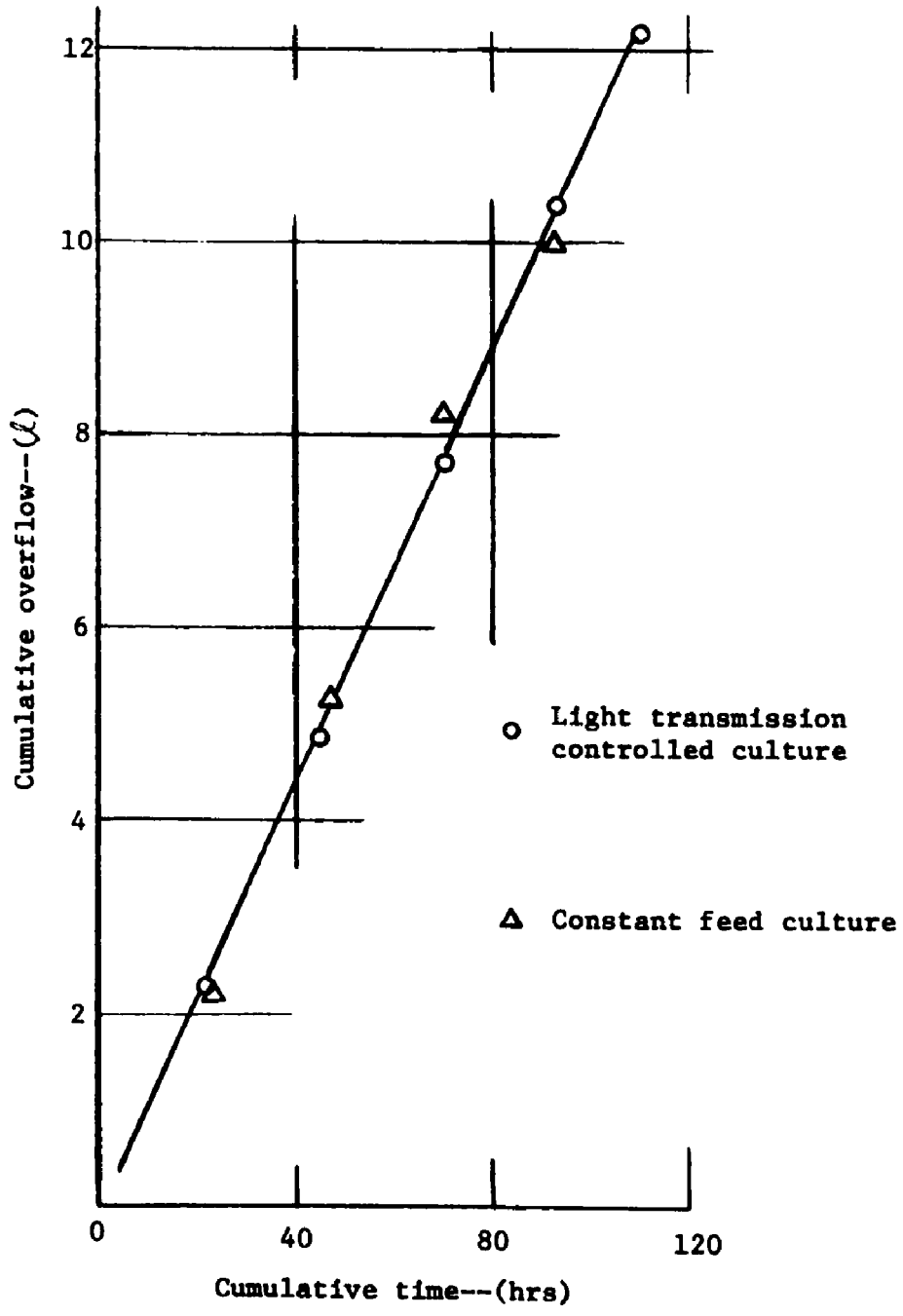


TABLE 13

CONSTANT FEED AND DENSITY CONTROLLED CULTURE RESULTS

| | Constant Feed | Light-Density Controlled |
|---|---|---|
| Light (ft.c.) | 2620 | 2440 |
| Speed (rpm) | 1726 | 1730 |
| Temp. (°C) | 20.0 | 19.9 |
| pH | 7.5 | 7.5 |
| % T | 58.9 | 56.2 |
| Density (mg/l) | 30.0 | 34.0 |
| TIN Removed (mg/l) | 5.3 | 8.0 |
| Orthophosphate Removed (mg/l) | 3.6 | 4.7 |

## NUTRIENT REMOVAL

During the course of these algal studies the nutrient observations have consisted of determinations to indicate the concentrations of nitrate, nitrite and ammonia nitrogen, all values expressed as N. The phosphorous determinations were made as ortho-phosphate. In all of the determinations the ortho-phosphate results were consistent for the various studies. The nitrogen determinations, for any one form, tended to be somewhat variable and thus for purposes of analysis the results have been reported and utilized as TIN (total inorganic nitrogen). Table 14 shows some typical average nutrient results for the various studies.

TABLE 14

TYPICAL AVERAGE REDUCTIONS IN NUTRIENTS AND ALKALINITY

| Component | Influent Concentration (mg/l) | Percent Reduction Through System | | | |
|---|---|---|---|---|---|
| | | 5.4% T | 20.8% T | 39.0% T | 57.0% T |
| $NH_3$-N | 45 | 73 | 39 | 22 | 15 |
| $NO_2$-N | 0 | - | - | - | - |
| $NO_3$-N | 10 | 18 | 20 | 10 | 9 |
| $PO_4$ | 28 | 31 | 24 | 11 | 15 |
| Alk. | 176 | 52 | 30 | 22 | 10 |

The influent concentration for all of this work was kept at a constant value with a calculated applied concentration of 55 mg/l of TIN and 28 mg/l of ortho-phosphate. Under these nutrient concentration conditions the removal for all of the studies was thus limited to light, velocity and density responses. In the culture density range utilized for these studies, about 30 to 175 mg/l, the variation for TIN removal was a straight line function as shown in Figure 43. This indicates that there was a mean TIN removal of 11.2 mg/l/100mg/l of algal dry weight. This is of course removal data for nonlimiting influent nutrient concentrations and thus variation should not be the result of nutrient limitations.

Figure 44 shows the percent removal of TIN with respect to culture density. This curve indicates that as the density increases the percent removal increases. Over the range of density conditions studied the removal was in the order of 29.8%/100 mg/l change in algal dry weight. This implies that for these culture conditions about 13% removal of TIN can be expected at a culture density of 30 mg/l and at a culture density of 175 mg/l the removal could be expected tp be 54%.

The effects of TIN removal are shown in Figures 45 and 46 for low density cultures under various dark to light ratios and for dark to total ratios. The removal results expressed in terms of gm/sq.m of lighted surface per day are similar to yield curves shown in the growth section. These results indicate that an optimum dark to light ratio for TIN removal would be in the range of 3 to 4 (0.75 to 0.80 dark to total ratio) for these low density cultures and the speed and light conditions indicated. The TIN removal based on the lighted area will vary according to the yield (gm/sq.m/da) as the amount of TIN removed is a function of

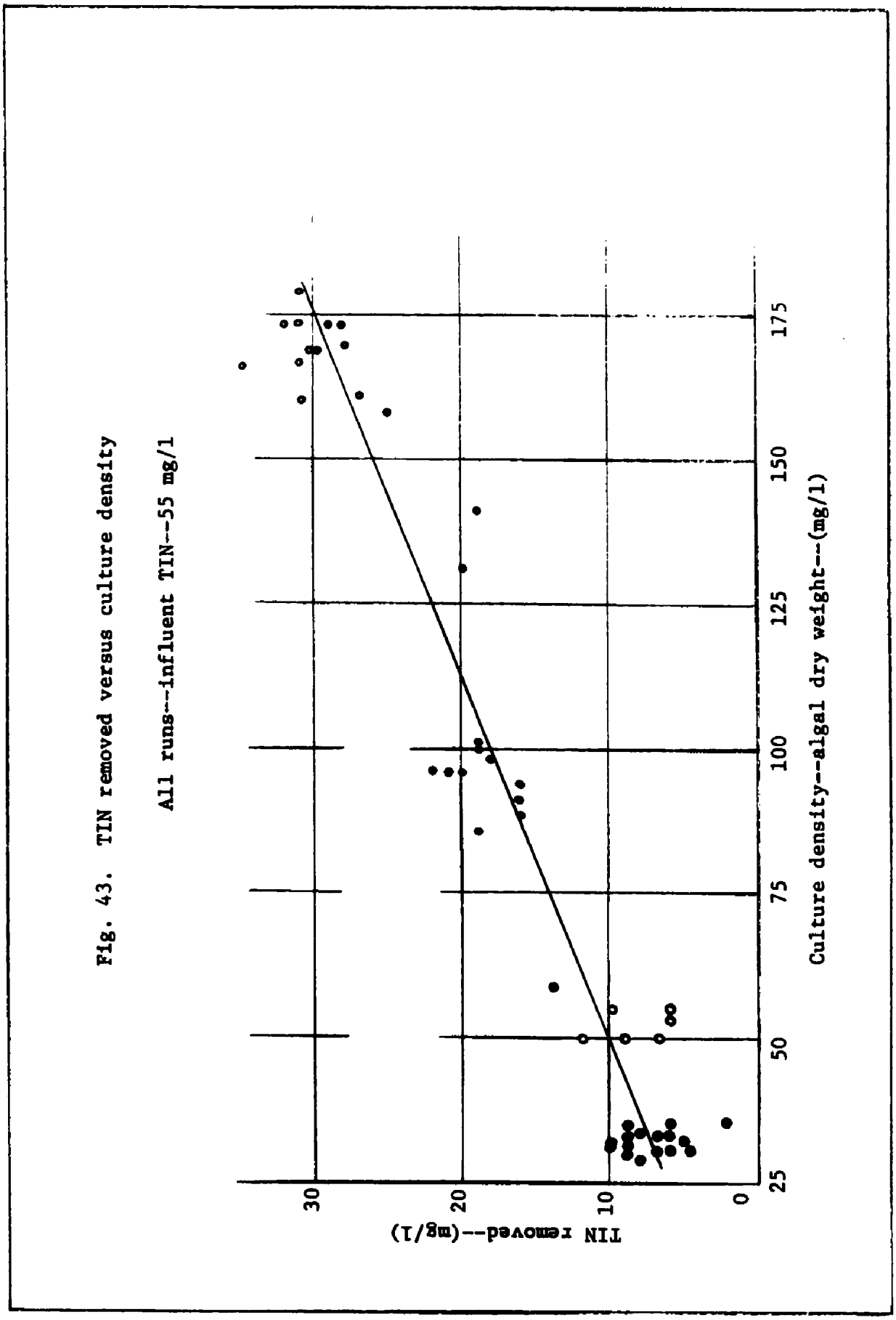



Fig. 43. TIN removed versus culture density

All runs--influent TIN--55 mg/l

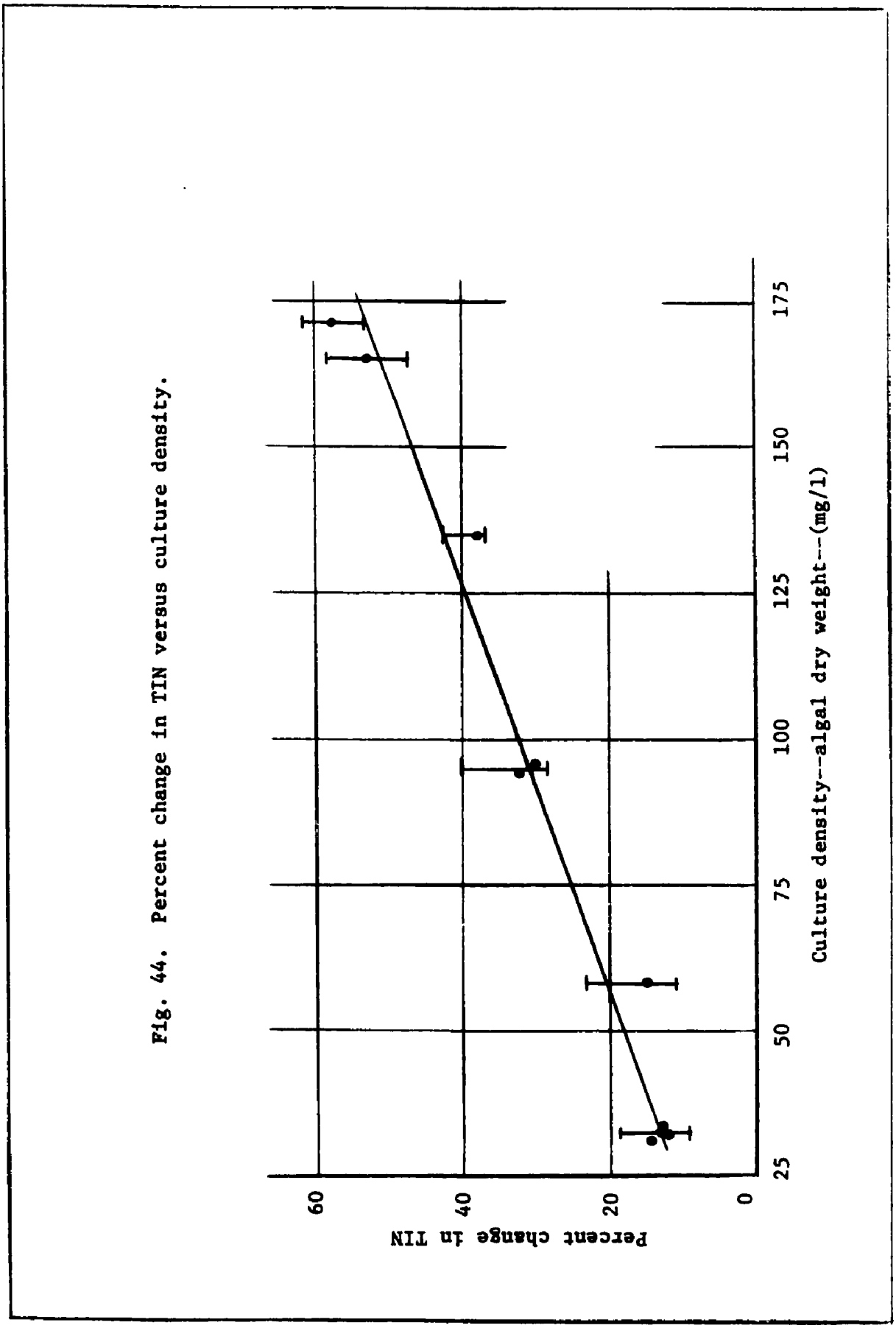



Fig. 44. Percent change in TIN versus culture density.

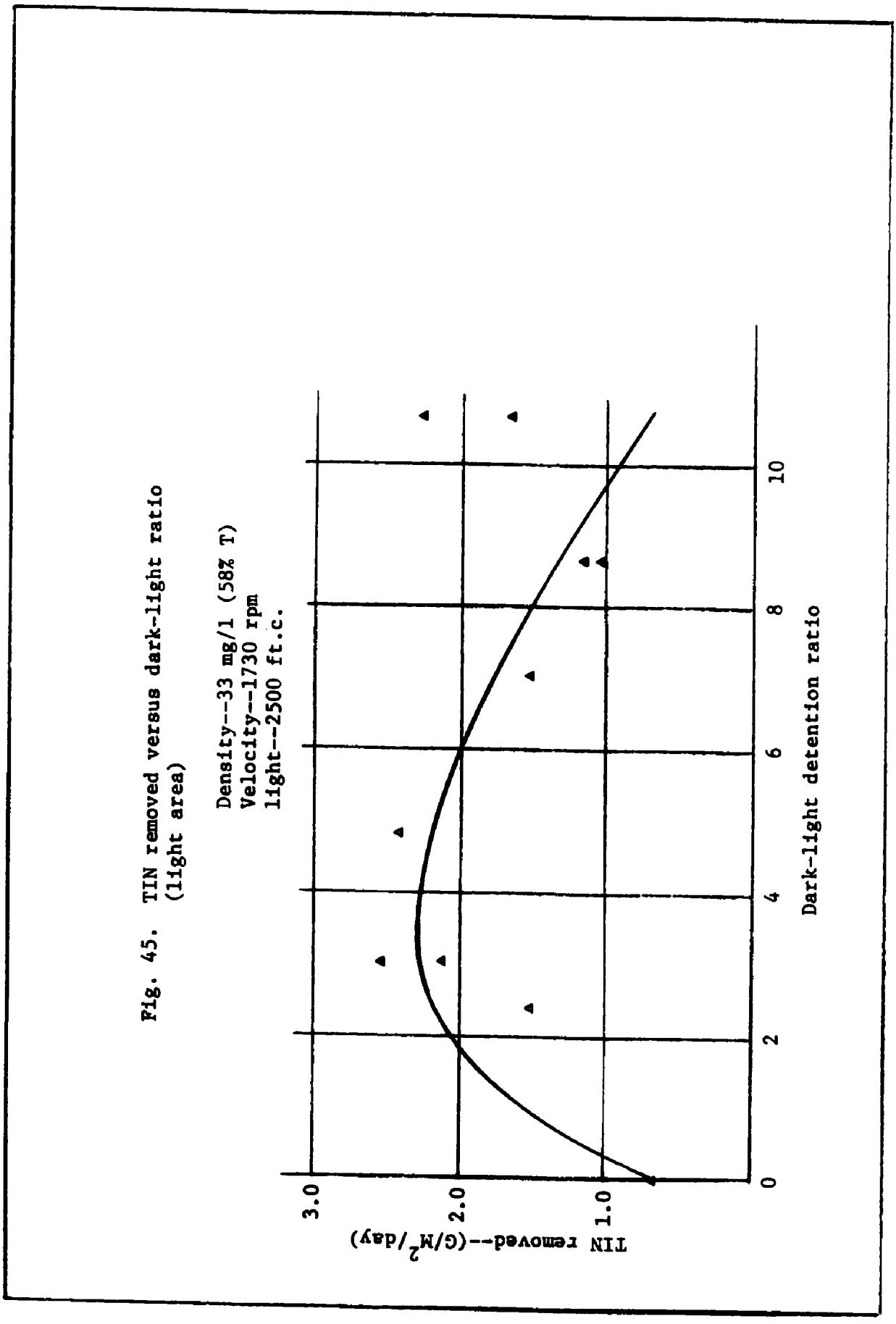



Fig. 45. TIN removed versus dark-light ratio (light area)

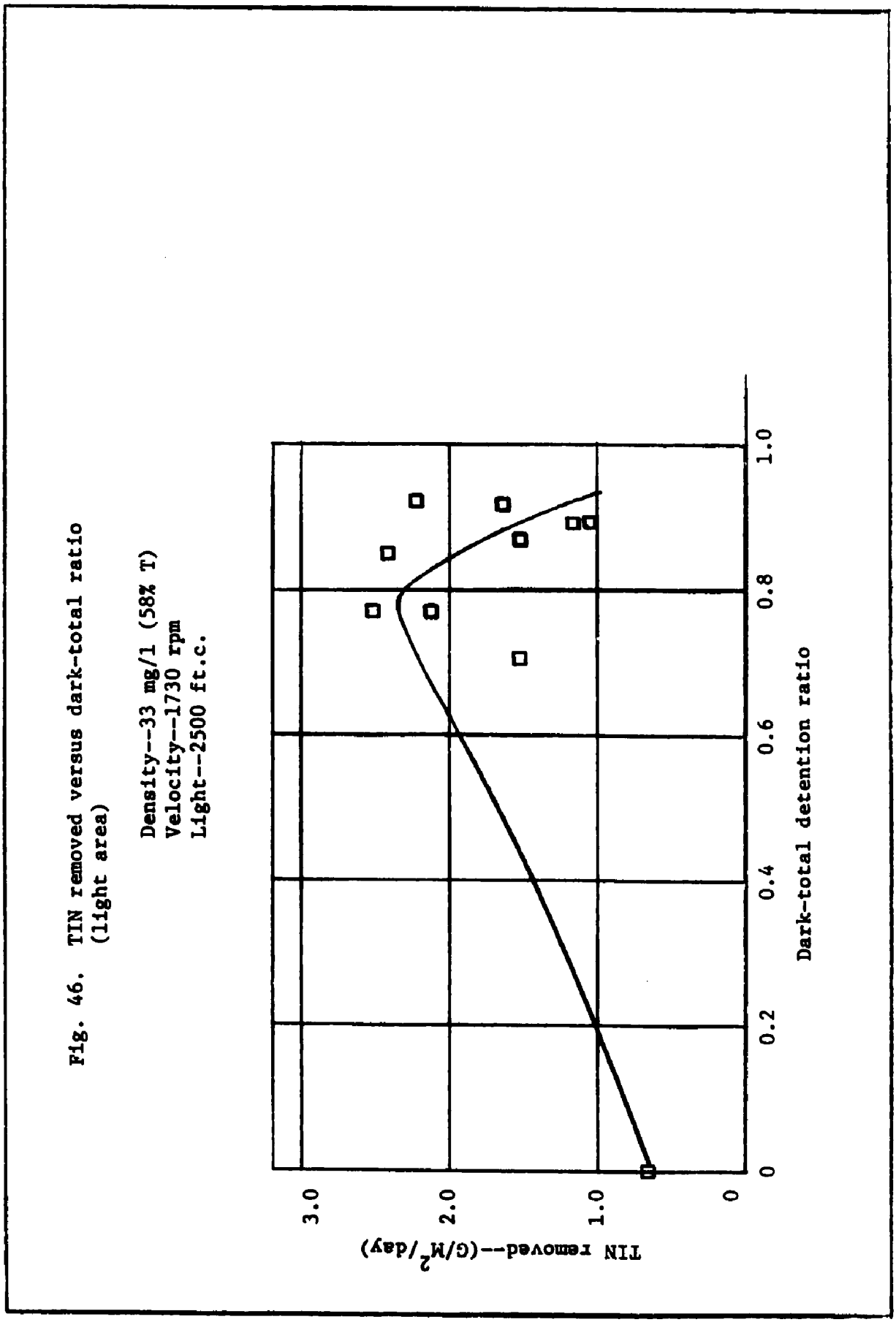



Fig. 46. TIN removed versus dark-total ratio (light area)

Density--33 mg/l (58% T)
Velocity--1730 rpm
Light--2500 ft.c.

the quantity of algae shown in the previous figures, Figure 43. Therefore, the amount of TIN removed, based on the lighted surface area, may be evaluated for various conditions by evaluating the yield relationship, Figure 47. This figure indicates that the amount of removal is directly proportional to the lighted area yield as might be hypothesized from Figure 43.

These figures show that there is little tendency for variation in TIN removal other than that shown by proportionality to the density of culture. These results could be expected from the information indicated by Gates for high nutrient concentrations. This probability could also be confirmed by the relationship of percent transmittance versus density in Figure 6 which confirms a relatively constant protoplasm content. This is also substantiated by the results shown in Figure 29 which shows the same relative spectral distribution of transmittance at various wave lengths for the various density conditions. These relationships show that there was no appreciable change in the algal characteristics from low density to high density cultures.

Phosphate removal exhibited the same general characteristics as the TIN within the range limits for these studies, for a condition of constant ortho-phosphate influent feed of 28 mg/l. In figure 48 it can be seen that the density range of 25 - 175 mg/l, the range of phosphate removal, was 3.6 mg/l/100 mg/l change in algal density. Figure 49 shows this relationship converted to percent of the influent for the phosphate content indicating only 34% removal at a culture density of about 175 mg/l, for conditions of a rather high ortho-phosphate media.

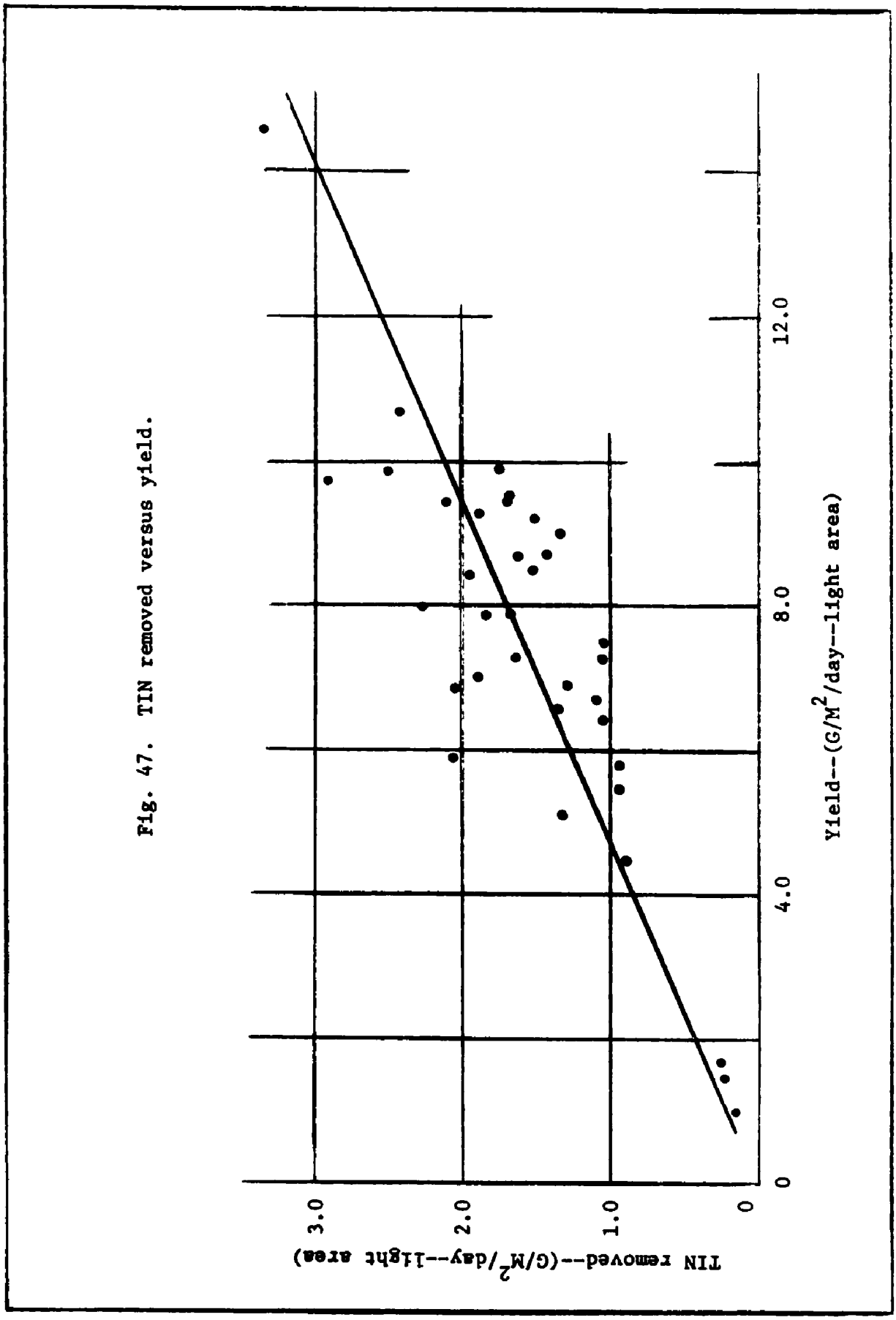



Fig. 47. TIN removed versus yield.

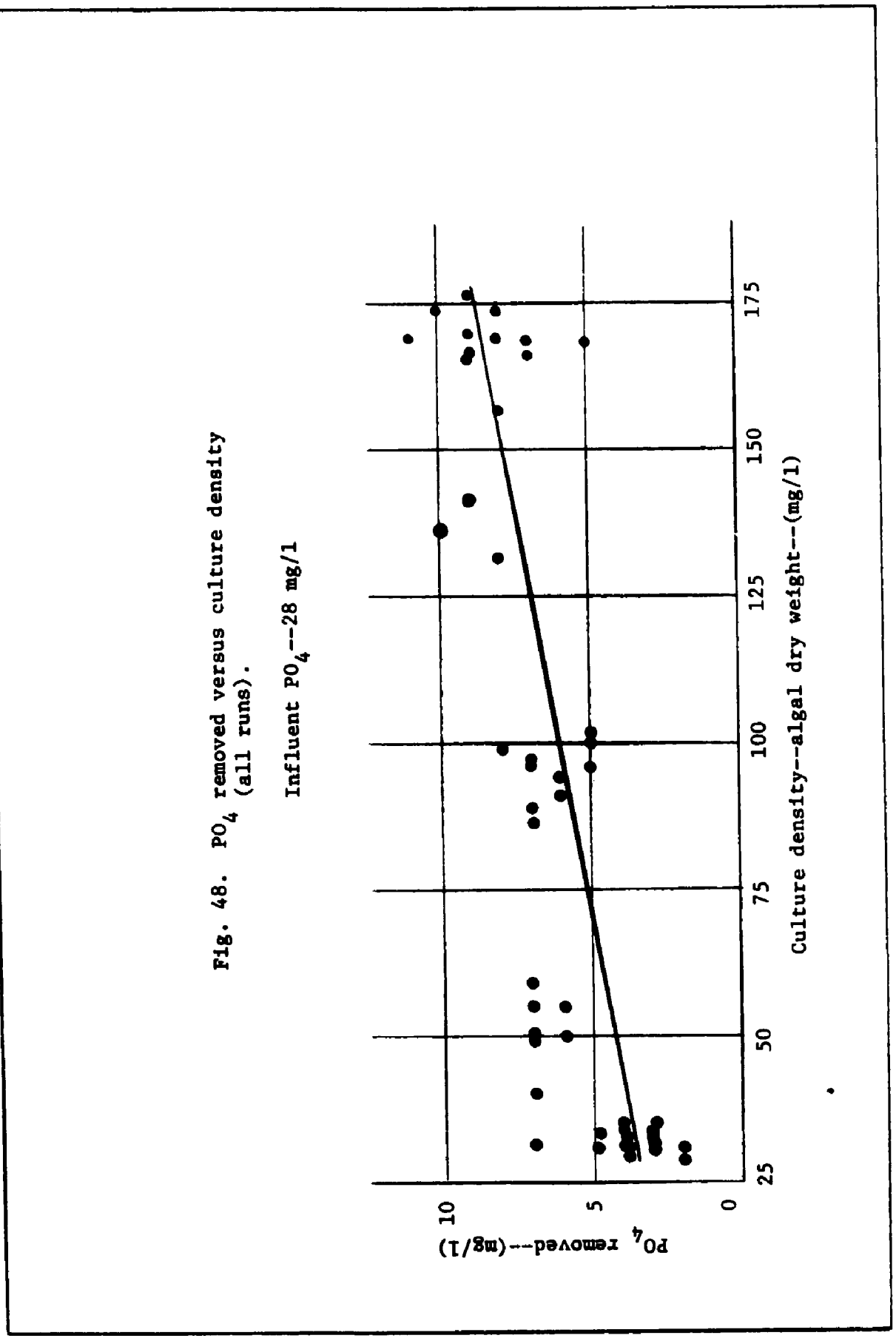



Fig. 48. $PO_4$ removed versus culture density (all runs).

Influent $PO_4$--28 mg/l

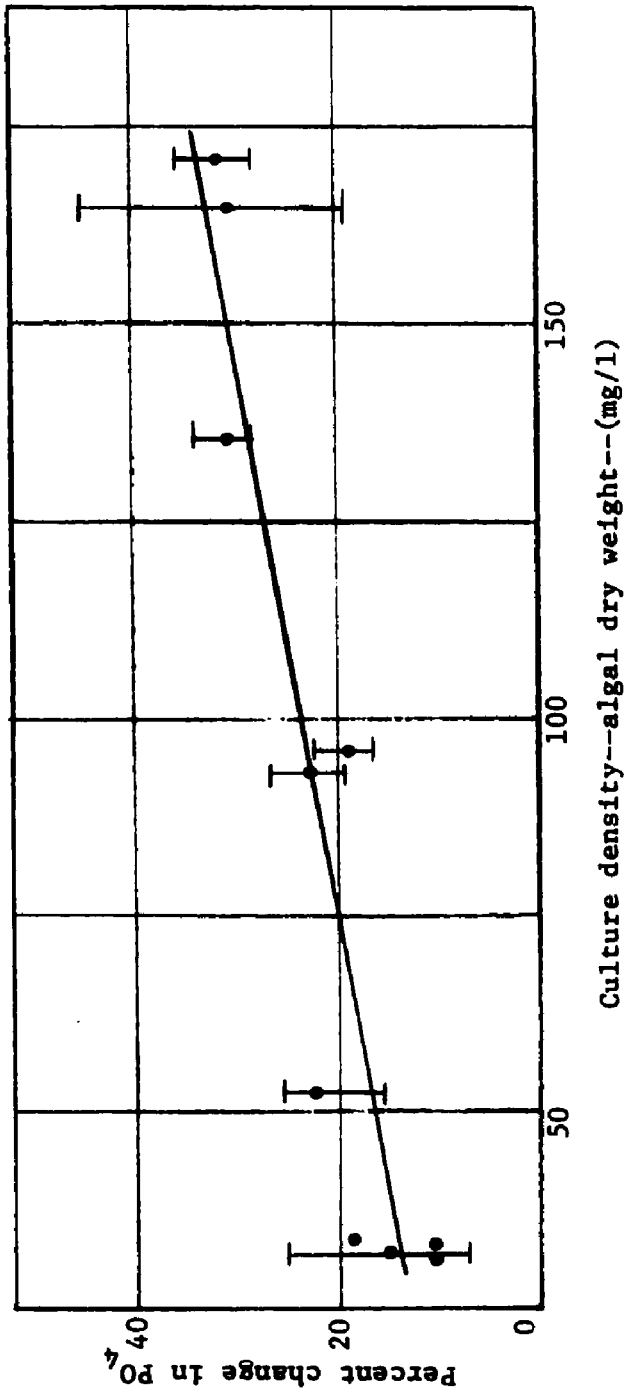



Fig. 49. Percent change in $PO_4$ versus culture density.

Figure 50 shows the correlation of removal of ortho-phosphate with respect to the removal of TIN. This curve indicates that under these conditions it can be expected that the change in ortho-phosphate decrease would be 2.75 mg/l of ortho-phosphate removed per 10 mg/l of TIN removed. This gives a N:P ratio of 11:1 which is much higher than the frequently quoted theoretical ratio of about 16:1 (33). This would indicate that under the high phosphate conditions the algae may have been showing luxury uptake of phosphate. However, under these conditions, Figure 50 shows that phosphate removal can be related to yield per unit of lighted surface in the same manner as that related for TIN. These data then give criteria which may be utilized in evaluating the nutrient removal characteristics expected of controlled dark and light culture systems with high nutrient content in the influent.

Velocity has some slight influence on nutrient removal as shown in Figure 51 for percent TIN removal versus mean velocity. This figure shows that as velocity increases from about 0.6 ft/sec to about 1.0 ft/sec the percent removal of TIN increases from about 10% removal to about 16% removal for the particular density condition. This would be the result of the fact that increased velocity tends to produce increased culture activity as may be shown in the relationship for growth rate versus velocity.

If increased culture activity is developed by increasing velocity it would be expected that there should be a relationship between growth rate and nutrient removal. This situation is shown to exist in Figure 52 for percent TIN removal versus growth rate. This relationship demonstrates a curvilinear response similar to the velocity response for TIN removal showing a growth rate of about 0.18 for a TIN

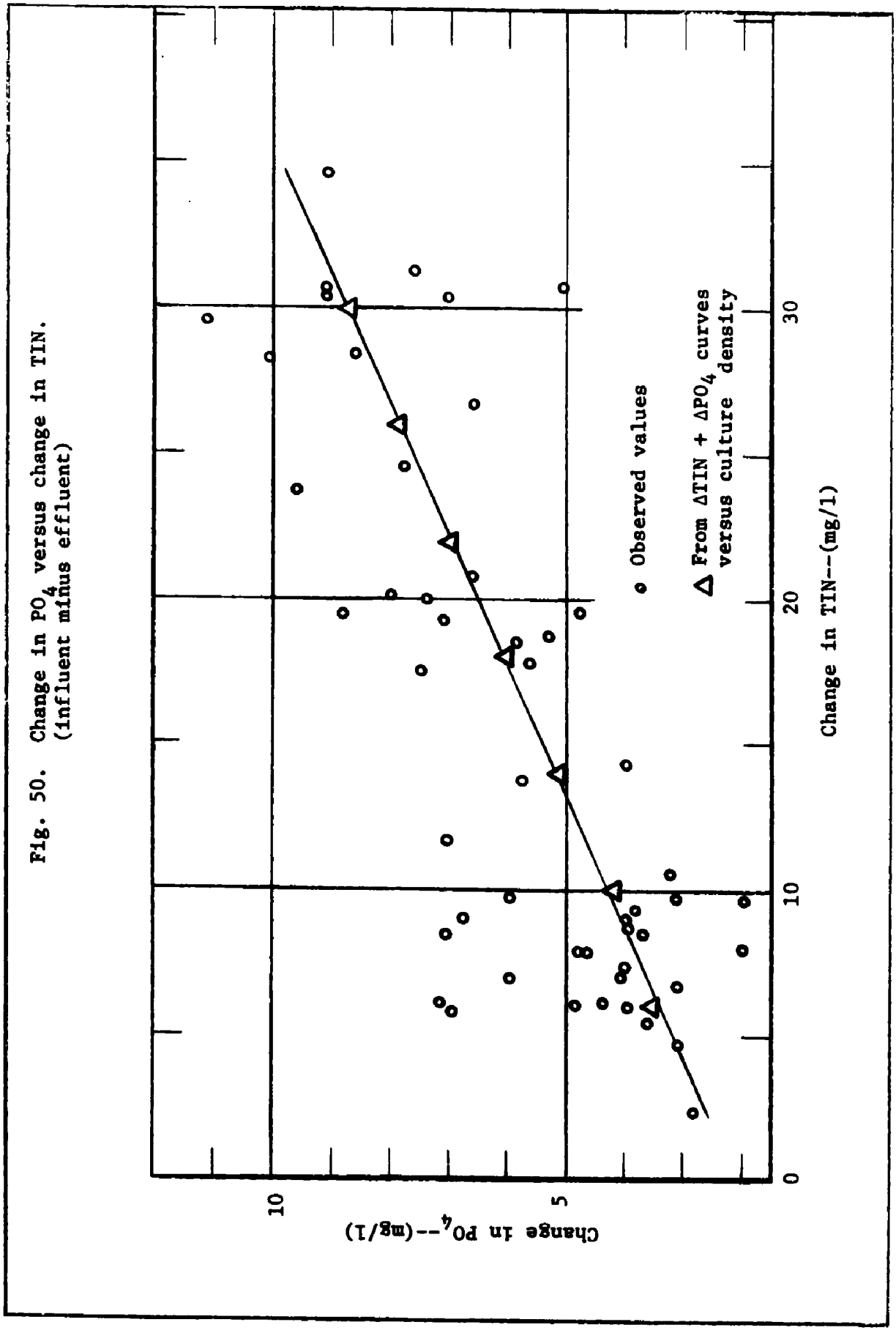



Fig. 50. Change in $PO_4$ versus change in TIN. (influent minus effluent)

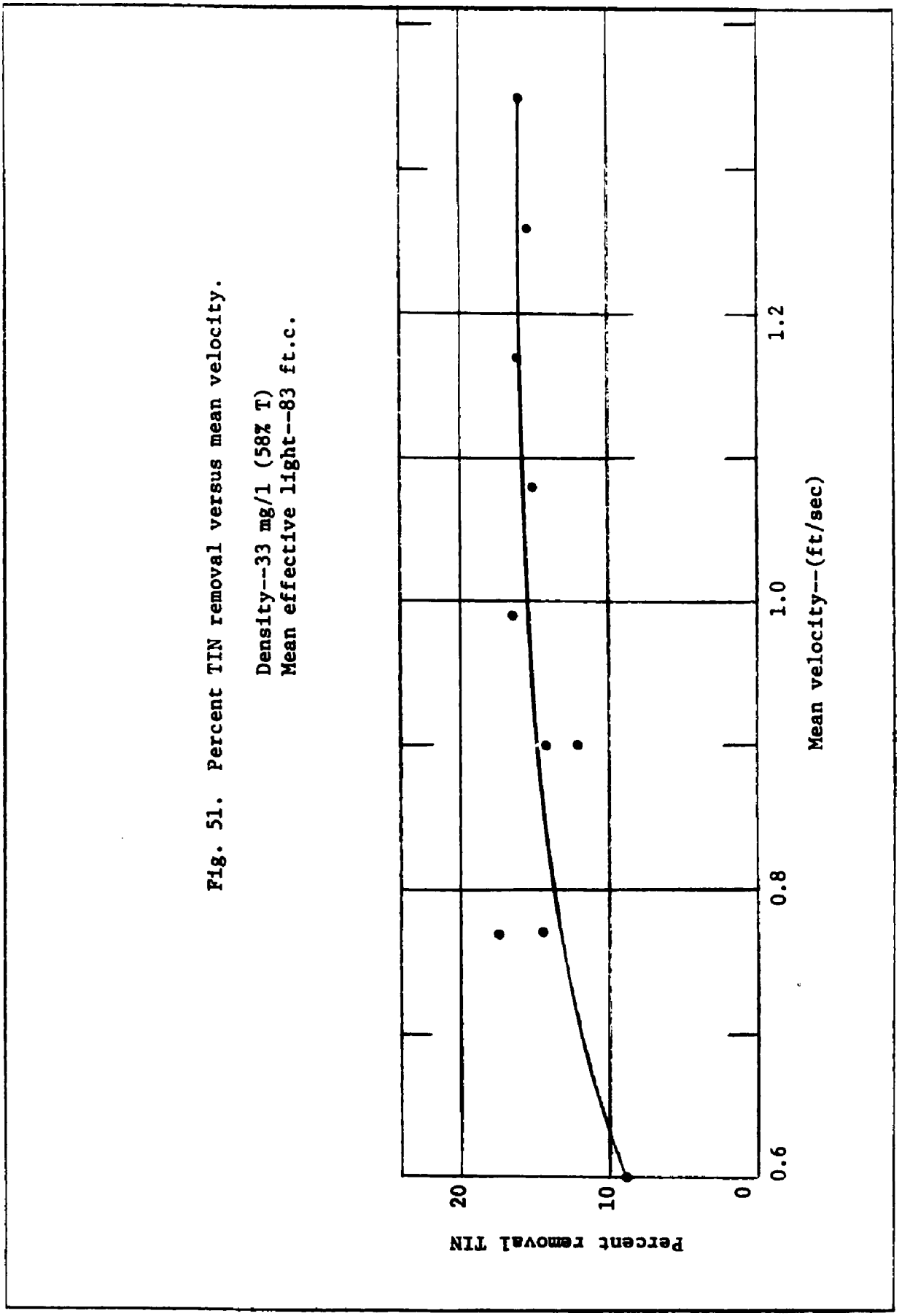



Fig. 51. Percent TIN removal versus mean velocity.

Density--33 mg/l (58% T)
Mean effective light--83 ft.c.

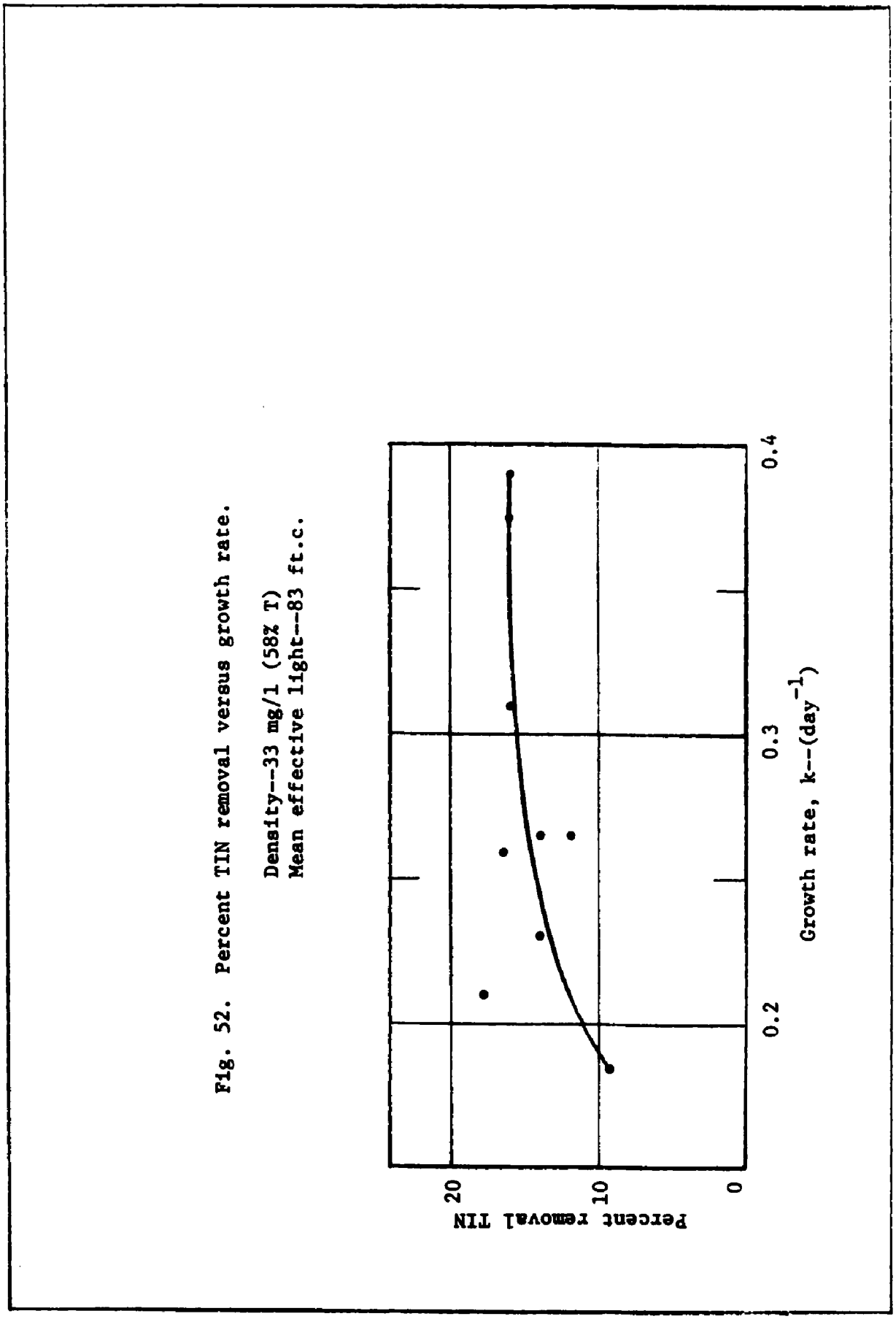



Fig. 52. Percent TIN removal versus growth rate.

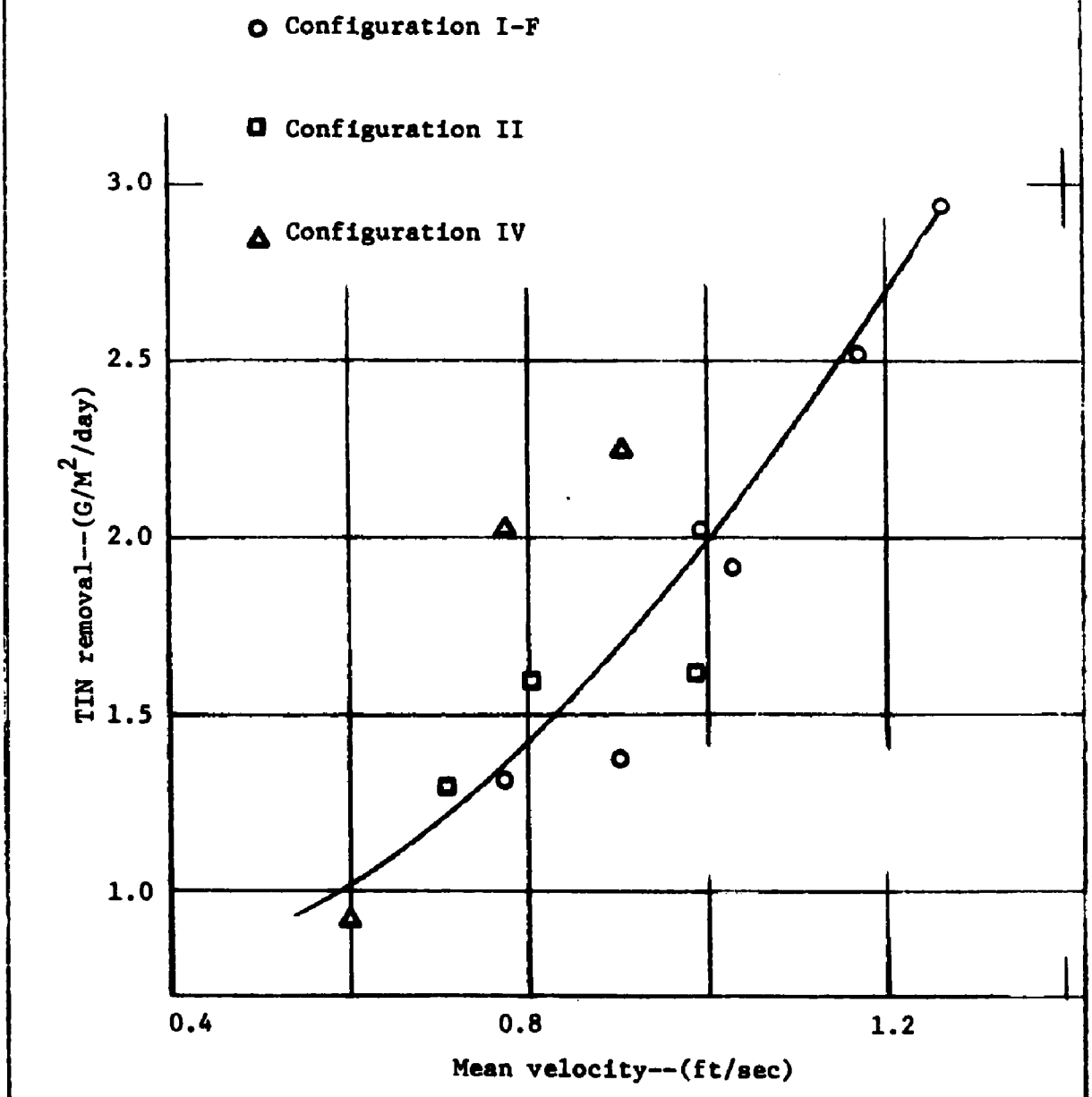
Density--33 mg/l (58% T)
Mean effective light--83 ft.c.

removal of about 10% increasing to a growth rate of about 0.3 producing a removal of about 16%. Stimulated activity beyond about 0.3 does not appear to produce any increased removal with increasing growth rate. This would then appear to show that the result of better mixing at the higher velocities produces some improvement in that a velocity of 0.6 ft/sec is less than optimum for mass culturing conditions of this type. For these conditions a minimum of 1.0 ft/sec was desired. This would mean that for any applied mass culturing system the minimum desired mixing situations should be evaluated for the particular configurations used.

This relationship of velocity in nutrient removal can be further amplified by considering the TIN removal in terms of gms/sq.m/d. This is demonstrated in Figure 53 for TIN removal versus mean velocity for the various velocity studies. This figure indicates a fairly consistent relationship in this range of study with removal going from about 1.0 gm/sq.m/d., at a velocity of about 0.60 ft/sec, to a removal of about 2.9 gm/sq.m/d., at a velocity of about 1.28 ft/sec, for the indicated culture density and light intensity conditions. This relationship is the result of improved yield as well as increased removal effectiveness with increasing velocity. Thus, this indicates that there is an improvement in efficiency, as the velocity is increased, as removal per unit of lighted area per day is a measure of the efficiency of light utilization. This advantage becomes relatively constant at velocities greater than 1.0 ft/sec.

If velocity has some effect on nutrient removal then there should be some effect with respect to the amount of time the algae are in the dark portion of the culture. This would be true because variation of

Fig. 53. TIN removal versus mean velocity.



velocity would increase or decrease the dark time. In Figure 54, for TIN removal (mg/l) versus dark time, there tends to be a curvilinear variation of removal over the range of dark time of 2 - 8 seconds. This curve confirms the demonstrated relationship for removal with respect to density as it is evident that there are three distinct curves: low density of about 33 mg/l, medium density of about 100 mg/l, and a high density culture of 170 mg/l. The most advantageous dark time for TIN removal was probably about 5 - 6 seconds regardless of culture density. This shows best in the curves for low and high density, where most of the values were obtained.

Since a linear relationship has been shown to exist for TIN removal versus culture density it should be possible to further amplify this relationship by considering a unit density relationship for TIN removal versus the dark time. In Figure 55 the removal of TIN per unit density of algal culture versus dark time shows a similar relationship by indicating a possible favored removal range of about 5 - 6 seconds. This indicates that the best removal was about 21 mg/l/100 mg/l of algal culture density.

The results for the ortho-phosphate removal tend to show similar relationships to TIN removal. This would be expected from the trend shown in Figure 50 which indicates a relatively linear relationship between change in ortho-phosphate to change in TIN. However, percent removal of ortho-phosphate with respect to velocity or growth rate, Figures 56 and 57, show considerably less variation with respect to these conditions than was shown in the TIN conditions. The variation appears to be so small that it could almost be stated that the result

Fig. 54. Change in TIN versus dark time.

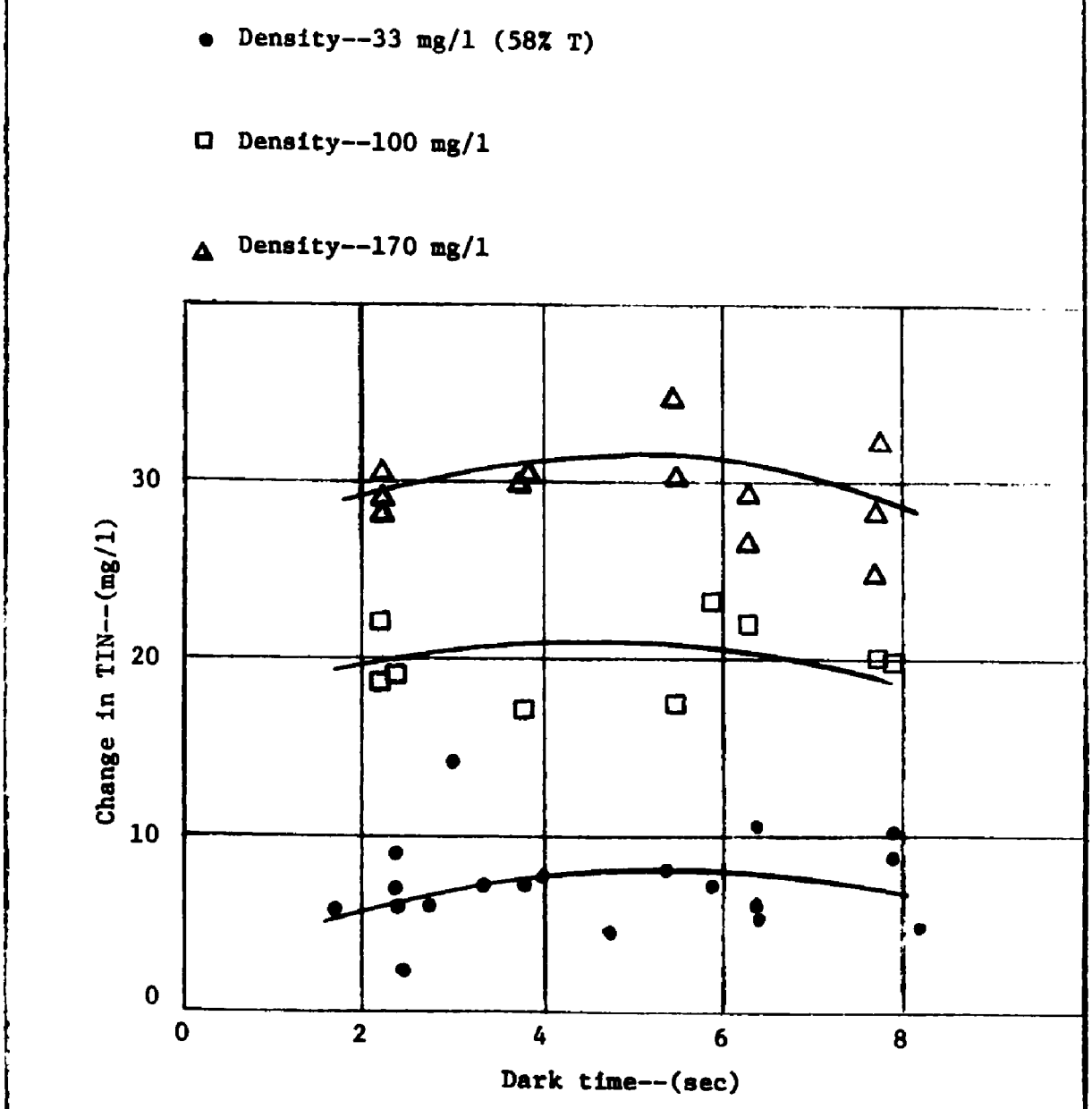


Fig. 55. Average unit change in TIN versus dark time for all culture densities.

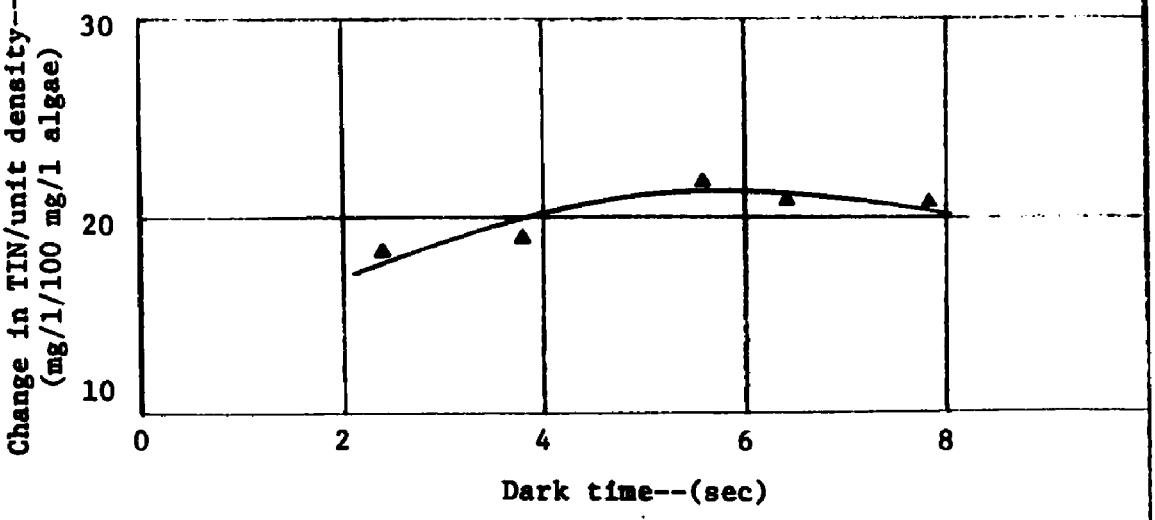


Fig. 56. Percent $PO_4$ removal versus mean velocity.

Density--33 mg/l (58% T)
Effective light--83 ft.c.

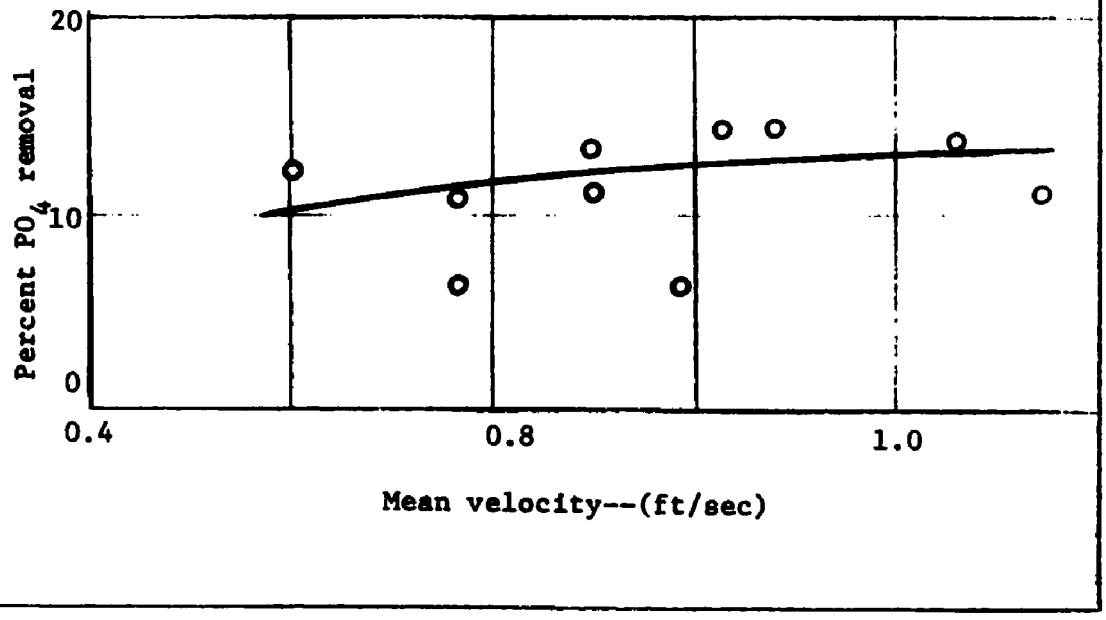
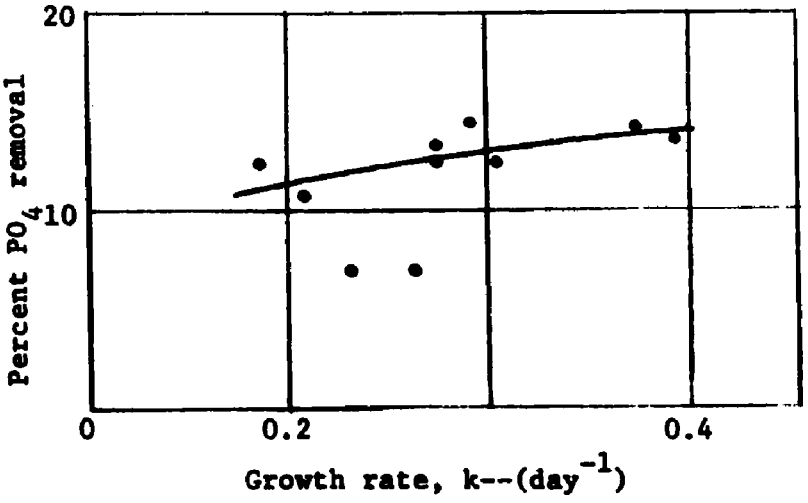


Fig. 57. Percent $PO_4$ versus growth rate.

Density--33 mg/l (58% T)
Effective light--83 ft.c.



is nearly a constant. This tendency would be further substantiated by Figures 59 and 60. These figures show a relatively constant relationship for removal with respect to dark time (seconds). This could be expected because the ratio of N:P appeared to indicate a condition of luxury uptake of the phosphate and if this is the case there would be less of a tendency for it to utilize more as a result of mixing stimulation.

The relationship of velocity in ortho-phsophate removal could be expected to be similar to that for TIN in terms of gms/sq.m/da. This relationship is demonstrated in Figure 58 for $PO_4$ removal versus mean velocity for the respective velocity studies. This indicates a relatively linear relationship in this range with removal being about 0.20 gm/sq.m/da. at a velocity of about 0.80 ft/sec up to a removal of about 1.35 mg/sq.m/da. at a velocity of about 1.28 ft/sec, for the indicated culture density and light intensity conditions. This increasing condition with velocity is primarily a result of improved yield with improved velocity conditions. Thus there is an improvement in light efficiency and thus light utilization with respect to phosphate removal. If the velocity appears to have little effect on the phosphate removal then there should be some similar relationship with respect to the amount of time the algae are in the dark portion of the culture system. This should be related because the variation in velocity would increase or decrease the amount of dark time for the velocity run conditions. It is shown in Figure 59 that there appeared to be a relatively constant removal over the range of dark times studied in the velocity determinations. These curves also confirm the demonstrated relationship for $PO_4$ removal with respect to density as it is evident that there are

Fig. 58. $PO_4$ removal versus mean velocity. (For velocity runs)

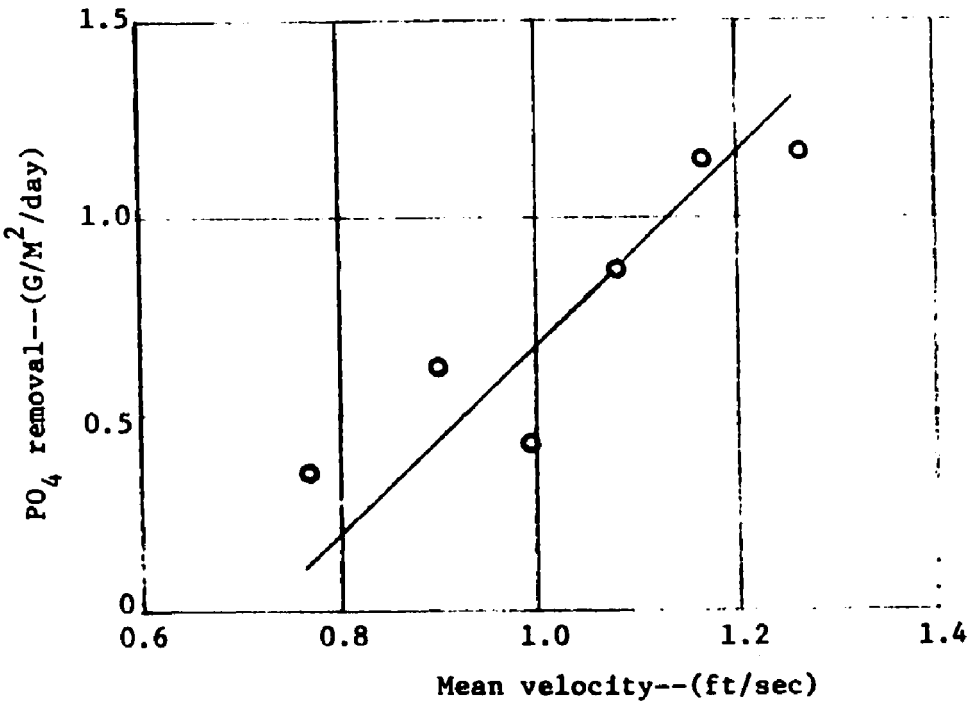


Fig. 59. Change in $PO_4$ versus dark time.

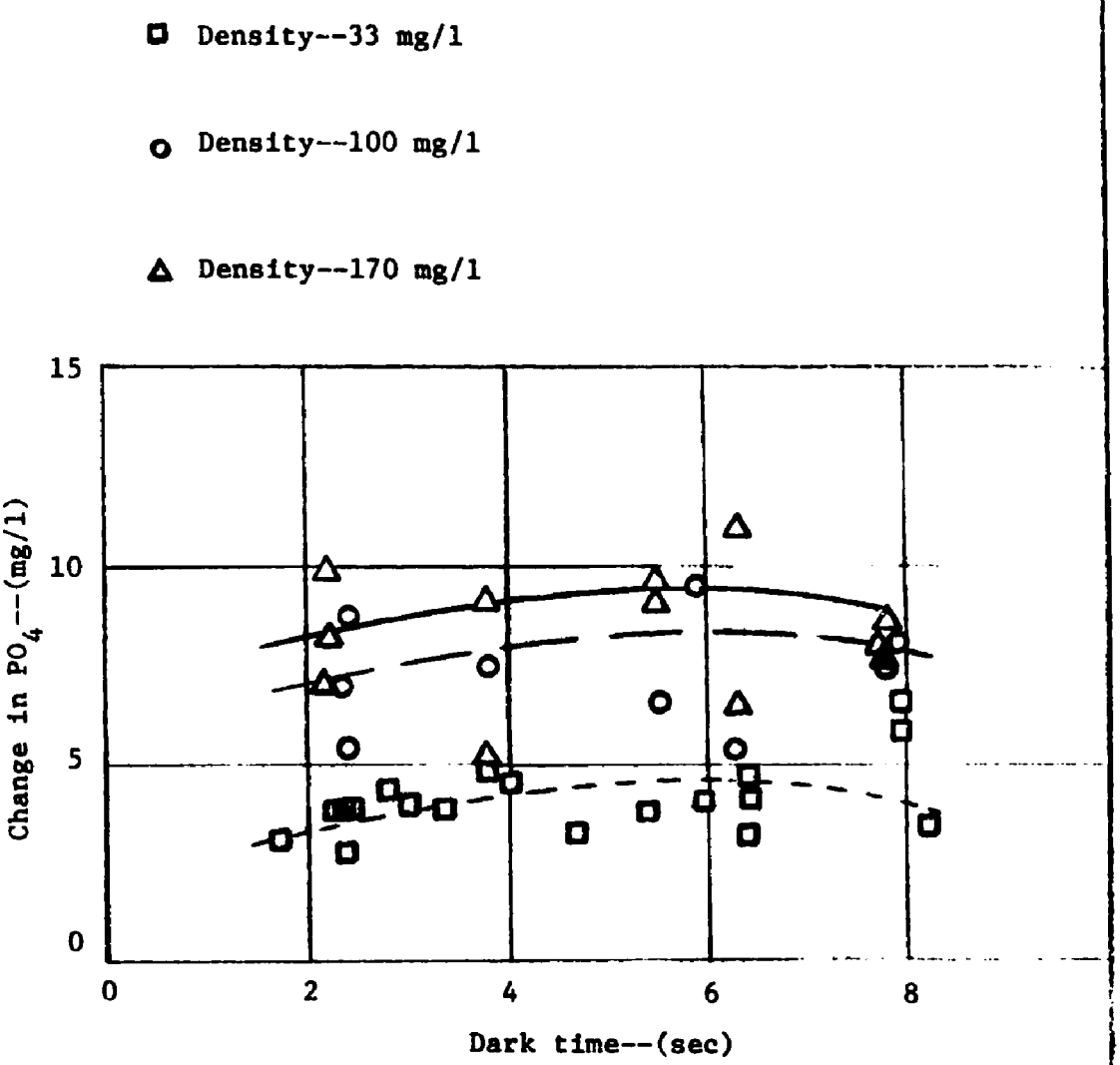
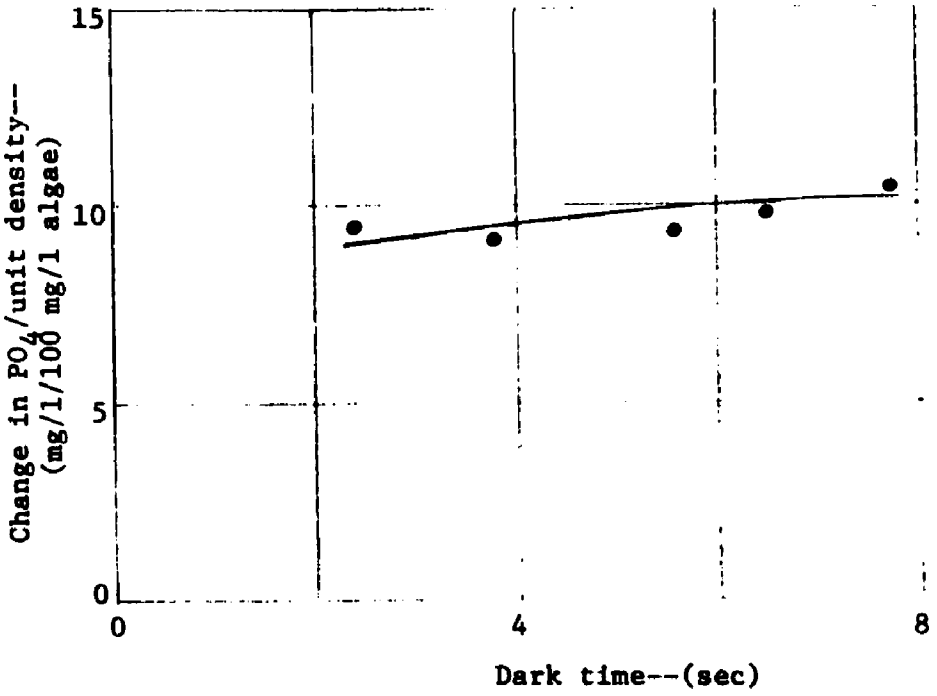


Fig. 60. Average unit change in $PO_4$ versus dark time for all culture densities.



three distinct curves for the ranges of culture densities. A consolidation of these results are shown in Figure 60 which shows a similar trend, relatively constant phosphate removal, with respect to dark time. This would be expected since with high phosphate concentration there would be nothing to tend to stimulate the algae to utilize more phosphate if they are already in a luxury uptake situation.

## CHAPTER IV

## ENGINEERING APPLICATIONS

The results of this study provide information which may be readily utilized in the design of systems for mass algal culturing. The major system limitation would be the requirement that the influent medium be similar in nutrient content to that used in the study.

This engineering design evaluation is based on a flow of 100,000 gal/d and an algal density of 174 mg/l in order to provide a dense culture condition. The dark-light detention ratio is assumed to be in the optimum range of 3.5. For the conditions studied this would give a detention time of about 9 days for the mean effective lighting conditions used during the study. For design purposes it would be desirable to try to obtain the maximum light and growth conditions and thus for such varying conditions the detention time may be related on the basis of the growth rate (Equation 27). The best growth rate was observed at a mean effective light intensity of 240 ft.c. and the best efficiency in the velocity studies was a growth rate (k) of 0.49 (day-1/100 ft.c. of effective light). If these conditions are obtained, this would produce a growth rate of 1.18. Such a growth rate would give a detention time of 0.85 days. For an inflow of 100,000 gal/d the volume then would be 85,000 gal. In order to obtain the corresponding growth the depth of the light section would be 10 centimeters and the minimum velocity in the light cell would be 1.25'/sec. This velocity would then give a flash time of 0.45 sec in a light exposure distance of 6.8 inches

measured along the line of flow.

For artificial lighting the lights would be placed 8.8" from the culture face in order to use the depth correction factor obtained for these studies. Considering such placement of the lights, a dark-light detention ratio of 3.5 and the algal density of 174 mg/1, the light intensity at the culture surface would have to be 22,300 ft.c. (shape factor = 15).

An adequate system would be to have 114 light-dark cells having a cell length of 120 ft, flow normal to the cell length. The cell system would be constructed on a slope of about 0.1% in order to produce the velocity of 1.25 ft/sec, as shown in Figure 61. This figure shows the typical arrangement of the cross-section (parallel to the flow) of the light-dark cells. This would require a plan area of 120 ft x 102 ft. The flow would enter at the top, flow through the cells, and be collected at the bottom and returned to the top by means of appropriate pumping.

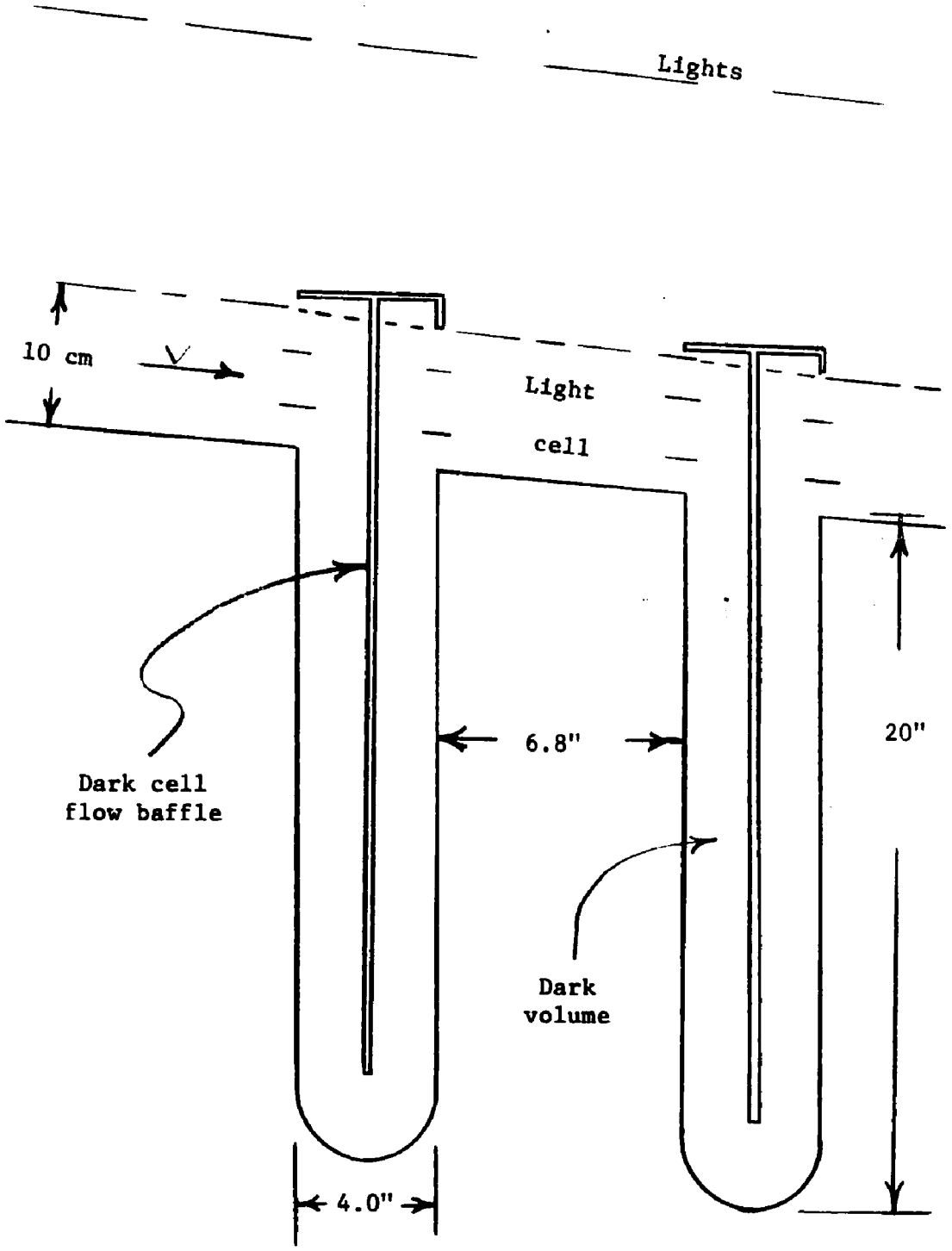
The expected yield can be evaluated by utilizing the data obtained in this study. The system would be expected to produce 66,000 gm algae/day or 145 lbs/day. This would amount to 93 gm/sq.m/d.

The expected nutrient removal would be 34 mg/1 of TIN under the velocity conditions of 1.25 ft/sec. The $PO_4$ removal would be 8.5 mg/1. Thus, this shows that the nutrient source must contain nutrient concentrations greater than this in order to maintain the design growth conditions. Nutrient concentrations close to these values would tend to produce some restrictions on growth and yield characteristics.

For the controlling conditions of this design, 85,000 gal detention volume, dark-light ratio of 3.5, the recirculation system would

Fig. 61. Schematic diagram of possible continuous dark-light algal culture cells.

S = 0.1%



require a pumping rate of 30 mgd to maintain the desired relationships. This pumping would be against a head of 3 - 5 ft depending on the friction losses in the circulation piping and the pump efficiencies. The pumping costs would be fairly reasonable for such a system. At 3¢/kw-hr, the cost would be about $10 a day for a pumping head of 3.5 feet.

Such a system would be costly with respect to light energy. If the light source used were similar to the lights used in the study, 1440 watts (12 bulbs) would give about 2500 ft.c. at the distance of 8.8 inches. In order to obtain 22,300 ft.c. it would be necessary to obtain a source which could provide about 9 times as much light intensity and would thus require about 1080 watts for a 4 foot length. To cover the whole cell area (12,200 sq. ft.) would require 2100 units or about 55,000 kw-hr/d. At a cost of 3¢/kw-hr this would amount to about $1640/day in order to provide artificial light.

An alternate engineering evaluation approach to such a system would be to consider growth under sunlight conditions. On a bright day the light intensity could be expected to be about 10,000 ft.c. at the culture face. In order to maintain the same detention time and thus the same growth rate, the culture density would have to be less. For such light intensity conditions available light energy would be able to support an algal dry weight density of 140 mg/l. The growth rate would then be $k = 1.18$ and the yield would be $5.3 \times 10^4$ gm/d or 115 lbs of algae per day, a 20% reduction in yield. This would provide 74 gm/sq.m/d.

Such growth and yield characteristics could not be maintained under natural conditions, without auxiliary lighting, as the periods of darkness and reduced intensity would have to be augmented with articicial light. This would again mean that the system would be extremely costly

from the point of view of light energy.

This discussion outlines a possible application. However, in any consideration of design, it must be recognized that there is usually a systems scale factor in going from research or pilot plant studies to actual applications. Therefore, the actual system would undoubtedly produce somewhat different results as compared to this theoretical evaluation.

If such a system design were based on the previous criteria, conditions involving extremely high light intensities would have to be given careful consideration. Such high intensities were not utilized in the actual studies and thus it might be that there would be some adverse effects on the cells exposed to the unshaded light. In the long run this would tend to produce a reduction in growth characteristics in a mass culturing system.

In an engineering application it would be possible to utilize some of the extrapolated characteristics not used in this evaluation. One extrapolation possibility which would help the high light intensity situation would be to increase the velocity and thus shorten the flash time. The results indicate that decreasing the flash time would give higher efficiencies in terms of growth rate/100 ft.c. of mean effective light intensity. Flash time could be provided in the order of 0.10 seconds by increasing the slope of the treatment area. The recirculation pumping would have to be increased considerably but it would not be prohibitive.

Eventual design would involve balancing decreasing flash time and the resulting increase in pumping costs against the decreasing light requirements for artificial lighting situations. More efficient light

sources would of course be beneficial.

It would appear that an engineering application of this type would be possible but probably not economically feasible. An alternate possibility, which would provide some improvement in feasibility and would be more consistent with current practice, would be to use tank cultures. In such a system appropriate verticle and horizontal baffles would have to be provided. These would have to be constructed so that the pumping device could provide light and dark flow patterns similar to the parameters outlined. Short circuiting could be a problem in a tank type of mass culture system.

# CHAPTER V

## SUMMARY

These studies were carried out to investigate three primary areas of concern: 1) to define more completely mass culture growth characteristics with respect to controlled light-dark detention systems under constant velocity, varied velocity and non-nutrient limiting situations; 2) to evaluate possible attainment of light integration in mass cultures under conditions of fairly long light exposure times, in the range of 0.3 - 1.0 seconds, and conditions of sharp light-dark transition; and, 3) to evaluate the subsequent effects of the growth conditions on nutrient removal under conditions of non-nutrient limitation.

## MASS CULTURE GROWTH CHARACTERISTICS UNDER CONTROLLED LIGHT-DARK DETENTION SYSTEMS

The objectives of this phase of the study were to determine what influence dark-light combinations would have on the growth characteristics of algae under various density conditions. In order to accomplish this the culturing conditions were carried out in a dark-light tank system with a light capacity of about 1.1 liters and a maximum dark capacity of about 12 liters. Density was maintained by constantly monitoring light transmission and by periodic dilution with nutrient media when density was of sufficient magnitude to require dilution. Mixing was accomplished by means of a plastic coated impeller pumping the culture through the respective compartments. Baffles were provided at the inlet

and outlet of the light cell to make the transition from one condition to the other as instantaneous as possible. Growth was measured and evaluated from overflow produced by the addition of dilution medium.

Nutrients were provided from artificial sources. Carbon and pH control were combined in that carbon-dioxide was added to maintain a pH of 7.5. The artificial medium was prepared fresh each day from reagent grade chemicals and distilled water. The concentrations of the nutrients were similar to a strong sewage plant effluent and sufficiently high to insure that there would not be any nutrient deficiency. The necessary light for growth was provided by a bank of special plant growth stimulating fluorescent tubes which could provide variable light intensity from about 250 ft.c. of light to about a maximum of 4000 ft.c. (3000 ft.c. for prolonged maximum conditions).

The recorded control observations were made daily for most studies. Density was evaluated on the basis of percent transmittance at 425 millimicrons, 20 millimeter depth, by dry weight on micro filters and by cell count. The chemical observations consisted of TIN (as nitrate, nitrite, ammonia nitrogen), $PO_4$, alkalinity and also a check on pH. The quantity and relative time of medium feed was recorded to provide correlation with overflow records. Light intensity was determined in terms of ft.c. on the outside center of the light cell.

The organism used in these studies was Schizothrix calcicola which was identified by Dr. Francis Drouet, Academy of Natural Sciences, Philadelphia, Pennsylvania 19103. This organism was dominant throughout most of the study, in the order of 99.99%.

The basic reference studies were made at constant velocity (based on observed rpm of 1730) and at a mean light intensity of 2500 ft.c.

These studies were carried out for four basic densities. The mean densities were 33 mg/l (58% T), 57 mg/l (39% T), 95 mg/l (21% T) and 174 mg/l (5.4% T). For each one of these density conditions six ranges of dark-light detention ratio were investigated. These ranges were 0, and mean dark-light detention ratios of 3.2, 4.7, 6.8, 8.5 and 10.6. Under these standard conditions of study increases in growth rate occurred with decreasing density and decreasing dark-light ratio. There was pronounced growth rate in the dark-light ratio range of 0 - 2.

In the high culture density range, 175 mg/l and 95 mg/l, the density tended to modify the mean effective light availability. Reduction of the dark-light detention ratio, from 10.6 to 3.2, primarily increased the mean effective light availability. Thus a decrease in density and/or dark-light detention ratio produced increases in the growth rate. The maximum growth rate was at dark-light ratio of zero and the maximum for this condition was observed at a density of 95 mg/l. The effect of culturing conditions on available light are very evident in comparing the results on a dark-total detention ratio relationship.

The low density cultures, 57 mg/l and 33 mg/l, showed a similar modification of the mean effective light but also appeared to provide improvement of dark-light reaction ratios. The improvement is evident in the dark-total ratio as the lighter density cultures tend to deviate from the linear relationship shown by the higher density cultures.

Velocity effects were found to be very critical and important in the light cell. In general, the velocities in the light cell were fairly constant with rpm. However, there was enough variation to make it necessary to study the rpm velocity relationship for the various configurations of dark-light ratio. This was necessary in order to provide

appropriate correction, correlation and evaluation of the mean growth results. At 1730 rpm, used as the basic reference study velocity control, the velocity results were fairly consistent. This is shown by the rpm velocity relationships in Table 15. Velocities above and below the standard mixing speed tended to deviate with configuration.

TABLE 15

EFFECT OF CELL CONFIGURATION ON CULTURE VELOCITY IN LIGHT CELL

| Configuration | Velocity |
|---|---|
| I - A | 0.79 ft/sec |
| I | 0.82 ft/sec |
| II | 0.70 ft/sec |
| III | 0.68 ft/sec |
| IV | 0.77 ft/sec |
| V | 0.77 ft/sec |

The velocity effects were primarily studied at 33 mg/1 (58% T) and at the basic light intensity of 2500 ft.c. The velocity relationships were obtained mostly for the main dark-light detention ratios of 4.8 and 8.6 and for a special velocity study utilizing a light cell filler which gave a dark-light detention ratio of 5.7.

Velocity in the light cell had pronounced effect on the growth rate, being the most pronounced under conditions of higher light availability. The increase of growth rate (k) with velocity was fairly constant, up to about 0.8 ft/sec. Above this velocity the growth rate increased rapidly. There was an increase in growth rate with decrease of dark-light detention ratio similar to the conditions evident in the constant velocity and constant light studies.

In the velocity studies it was necessary to evaluate the light energy availability in order to correctly interpret the effects. It was

necessary to consider the light effect with respect to the square of the distance from the light source in order to provide an overall correction. This was found not to be a direct comparison because the results depended on the bulb arrangement with respect to the light cell. The filler system used in the high velocity studies demonstrated a most pronounced light restriction because of its construction. There was about 33% less light available with the filler as compared to the situation without the filler.

Yield was evaluated and expressed in terms of grams per square meter of lighted surface per day. The largest yield in the dark-light detention range was a mean value of about 13 mg/sq.m/da. for the 57 mg/l culture (39%T). This result was similar to the less dense culture of 33 mg/l (58% T) where there was a mean yield of about 10 mg/sq.m/da. at a dark-light detention ratio between 3 - 5 (dark-total ratio of 0.7 - 0.8). There was a very marked decrease in yield on either side of this range in the dark-light detention ratio comparison or in the dark-total detention ratio comparison. There was a relatively constant yield (about 10 gm/sq.m/da) in the 3 - 5 dark-light detention range for the high density cultures. The high density cultures also showed an increase in yield to the full light intensity condition (zero dark time) or 95 mg/l (21% T) and 174 mg/l (5.4% T). This demonstrated that there was more light available under these conditions.

Velocity showed a pronounced increase on yield as the velocity increased above the value of 0.8 ft/sec. There was a slight advantage in reduction of dark to light ratio as a result of configuration change. The 33 mg/l (58% T) study system indicated a similar possibility.

Under full light conditions the high intensity continuous light produced higher growth rates at all densities when compared with the dark-light detention ratio results. However, these culture conditions varied the algal characteristics considerably. The percent T versus dry weight relationship shifted. The organism response tended to be one which required greater density to produce the same light absorption. This variation was also evident in the physical appearance as the cells appeared to have a lighter and somewhat bleached look.

The growth rate under continuous light conditions, compared on a mean effective light basis, produced different results from the dark-light detention ratio basis. The maximum growth rate reached was 1.84 (57 mg/l culture). For the lowest density culture (33 mg/l) the light had an adverse effect on the growth characteristics. This adverse effect was evident from the low growth rate, the extremely bleached appearance of the algae, difficulties encountered in obtaining any growth, and also the pronounced variation in the percent-T-density relationship.

These comparisons showed that controlled dark-light ratios are advantageous to algal growth. Such ratios produce consistent growth relationships. The dark-light detention ratio is also necessary if high light intensities are available.

Velocity and mean effective light utilization are related. There is more effective use of light with an increase in velocity or decrease in light exposure time. This was shown for the 33 mg/l (58% T) cultures. At a velocity of 0.8 ft/sec or less, growth rate, k, per unit of light intensity tended to be relatively constant at about 0.25. This velocity produced a flash time of about 0.72 seconds. At a velocity of 1.2 ft/sec the growth rate increased markedly to a value of about 0.47, about double

the growth rate. This produced a flash time of 0.46 seconds. The constant relationship with light (on a unit light utilization basis) at 1730 rpm confirms the relationships shown in the normal reference studies in that they showed a tendency for increasing growth rate with increasing light availability. At these velocities the unit light utilization produced a relatively constant growth value. Routine light determinations were taken at the front of the culture but mean effective light had to be evaluated to determine the real effect. The loss through the light cell window as a result of absorption and also physical displacement amounted to 12 - 19% depending on the light arrangement. The depth-distance effect was studied along with the velocity studies and it was found that in the full light cell there was a variation for depth of about 0.63 of the inside corrected intensity. With a filler in the light cell this intensity was in the order of 0.42 of the inside.

Another important effect on light intensity was the mutual shading effect. This organism has a light transmission characteristic through one cell in the order of 84% or about 16% absorbed per cell thickness. Statistical evaluation produced a mean length of 47 $\mu$ (range 3 $\mu$ - 100 $\mu$). From this information the extinction coefficient averaged about $6.16 \times 10^{-3}$(1/mg-cm) for most densities. The culture density did not seem to produce any appreciably variation in the extinction coefficient or in the spectral analysis in the wave length range of 400 to 700 millimicrons.

This study system provided a means for more complete and definite evaluation of mean probable light intensity. The probability of the exposure as the result of shading was used to indicate a mean effective light intensity. For long filamentous algae, such as this one, it was

necessary to correct for the length by providing a shape factor. A shape factor of 15 was used on the basis of the ratio of the mean diameter to the mean length, which was 15. Using this factor the relative average fractional light intensity (based on intensity on the inside face of the cell) provided a value of 0.50 for the 33 mg/l (58% T) culture, 0.32 for the 57 mg/l (39% T) culture, 0.18 for the 95 mg/l (21% T) culture, and 0.07 for the 174 mg/l (5.4% T) culture.

Considering all light variations, the growth characteristics, under constant velocity conditions, tended to vary in proportion to the mean effective intensity. This intensity or availability varied with the outside intensity as well as the dark-light ratio in the same manner as varying density. For any given configuration reduction of density produced pronounced increase in mean effective light. For any particular configuration mean effective light was constant when corrected for density. Thus the mean effective intensity may be predicted from culture density, spectral transmittance, statistical culture distribution, light-dark detention ratio, physical configuration and algal size for any algal culture.

Constant density culturing was utilized in these studies and evaluation of the results indicate that the data may be used to predict the growth characteristics in continuous flow systems where dark-light detention ratio configurations are used. Dense cultures show longer detention times, such as 10.5 days for a dark-light detention ratio of 4.0 (174 mg/l culture density) and only about 1.9 days for a 33 mg/l culture, at the same dark-light detention ratio. Under continuous flow conditions the detention time is established by growth rate and the value in days is equal to the reciprocal of the growth rate, $\frac{1}{k}$. Actual constant feed experiments confirmed the predicted condition by producing a density and

growth rate relationship, for a given dark-light detention ratio and detention time, which compared with the predicted results.

## ATTAINMENT OF INTEGRATION

Light integration, Myers (71) and Kok (72), definition, was a boarderline characteristic at 1730 rpm, for the 33 mg/1 (58% T) and the 57 mg/1 (39% T) cultures. At a dark-light ratio of about three, this integration was evident when compared with the zero integration relationships. This was possible because these studies provided an analytical approach to identify possible integration and more complete light evaluation. Light integration was not shown in the higher densities because the reduced mean effective light intensity was too low to obtain integration effects.

Constant velocity conditions demonstrated possible integrating yield in the 10 - 13 gm/sq.m/da range (dark-light ratio 3) with reduction to less than integrating results on either side of the favorable dark-light ratio. Density increase from 33 mg/1 to 57 mg/1 produced an increase in yield from a mean of 10 gm/sq.m/da to 13 mg/sq.m/da, thus demonstrating light integration trends. Velocity increases produced appreciable effect on the growth rate above 1730 rpm and thus pronounced effect on probable integration. This was especially shown by the more effective utilization of light (on a unit basis) with respect to velocity or flash time.

## NUTRIENT EXTRACTION EFFECTS

Nutrient removal evaluation primarily consisted of determining ortho-phosphate and ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen reductions. The ortho-phosphate results tended to be fairly consistent for any one configuration. The individual nitrogen determinations appeared to be somewhat variable but when expressed as total inorganic nitrogen, TIN, the results appeared to be somewhat more consistent. The reduction evaluation was based on a synthetic medium as influent. This medium was made up with high alkalinity and contained a constant amount of nutrients, 55 mg/l of TIN and 28 mg/l of $PO_4$.

For all of the constant velocity studies the TIN removal was proportional to the density, both on an absolute and percent change basis. At a concentration of 30 mg/l algal dry-weight density there was 13% removal and at 175 mg/l culture density there was 54% removal. This gave a removal of 11.2 mg/l/100 mg/l of algal dry weight density or 29.8% removal per 100 mg/l of algal dry weight.

The TIN removal was maximum at a mean value of 2.3 mg/sq.m/da at a dark to light ratio of about 3. This was similar to the yield results obtained for the 33 mg/l (58% T), 1730 rpm, 2500 ft.c. cultures (dark-total detention ratio of 0.78). This proportionality was related as the consideration of TIN removal in gm/sq.m/da produced a linear relationship with respect to algal yield in gm/sq.m/da.

The ortho-phosphate removal was similar to the TIN relationship in the range of algal culture density of 25 - 175 mg/l; the $PO_4$ removal was 3.6 mg/l/100 mg/l of algal density. This produced a reduction of 13% in $PO_4$ at a culture density of 25 mg/l to a value of 34% at a culture

density of 175 mg/l.

The ortho-phosphate removals compared with the TIN removal. This produced a linear relationship of 2.75 mg/l of $PO_4$/10 mg/l of TIN removed. This relationship indicated that there was a possibility of luxury uptake under this high ortho-phosphate condition in that the N:P ratio was in the order of 11:1. In considering the $PO_4$ removal, algal yield relationship, it was found that the relationship was similar to the yield value for TIN, thus demonstrating the same pattern.

The nutrient removal values were analyzed with respect to possible velocity effects. It was found that velocity had some slight effect on TIN in that in increasing velocity from 0.6 ft/sec up to 1.0 ft/sec the percent TIN removal went from a mean value of about 10% to a mean value of about 16%, for the 33 mg/l culture. This would appear to be the result of stimulating the growth to a more active level. The removal tended to become constant beyond the 1.0 ft/sec value for these study conditions and configurations. This tendency was confirmed in the TIN removal in terms of gms/sq.m/da. of lighted surface in that the removal increased with the velocity with a somewhat greater removal at the higher velocities. This was for the 33 mg/l culture and 2500 ft.c. situations. This indicated improved efficiency with velocity as removal per unit of lighted area per day is a measure of efficiency of light utilization.

The best TIN removal for the 33 mg/l culture and 2500 ft.c. situation appeared to occur in the dark time range of about 4 - 6 seconds, for the densities studied, in the order of 33-174 mg/l. The $PO_4$ removal did not appear to be influenced by velocity. It appeared that there was a linear relationship of $PO_4$ removal (gms/sq.m/da.) with

velocity. At a velocity of 0.8 ft/sec there was a removal of 0.2 gm/sq.m/da and at a velocity of 1.28 ft/sec a removal of 1.35 gm/sq.m/da This linear relationship was expected as the only effect that the velocity had was on growth rate. Therefore, increasing velocity s[illegible]d more efficient utilization of light energy involved in the removal of the phosphate because of more efficient growth rate. The average dark time had very little influence on the phosphate removal.

# CHAPTER VI

## CONCLUSIONS

1. Varying culture density produced an effect on growth rate equivalent to varying source light intensities as density moderated the effective light intensity.
2. Decreasing dark-light detention time ratio increased growth rate because mean effective light energy was increased.
3. Dark-light detention ratio was advantageous for algal culturing under conditions of high light intensity.
4. Growth rate under continuous light conditions produced the highest growth values but light energy was not utilized efficiently.
5. Increasing velocities produced improved growth and more efficient light utilization. There was a minimum mean velocity of 0.8 ft/sec for this system. Above this value there was significant and linear growth improvement with velocity.
6. Velocity increase produced marginal possibilities of light integration, as defined in this study, under conditions of high light intensity, low culture density and at a dark-light ratio of about 3.5.
7. Mean effective light intensity provided the most meaninfgul method of expressing available light in mass culture.
8. Apparent light integration appeared to be evident in the dark-light ratio ranges of 3 - 5, where the greatest yield was reached, with less than yield on either side of these values.

9. Decreasing flash time improved growth rate per unit of light intensity with a critical flash time of 0.7 seconds. Light utilization was relatively constant from 1.0 - 0.7 seconds with pronounced increase in effectiveness from 0.7 - 0.45 seconds.
10. Probability expressions are important in evaluating effective light in mass culture systems.
11. Detention time was related to growth rate on the basis of the reciprical of the growth rate.
12. The density controlled system provided detention data which was directly comparable to that obtained in constant feed systems.
13. TIN removal was a linear function of density under the nonlimiting nutrient growth conditions.
14. TIN removal was slightly improved by velocity up to a critical velocity of 1.0 ft/sec. Removal with velocity remained constant with density beyond this value.
15. Dark time might favor 5 - 6 seconds for TIN removal.
16. $PO_4$ removal was a linear function of density under the nonlimiting nutrient conditions.
17. The algae demonstrated borderline possibilities of luxury uptake of phosphate under the high phosphate nutrient conditions.
18. Velocity had little effect on $PO_4$ removal for any given density condition.
19. Dark-light culturing conditions produced predictable and uniform algal growth characteristics with respect to percent transmittance and algal dry weight.
20. High continuous light intensity produced a variation in algal growth response from the algal cells grown in dark-light systems. High

intensity, continuous light produced cells requiring greater cell density for a given percent transmittance when compared with the cells grown under dark-light conditions.

# APPENDIX I

## UTILIZATION OF NITRATE AND AMMONIA NITROGEN MEDIA

## UTILIZATION OF NITRATE AND AMMONIA NITROGEN MEDIA

During the course of these studies it was observed that there appeared to be an anomoly in the pH-alkalinity response relationship with growth under conditions utilizing $NO_3$-N media versus $NH_3$-N media. It was observed that when $NH_3$-N was the only form of nitrogen available pH would tend to rise during growth but the alkalinity (titratable) would decrease (see Figure 62) approaching a value of zero in about 120 hours, with an initial alkalinity of 185 mg/l.

When the algae were grown in a $NO_3$-N media, as the only source of nitrogen (see Figure 63) the pH tended to rise during growth. This rise was somewhat sharper than for the $NH_3$-N medium, but also the alkalinity (titratable) appeared to increase as well. Both of these changes appeared to be approximately linear with respect to time. In the case of the nitrate medium the apparent alkalinity would tend to rise from a value of about 175 to 430 mg/l in about 110 hours.

Some of these relationships could be expected because of the reactions involved in the utilization of the respective media. When algae use a nitrate medium $OH^-$ are produced according to the following reaction (159):

$$1.0\ NO_3^- + 5.7\ CO_2 + 5.4\ H_2O \longrightarrow \qquad [28]$$

$$C_{5.7}\ H_{9.8}\ O_{2.3}\ N_{1.0} + 8.25\ O + 1.0\ OH^-$$

This would indicate that when nitrate medium is used the pH should rise. When a medium using the ammonium ion is provided for growth, the ion may be utilized according to the following reaction (159):

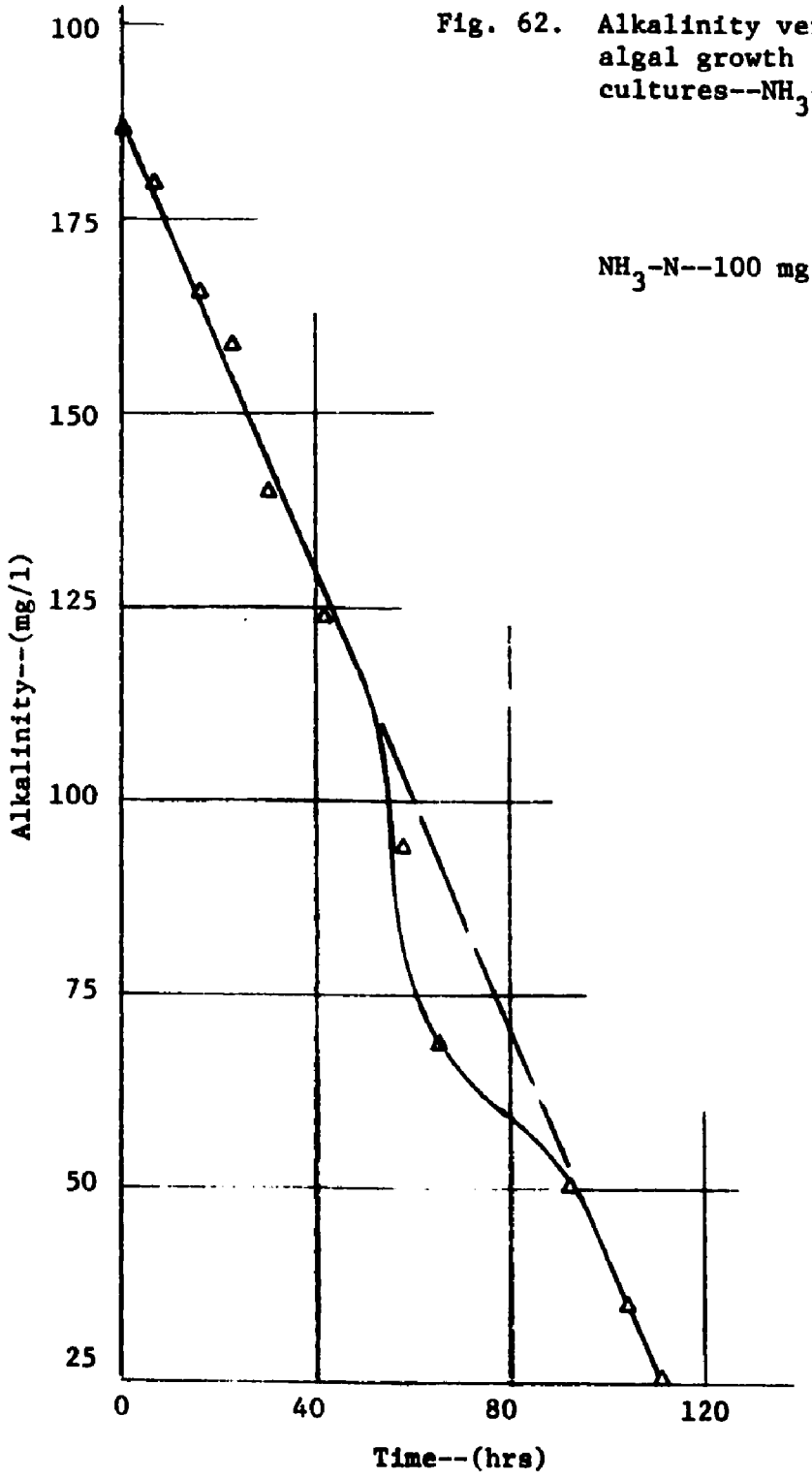
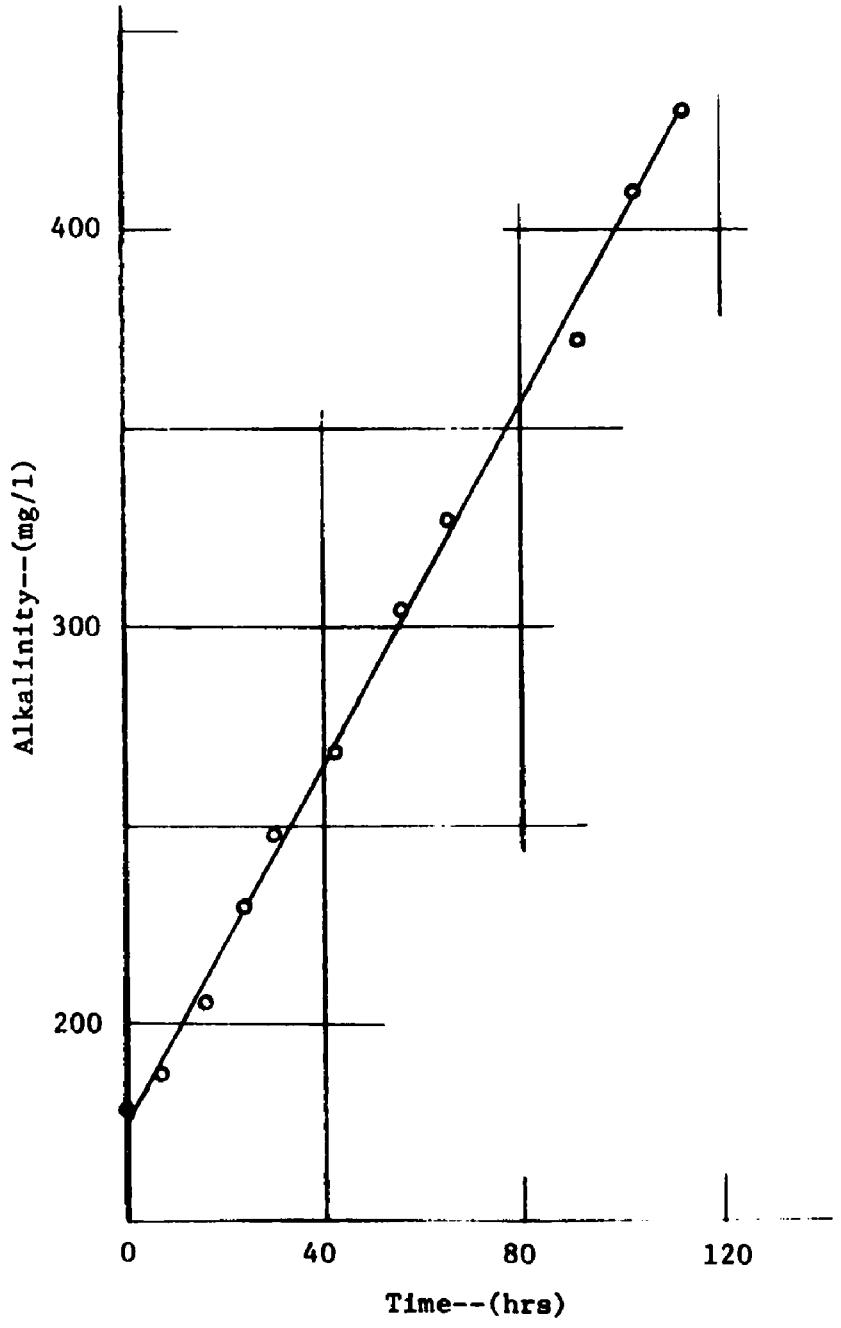



Fig. 62. Alkalinity versus time for algal growth in batch cultures--$NH_3$-N medium.

$NH_3$-N--100 mg/l (at start)

Fig. 63. Alkalinity versus time for algal growth in batch cultures--$NO_3$-N medium.

$NO_3$-N--100 mg/l (at start)



$$1.0\ NH_4^- + 5.7\ CO_2 + 3.4\ H_2O \longrightarrow \qquad [29]$$

$$C_{5.7}\ H_{9.8}\ O_{2.3}\ N_{1.0} + 6.25\ O_2 + 1.0\ H^+$$

According to this reaction the pH of the medium will then decrease as this nutrient is utilized by algae. These trends have been observed in many culturing situations. The characteristic has been utilized to try to maintain a constant pH by appropriate balancing of nitrate and ammonia nitrogen sources, which are readily used by the algae, so that the amount of $OH^-$ produced will just equal the amount of $H^-$ produced (112).

This type of response has been indicated to occur in oxidation ponds (105) when utilizing ammonium ions. With low alkalinity (50 mg/1) the pH and alkalinity decreased with growth. With higher alkalinity (about 115 mg/1) there was a reduction in the alkalinity but an increase in pH. It was thought that acid may be produced in the growth process which would tend to reduce the alkalinity and the pH decrease or rise would be related to the quantity of acid produced (105).

In order to evaluate this system more completely additional batch flasks with magnetic stirring were set up to study the growth changes in the two types of medium. The additional studies were carried out using about the same media as in the past. The only change in media was to provide increased $CO_2$ by adding an excess of $NaHCO_3$. Adjustment of pH was obtained by adding an HCl solution (0.0945 N) each day rather than use $CO_2$ for adjustment. An equal amount of NaCl solution was also titrated into the culture. The culture growth characteristics were evaluated as density, percent transmittance and dry weight; pH and other characteristics were observed during growth. At the end of the growth period the mixture was filtered through micro filters and the substrate analyzed

for alkalinity (as $CaCO_3$), total carbon dioxide and C.O.D. These determinations were made on the initial medium used for each culture. The media used for the various studies are shown in Table 16.

TABLE 16

MEDIA USED IN BATCH GROWTH STUDIES--
MEDIUM A AND B

| Chemical | Bottle Number | Ml/l ($NO_3$-N) | Ml/l ($NH_3$-N) |
|---|---|---|---|
| | | **Medium A** | |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 1 | 10.0 | 0*($CaCl_2$-5.0) |
| $KNO_3$ | 2 | 10.0 | 0 |
| $MgSO_4 \cdot 7H_2O$ | 3 | 5.0 | 5.0 |
| $K_2HPO_4$ | 4 | 2.0 | 2.0 |
| EDTA-$F_e$ | 5 | 1.0 | 1.0 |
| Microelements | 6 | 1.0 | 1.0 |
| $NH_4Cl$ | 7 | 0.0 | 10.0 |
| $NaHCO_3$ | 8 | 10.0 | 10.0 |
| | | **Medium B** | |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 1 | 10.0 | 0*($CaCl_2$-5.0) |
| $KNO_3$ | 2 | 10.0 | 0 |
| $MgSO_4 \cdot 7H_2O$ | 3 | 5.0 | 5.0 |
| $K_2HPO_4$ | 4 | 2.0 | 2.0 |
| EDTA-$F_e$ | 5 | 1.0 | 1.0 |
| Microelements | 6 | 1.0 | 1.0 |
| $NH_4Cl$ | 7 | 0 | 10.0 |
| $NaHCO_3$ | 8 | 20.0 | 20.0 |

*$CaCl_2$ for $Ca(NO_3)_2 \cdot 4H_2O$

Studies were made to evaluate the alkalinity changes, $CO_2$ changes, and C.O.D. with the pH being adjusted by additions of HCl rather than by adding carbon dioxide. Table 17 shows the results of these studies. In Medium A it should be noted that the alkalinity is somewhat lower in the starting medium than the alkalinity in Medium B. This is the result of doubling the concentration of $NaHCO_3$. This increase was provided because, as shown by Medium A, the growth characteristics were somewhat inhibited, toward the end of the growth period, by a shortage of carbon dioxide. As shown by Medium A the remaining carbon dioxide was almost zero. In Medium B the $CO_2$ and C.O.D. of the media show a similar increase. The results for the substrate after growth show similar characteristics but with the advantage of adequate carbon dioxide remaining at the end of the run.

TABLE 17

CHEMICAL CHANGES IN BATCH GROWTH STUDIES--
MEDIUM A AND B

| Medium | Alk* (mg/l) | $CO_2$ (mg/l) | C.O.D. (mg.l) | Medium | Alk* (mg/l) | $CO_2$ (mg/l) | C.O.D. (mg/l) |
|---|---|---|---|---|---|---|---|
| Initial Medium A | | | | Initial Medium B | | | |
| $NO_3$-N | 352 | 266 | 14 | $NO_3$-N | 623 | 530 | 24 |
| $NH_3$-N | 346 | 288 | 10 | $NH_3$-N | 646 | 522 | 46 |
| Substrate at End of Growth | | | | Substrate at End of Growth | | | |
| $NO_3$-N | 28 | 4.7 | 32 | $NO_3$-N | 134 | 73 | 44 |
| $NH_3$-N | 24 | 3.6 | 30 | $NH_3$-N | 91 | 45 | 64 |

*Alkalinity as $CaCO_3$

In both cases (two different kinds of medium) the titratable alkalinity tended to decrease and the pH tended to increase during growth, which would indicate that the condition of increasing pH and alkalinity with the nitrate medium and increasing pH with decreasing alkalinity with the ammonium nitrogen medium was a difference which was more apparent than real when pH adjustment was accomplished with carbon dioxide, which would also serve as source of food. However, the pH in the ammonium nitrogen medium did not rise as high in the same period of time as that from the nitrate media. This would be expected and may be explained by the fact that as bicarbonate is used $OH^-$ will become available according to the relationship $HCO_3^- \longrightarrow CO_2 + OH^-$. Thus the $OH^-$ from the $HCO_3^-$ and from the utilization of nitrate should produce an accelerated increase in pH. In the case of the ammonia nitrogen situation the $H^+$ will tend to conteract the $OH^-$. However, it would be expected that the pH might rise when bicarbonate is being used along with the ammonium ion because the amount of $OH^-$ should exceed the amount of $H^+$ since the amount of carbon required is theoretically in the order of three to four times the amount of nitrogen required. If we consider the relationship previously mentioned concerning 1.0 $NH_4^-$ reacting with 5.7 $CO_2$ and producing one $H^+$ it is necessary to provide 5.7 $CO_2$ and thus 5.7 $OH^-$ would be produced for every $H^+$, thus pH would increase.

In order to evaluate this relationship more completely additional studies were made to determine the unit requirements of acid (milliliters per liter) versus the unit relationship of algae produced as cumulative yield (mg/l); the results of such a study are shown in Figure 64. In this figure the cumulative yield, above the initial innoculum concentration, shows that the amount of titration required to maintain a pH of 7.5

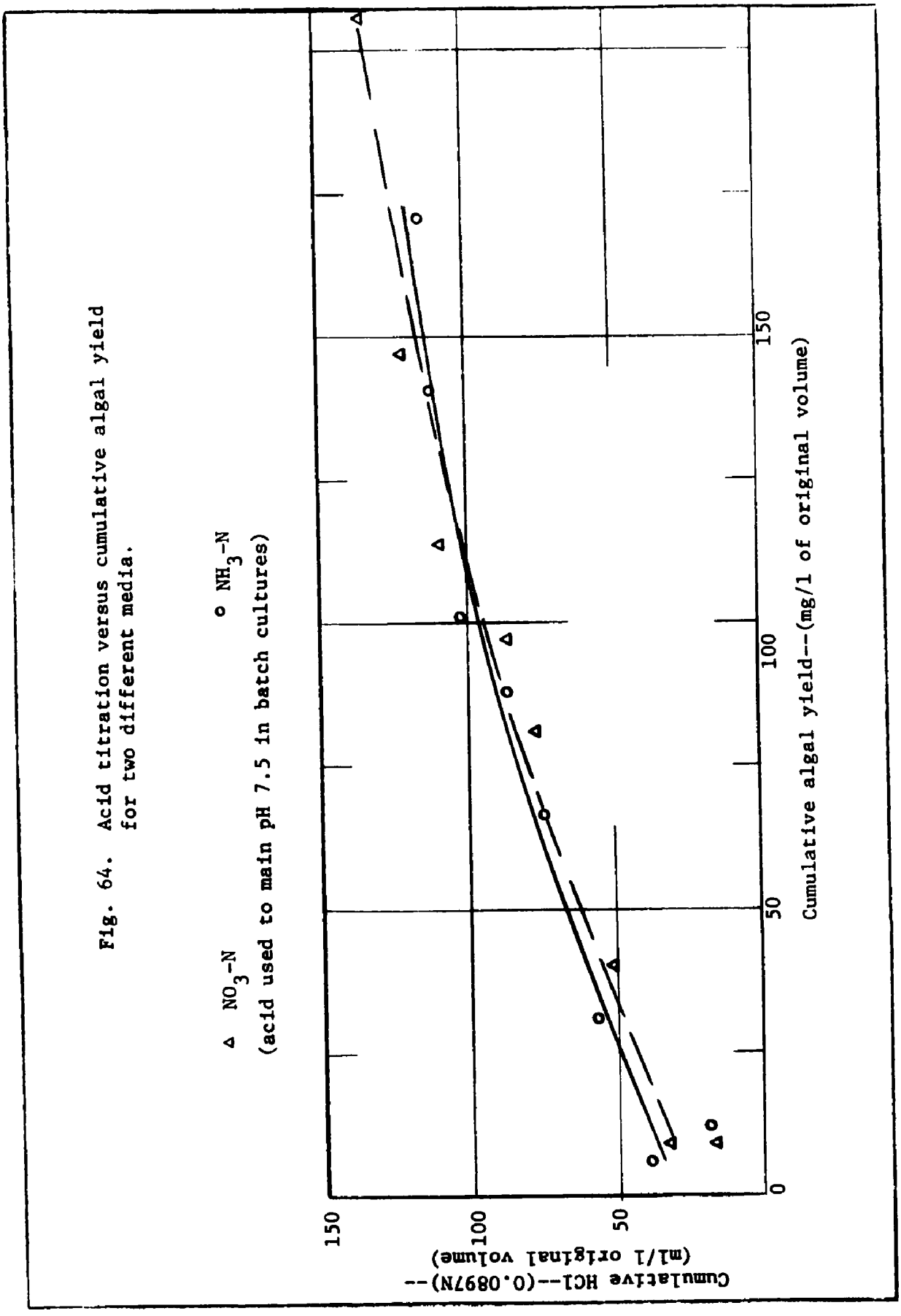



Fig. 64. Acid titration versus cumulative algal yield for two different media.

is relatively a function of the amount of algae produced. The first observations for both cultures were somewhat below the rest of the values as this initial information was obtained during the period of adjustment establishing uniform growth relationships. This is shown by the fact that there was very little growth, if not some decrease in density, during this time. Both cultures showed conditions of algal growth and production dependency with almost the same response to the point where growth inhibition begins to occur as a result of nutrient limitations. This deviation could be explained on the basis that as high density is obtained, as the result of prolonged growth, acid formation (105) may become more pronounced as a result of utilizing ammonium ion according to Equation 30 (112)

$$\begin{array}{ccccc} 2NH_4Cl & \rightleftharpoons & 2NH_4^+ & + & 2Cl^- \\ & & + & & + \\ 2HOH & \rightleftharpoons & 2OH^- & + & 2H^+ \\ & & \Downarrow\Uparrow & & \\ & & 2NH_4OH & & \\ & & \Downarrow\Uparrow & & \\ & & 2NH_3 & + & 2H_2O \end{array} \qquad [30]$$

An additional factor would be the tendency of the ammonium ion to become limiting as a nutrient as the result of the high pH conditions encountered during some of the growth periods. This high pH condition would tend to dissipate some of the $NH_4^+$ as $NH_3$. In some of the initial growth studies this characteristic curve was found to be typical of nutrient deficiency as it was observed for conditions of carbon deficiency. There appeared to be an increase in dry weight without appreciable growth activity. This could be associated with luxury uptake of phosphate.

There may be some organic compounds formed during the growth process as the C.O.D. values show an increase of about 20 mg/l in all cases. However, there does not appear to be any significant difference in the C.O.D. values with respect to either nitrate media or ammonia media as there appears to be about the same amount of increase in C.O.D.

# APPENDIX II

## NUTRITION

## NUTRITION

### Carbon

One of the most important nutrients in any algal system is carbon, as it is the major component of the cellular material produced during photosynthetic activity. During algal growth carbon may be used as free carbon dioxide, combined carbon dioxide, or organic carbon. Free carbon dioxide may be obtained from air, as the pure gas, and from respiration and other oxidation processes. Frequently there is not enough carbon dioxide available in a medium in which algae are growing rapidly, and then many algal forms utilize carbon from bicarbonate. This occurs in natural waters and has been utilized in synthetic laboratory media. This has been an advantage in laboratory cultures because it reduces the necessity of adding carbon by other means. If carbon is not available from carbon dioxide or from bicarbonate some algae can utilize organic compounds, such as glucose (129), to obtain the requisite amount of carbon.

Free carbon dioxide is the primary source (95) of carbon for most algae. In stabilization ponds the limiting nutrient (94) has been carbon dioxide. Ludwig and Oswals (130), in their studies with Euglena gracilis, showed limiting carbon dioxide in many situations, especially in oxidation ponds. They indicated that with normal atmospheric aeration ($CO_2$ concentration 0.03%) short detention times produced normal, dark green algae and long detention times produced yellowing effects indicating a carbon deficiency. When enriched air (carbon dioxide added) was used short detention times produced dark green algae with increased rates of growth and long detention times did not show the yellowing effect. Injuries seemed to occur in extremely long detention times as the result of

exposure to an excessive amount of carbon dioxide. Krauss (107) tended to confirm these carbon requirements as he indicated that the best carbon dioxide concentration would be about 0.03%, about the concentration found in air. Tamiya (85) indicated that low density cultures of Chlorella showed growth characteristics independent of carbon dioxide until the concentration in the medium was down to 0.01 - 0.03, air bubbling through the culture. Concentrations of $CO_2$ in air of 0.1% have been found to be satisfactory for providing carbon although as much as 20% has been used. Nielsen and Jensen (137) report that carbon concentrations as free $CO_2$ have shown saturating effects on the photosynthetic mechanism at concentrations of $90 \times 10^{-6}$M (half saturation at $3 \times 10^{-6}$M). Half saturation has also been reported in the range of $0.5 \times 10^{-6}$M to $1.0 \times 10^{-4}$M.

The desired concentration of carbon dioxide may vary with other conditions and Osterline (141) indicated that at low pH values high carbon dioxide concentration was the inhibiting factor in growth of Scenedesmus. Toxic effects of carbon dioxide appeared at concentrations of $2 \times 10^{-3}$M. The high pH conditions in growth rate reduction occurred (141) as a result of interference with photosynthesis by either the carbonate ion or the hydroxyl ion, since the system provided enough carbon dioxide in the substrate. High carbon dioxide concentration (above 1%, by volume) have demonstrated toxic effects in photosynthesis. From studies on Chlorella (142) it appeared that the explanation for this effect was that the photosynthetic process was blocked by the $CO_2$ concentration. This would appear to be a contradiction as Myers (133) reports that in growth studies using Chlorella pyrenoidosa, 5% and 100% carbon dioxide, no observable differences were obtained in the growth rates. This indicated there was no inhibition in growth with the 100% carbon dioxide. Thus, it

appeared that the main concern is not carbon dioxide concentration as applied but the carbon dioxide concentration maintained in the medium. This is substantiated by work done by Eddy (89) in which he indicated (special emphasis on Dunaliella bioculata) that 5% $CO_2$ with air, bubbled through samples at the rate of 7 vol/hr did not produce any carbon limitation or other adverse effects.

There are situations where bicarbonate sources of carbon may be advantageous, desirable or the only source of carbon. Krause (107) indicated it would be advantageous to use algae in cultural studies which could utilize both or either source of carbon, $CO_2$ or $HCO_3$. Blue-green algae are desirable for study purposes as most of them can use both sources of carbon as they grow rapidly under alkaline conditions. Tamiya (85) indicated that there have been reports of Scenedesmus quadricauda utilizing bicarbonate more readily than carbon dioxide. However, in analytical evaluation the carbon dioxide, bicarbonate possibilities must be studied carefully. Briggs (139), in theoretical analysis of carbon dioxide, bicarbonate ion balance, indicated that changes in the bicarbonate ion will tend to increase the carbon dioxide content in a system and he implied that this increase in the carbon dioxide would be the controlling factor.

Organic carbon sources appear to be utilized less frequently. Cramer and Myers (97) in studying Euglena indicated that up to 5% $CO_2$ concentrations in the air, bubbled through cultures, improved the ability of the organisms to utilize glucose. Higher growth rates and population densities were obtained by increasing the glucose from 0.1 to 1.0%. Myers (140) reports that Chlorella can utilize carbon dioxide during illumination or glucose during the dark period. In a light saturated

environment about 50% of the glucose was utilized and 50% carbon dioxide. However, under certain circumstances glucose may suppress the utilization of carbon dioxide in the photosynthetic process.

Krauss (134) confirms this observation in that he indicated that glucose may inhibit carbon dioxide utilization by Chlorella in the presence of light. Inhibition may not be obvious as the growth rate may remain the same, as with the experience using $CO_2$ alone, because both $CO_2$ and glucose may be used simultaneously as carbon sources. Nine types of algae were reported to exhibit ability to grow in the dark on various sugars, such as glucose, fructose, mannose, galactose, sucrose, maltose, raffinose, starch, glycogen, glycerol and ribose. This capacility has been considered to be rather important as many of these sugars may be used as well as organic acids, such as acetic, lactic acids, amino acids and also nitrogenous compounds (129). Many of these compounds are commonly found in sewage and thus would be available as sources of carbon in treatment processes or in stream pollution problems, such as in situations where carbon dioxide might be a limiting growth factor. Kuentzel (168) indicates that $CO_2$ is the major nutrient in natural bodies of water and thus it is the most important limiting nutrient.

Light and other environmental conditions have appeared to have significant bearing on the ability of algae to utilize carbon sources. Golueke (96) reported that in studies of mixed cultures of Chlorella, Schenedesmus and bacteria, periodicity of 80% increased efficiency 12 - 15%. These conditions were studied with 2% carbon dioxide and a variable light duration (periodicity) expressed as percent [(light hours ÷ 24) x 100]. Increased efficiency was primarily the result of increasing carbon dioxide concentration to 3% and 4%. At 60% periodicity 1%

to 2% carbon dioxide concentration appeared to be the best concentration with 2 days detention time. Photosynthetic efficiency of 23% was obtained under light energy conditions of 8.1 cal/l/min using 1% $CO_2$. Increasing the concentration of carbon dioxide has been shown to increase the light saturation level (36,136). Under flashing light conditions the amount of photosynthesis per unit of flash time has been shown to increase with an increase in carbon dioxide when growing Chlorella (82). Osterlind (115), in experiments on Scenedesmus quadricauda, indicated that carbon dioxide utilization begins when the algal system is exposed to light. If bicarbonate is being utilized, there is usually a light lag period, of approximately ten minutes, before the bicarbonate is used as a carbon source. This is similar to the effects noted in Chlorella (137) in that it can use bicarbonate as a source of carbon but only very slowly.

The utilization of bicarbonate appears to be most favorable when pH is around 8.5 and the carbon dioxide content is low. This tends to substantiate the claim that the algae will use carbon dioxide preferentially. This is reinforced by other experiments (138) in which inhibited green algae demonstrated an ability to utilize carbon dioxide immediately on exposure to light, but the utilization of bicarbonate showed effects of inhibition. The results of these studies were expressed in terms of percent light efficiency.

Frequently the process of photosynthesis is evaluated in other than percent efficiency in order to express a constant relationship for photosynthesis. The term which is frequently used to express a comparable photosynthetic relationship is the photosynthetic quotient (36). This is an expression which relates the ratio of the volume of $CO_2$ released divided by the volume of $CO_2$ utilized.

Algal growth and carbon requirements have been studied extensively in the natural environment. McDonnell and Kountz (27) reported values of $HCO_3$ in sewage plant effluent in the order of 140 mg/l with concentrations of 155 to 175 mg/l ($HCO_3$) in stream samples below a sewage plant outfall. Bush, et al. (101), reported that from studies on algal ponds it has been found that bicarbonate concentrations in the influent waste waters may be in the order of 200 to 320 mg/l. This was indicated to be a rather high concentration but at the rate of influent flow, under the conditions being studied, the carbon requirement in the pond could not be met. Higher rates of influent feed tended to maintain more constant pH values, indicating that the carbon requirements could be met in this manner. When sewage is used as a culturing medium, carbon may be a limiting nutrient because there tends to be a limited quantity available in sewage plant effluents (29). This has been conformed by Stumm and Morgan (33) in that they indicated that the nutrient composition of sewage is deficient in organic carbon in ratio to the nitrogen and phosphorous normally available and required for complete heterophic utilization of all three nutrients. This tends to agree with Kuentzel's statement of carbon limitation (168). Therefore, with sewage, a balance of carbon dioxide in the air must be maintained with the bicarbonates, hydroxide ion and carbonate ion (25) with best results obtained at about 2% carbon dioxide in the air being introduced into the systems (51). Higher concentrations tended to produce a decline in the efficiency (51). However, addition of carbon dioxide may be beneficial in oxidation ponds as Fitzgerand and Rohlich (132) indicated that phosphorus removal was improved by adding carbon dioxide to cultures of Scenedesmus growing in sewage. Under normal growth conditions there was 1.2 mg/l P present in the substrate, but

when additional $CO_2$ was added there was only about 0.3 mg/l P remaining.

Under natural conditions productivity is a function of many factors and may be measured by the amount of carbon being fixed. Goldman and Carter (21) indicated some productivity values for Lake Tahoe with a maximum of 133 mg and a low of 28 mg carbon (mg per day per square meter). Burkholder and Mandelli (53) reported on the productivity of micro algae of antartic origin. Their studies reported maximum assimulation numbers of about 2.6 under conditions of 10 to 15 Klux. The assimulation number is the milligrams of carbon per hour per milligram of chlorophyll. Maximum carbon fixation was reported to be about 1200 mg per cubic meter per hour at a light intensity of 10 to 15 Klux.

Phosphorus

Phosphorus is a primary nutrient. It has frequently been referred to as the most critical as it is generally in limited supply in most natural environments (25). It cannot be obtained from many sources and under algal culturing conditions it must be included in all media formulations. However, difficulty in providing it or its tendency to be the limiting factor does not mean that algal growth, when growth is not desirable, can be easily controlled through this element as concentrations do not have to be very high to support significant algal populations.

Kuhl (151) indicated that phosphorus is important because of its significance in the photosynthetic process. It is primarily involved in energy transfer relationships and the reaction of phosphorus related compounds during photosynthesis. Inorganic ortho-phosphate is the primary form used by algae (17) and thus has been of major concern in eutrophication since blue-green algae can obtain nitrogen from other sources. This

explains why treated sewage plant effluent discharge has been limited in some areas unless phosphate is removed from the effluent as part of the treatment process.

As a result of this importance, phosphorus concentrations under natural conditions has been given considerable attention. Ferguson (34) reported extensive variations in soluble or inorganic phosphorus requirements. Concentrations of 0.001 to 0.01 mg/l produced algal blooms during favorable growing seasons. However, concentrations of 0.05 mg/l have not always produced algal blooms. These values are variable but they give the order of magnitude of critical levels. Stumm and Morgan (33) indicated that if deoxygenation of the hypolimnion is to be avoided the concentration of inorganic phosphorus in the hypolimnion, under stagnant conditions, should never exceed 0.06 mg/l. Lake Tahoe (21) studies reported ortho-phosphate concentrations of 4.0 to 7.5 ugP/l, maximum value 25 ugP/l. This would be enough phosphorus to stimulate luxurious growth of algae as 7 ugP/l would adequately support more growth than was observed, but nitrogen and other nutrients were limiting.

Other surface water studies were reported by Mackenthun, et al. (131), in their report on algal concentrations and nutrients in Lake Sebasticook. Concentrations of soluble P were reported to vary from 0.01 mg/l up to 0.33 mg/l. These values varied from season to season and with respect to depth. Concentrations were higher near the bottom (50' to 60' depth) as compared with depths of 0' to 10'. Low concentrations appeared to be present in the summer months when the highest algal production was reported. However, in the fall and winter most of the total and soluble phosphate content tended to disappear. This appeared to be the result of the algae dying, settling to the bottom, and becoming

part of the sludge deposit. Most of the phosphorus tended to re-occur in high concentrations in February as the soluble form.

Concentration changes have also been shown to occur with respect to time, as Edmondson et al. (23) indicated that dissolved phosphates in Lake Washington were about 0.02 mg/1 (P) from 1933 to 1950, but in 1955 pronounced changes were recorded with values of 0.038 mg/1 (P). This indicated an important change in fertility. This may be compared with a very fertile lake, Lake Minnetonka, which was studied (10) and found to contain 0.14 mg/1 total phosphorus, and 0.013 mg/1 inorganic phosphorus.

If phosphorus tends to be limiting in the natural environment, it would be desirable to reduce inorganic phosphorus content and thus help prevent excessive algal growth. To accomplish this it is necessary to consider the origin of inorganic phosphorus. The most common sources are rain, mud and sludge deposits, surface runoff and sewage (34). There have been estimates that surface waters in the United States receive about $0.9 - 1.7 \times 10^9$ lb P/yr from natural and man-made contributions. Man's direct contribution is about 75% of this amount, a large portion being from the phosphates found in household detergents. Sawyer (25) indicated sources of phosphorus as ground water, storm water and waste waters. In general, the ground waters are low in phosphorus with the storm waters and waste water being the most important sources. Some typical characteristics were reported for orthophosphate concentrations, ground water 0.0 mg/1, surface runoff 0.2 mg/1, domestic sewage 8.0 gm/1, fertile lake (poor water quality) 0.2 mg/1 and nonfertile lakes (good water quality) 0.0 mg/1. These values agree with other studies (27) in which the orthophosphate concentrations for streams have been

indicated to be in the range of 0.1 - 1.2 mg/l in the Pennsylvania area. However, sewage plant effluent has been indicated to be as high as 24 mg/l. Fruh (12) indicated values from forests and streams as ranging from 19 - 140 PPB as total P and 0 - 23 PPB as soluble P. Some values as high as 200 PPB as total P have been reported.

Laboratory studies of algal growth provide phosphorus concentrations much higher than the border-line concentrations found in natural waters. In order to evaluate the amount which may be required for desirable culturing conditions, it is necessary to determine the limiting concentrations under favorable nutrient conditions. Al Kholy (147) indicated that the absolute minimum phosphorus content for Chlorella cells is $0.10 \times 10^{-6}$ ugP/cell thus giving a base from which to calculate limiting phosphorus concentrations. Thus, if an algal bloom is in the order of $0.5 \times 10^{6}$ cells/l, this should mean the limiting phosphate concentration would be about $5 \times 10^{-2}$ µg/l as P. Krauss (134) agreed with this in that he indicated that algae may utilize organic as well as inorganic phosphorus compounds or at least it may take such compounds into their system. Limiting phosphorus content was indicated to be in about 0.06 µg P/$10^{6}$ cells. However, concentrations may not be well defined with respect to critical level, as Asterionella has demonstrated the ability to remove and store phosphorus from a medium with concentrations of less than 1 mg/l.

These reported studies have indicated the minimum but not a practical range. The practical limitation would be phosphate concentrations in the order of 0.1 mg/l as this will tend to produce conditions in natural bodies of water whereby they will be free from objectionable quantities of algal growth (149). The threshold level appears to be about 0.3 - 0.4

mg/l as $PO_4$ as higher concentrations will produce algal blooms. Abbott (143), in microcosm studies of estuarine waters from the Gulf of Mexico, indicated that by adding slugs of phosphate to samples of water algal growth was stimulated with ultimate consumption, and a maximum limiting rate of utilization, of about 5 to 6 micro moles/da/l of mixture. The utilization of the phosphate under these conditions appeared to be independent of the nitrate concentration in that there was not any evidence of inhibition or enhancement of one with respect to the other.

Time may be of concern in evaluating limiting concentrations of phosphorus. Oswald and Goleuke (19) in reporting on the AGT determination utilized $PO_4$ concentrations of 0.0001 to 10 mg/l $PO_4$ in samples of drainage water. Higher ortho-phosphate gave considerably larger yields, the most rapid occurring within about 10 to 12 days. Other experiments on phosphorus concentrations in natural water (145) have indicated a similar time relationship. In these experiments concentrations of $PO_4$ - P in the order of 0.037 mg/l supported about 100 mg of dry weight in about 9 days time. Higher concentrations, 2.10 mg $PO_4$ - P/1 produced a dry weight density of green and blue-green algae of about 350 - 450 mg/l. In the green algal case the yield in 9 days time tended to show a progressive increase with concentration of phosphate, the phosphate being obtained from sewage plant effluent which contained other nutrients. In the case of blue-green algal studies the maximum production was obtained in 9 days when the phosphorus concentration reached 0.553 mg $PO_4$ - P/1. These studies showed that the most important component of the sewage in stimulating blue-green algae was the $PO_4$ - P concentration.

Maloney (146) reported that synthetic detergents support algal growth by providing a source of phosphorus in the form of sodium

triphosphate. The ABS associated with detergents tended to produce some inhibition at a concentration of about 3.6 mg/l of ABS, complete inhibition at about 30 mg/l ABS. Phosphorus was reported to be absorbed by the cells, especially when it was in high concentrations. This intracellular phosphorus could then be available for growth when phosphorus tended to be depleted in the surrounding media. Sorbed phosphate tended to run from 3% to 9% in 3 days time to around 40% in 26 days time, depending on the concentration.

To stimulate algal growth or provide concentrations for algal culturing the concentrations must be well above any of these limiting values. Krauss (143) indicated that the composition of nutrient media for algal growth does not differ materially from the concentrations necessary for higher plants. Phosphorus requirements for adequate growth range from 0.1 to 1.8 mg/l. These may be somewhat on the low side as Kuhl (151) indicated that the phosphorus requirements for Asterionella formosa appeared to be 0.018 - 17.8 mg/l with inhibition appearing at concentrations below 0.05 mg/l or greater than 20.0 mg/l of phosphorus. The phosphate in these cases appeared to be important in both the light and dark reactions of photosynthesis. Ortho-phosphate is utilized in the substrate by the algae converting it to intracellular polyphosphate. For blue-green algae the requirements would appear to be about the same in that Kratz and Myers (43) indicate a desirable medium concentration for blue-greens of 1 g/l. The source of the phosphate was either from $KH_2PO_4$ or $K_2HPO_4$. These would also contribute to pH control.

There has been much interest expressed in the effect of culturing algae in a medium to remove the phosphorus content. This has been especially true in the case of treated sewage since the objective of removal

efforts have been to accelerate the development of methods to insure maximum phosphate elimination in, or as part of, the normal waste treatment process (113). Bush, et al. (101), indicated phosphorus content in algal pond influent of 5 - 15 mg/l and effluent content of 2 - 9 mg/l. These situations gave percent removals in the order of 19% to 68% with a maximum of 76%, and the removal effects were the result of algal growth. In other cases (149) reductions of 45% in ortho-phosphate concentrations were obtained in oxidation lagoons receiving inadequately treated wastes, waste activated sludge and even some supernatant liquor. Reid and Assenzo (148) in their studies on attached algae grown in sewage indicated that it might be possible to remove up to about 66% of the phosphate with a 5 hour contact time using various shallow depth cascading systems.

Some of this reported removal may not have been entirely the result of metabolic utilization. Fruh (12) indicated that phosphorus removal from solution during photosynthetic activity was the result of precipitation under high pH conditions rather than biologic removal. Maloney (146) reported that precipitated phosphorus tended to be 6% - 18% for 3 days contact time to about 50% in 26 days. Precipitated phosphorus, along with the sorbed phosphorus, tended to produce removals in the order of 12% - 26% in 3 days to 90% removal in 26 days of contact time. The precipitation in the 3 day period appeared to be the major portion of the removal rather than metabolic removal. The longer contact time (26 days) appeared to provide a slight increase or dominence of precipitation removal over that obtained by algal utilization. Witt (150) indicated somewhat variable results in removing phosphorus with values as high as 95% being obtained at detention times in the order of 30 hours. Gates (76) reported removals as high as 65%, but the results

were rather variable depending upon cultural conditions.

Another area of biologic removal of phosphorus, by metabolic or intracellular removal, involves phosphorus deficient cells. Kuhl (151) indicated that phosphorus deficient algal cells remove phosphate more readily in light than in the dark. Normal cells showed an initial acceleration of uptake of phosphate in the light. Utilization of phosphate by algal cells in the light is a function of the concentration but where phosphate deficient cells have been involved it has been shown that the uptake tends to be independent of the concentration. The effect of pH on phosphate utilization by Asterionella formosa showed best phosphate utilization in a pH range of 6 to 7. Other studies on P deficient algae have indicated that a favorable starvation can be obtained (152). Under such conditions almost instanteous removal may be obtained up to an amount about equal to 20% of the cells' dry weight.

Nitrogen

Nitrogen is most important in the development of algal protoplasm, especially production of proteinaceous material, even though the production of protein requires other compounds such as oxygen, hydrogen, and carbon (101). The requisite amount of nitrogen may be obtained in many ways, such as from effluent from activated sludge (101).

The type of algae and the form of nitrogen have considerable significance as algae may utilize nitrogen in various forms. Scenedesmus have used organic nitrogen by converting it to ammonia or have used the nitrate form (101). Chlorella has demonstrated a preference for the ammonia form of nitrogen (101) However, many algae may remove nitrogen from the atmosphere as well as from the substrate. Shapiro and Riberiro

(145) in their studies on the effects of diluting sewage effluent in river water indicate that nitrogen removal had a controlling effect on green algae but not on blue-green as these organisms were able to fix nitrogen from the air. Most natural waters contain the requirements to support algal bloom except nitrogen and phosphorus. Lake Minnetonka is an example of a very fertile lake with 2.06 mg/l total N and 0.56 mg/l $NO_3$ - N (10).

Other natural waters have been reported to contain various amounts of nitrogen. Fruh (12) reported nitrogen values in streams from forested areas as $NO_3$ - N, 0.03 - 0.50 mg/l; total N 0.19 - 0.28 mg/l as N. Surface irrigation drainage had higher concentrations, 0 - 5.7 mg/l $NO_3$ - N; total N, 0.6 - 2.2 mg/l.

Nitrogen may come from various sources, but much of it originates with sewage and sewage plant effluent. McDonnell and Kountz (27) indicated that in their studies of natural surface water sewage plant effluents have shown concentrations of 30 mg/l of N and stream concentrations of 0.8 - 3.0 mg/l of N. Fitzgerald and Rohlich (132) compiled lists of sewage analyses and indicated that total nitrogen concentration was primarily of organic nature or $NH_3$ - N. Values for organic nitrogen were 10.9 - 41.7 mg/l; $NH_3$ - N, 12.0 - 40.0 mg/l; $NO_3$ - N, - 3.5 mg/l. Concentrations of $NH_3$ - N of 0.33 mg/l have appeared to be adequate to support blue-green algae, and phosphate additions have stimulated growth far beyond the amount expected for such nitrogen concentrations (145). Gotaas, et al. (155), indicated that settled sewage has served as a very satisfactory nutrient source in that in 50 mg/l total nitrogen in sewage 13 mg/l was organic N, 37 mg/l was ammonia N and there were traces of nitrate N.

Under natural environmental growth conditions algae will utilize nutrients and preferential use may be seasonal. Dugdale and Dugdale (156) studied nitrogen metabolism in natural bodies of water and they indicated that spring algal blooms primarily utilized $NH_3$ - N, $NO_3$ - N was secondary and $N_2$ - N (N fixation) was tertiary. Mid-summer nitrogen utilization was much smaller in quantity and of the three nitrogens mentioned, $NO_3$ - N was not utilized. The preferential use of nitrogen in the fall was $N_2$ - N, $NH_3$ - N and then $NO_3$ - N. The three forms of nitrogen were all available during the periods of the growth studies.

Seasonal variations have been reported by other investigators. Mackenthum, et al. (131), reporting on nutrients in natural bodies of water, indicated total nitrogen concentrations of 3.3 - 0.92 mg/l in surface waters from February to October, at depths of 50 - 60 feet, total nitrogen about 6.2 mg/l in February and about 1.1 mg/l by fall. Most of the nitrogen was either organic nitrogen or $NH_3$ - N. Most of the time the organic nitrogen was about 50% of the total nitrogen, and $NH_3$ - N made up about 35% of the total nitrogen. This type of variation has been characteristic of algal activity in oxidation ponds. Varma and Wilcomb (45) in their studies on oxidation ponds indicated that total nitrogen in raw waste influent varied from 46 - 104 mg/l, and effluent values have been 12 - 64 mg/l. This indicated removal of 29 - 65 mg/l with percent removals of 38% - 80%.

Nitrogen has been supplied to algae in many forms in laboratory and artificial culturing. Syrett (154) indicated that algae can utilize nitrogen as nitrate or ammonium salts. Frequently ammonium nitrogen is used preferentially when nitrate is present. Some algae use ammonium nitrogen and nitrate at the same time. Nitrite has inhibitory action

when in 0.001 molar concentration or higher. Other sources of nitrogen may be utilized by algae, such as urea, amino acids, amides and uric acid. Gerloff, et al. (118), in experiments using the blue-green alga Microcystis aeruginosa, indicated that under controlled pH conditions this organism utilized nitrate, nitrite, and ammonium nitrogen equally well, the ammonium ion coming from $NH_4Cl$. This confirmed the indication by others (134) that the preference for $NH_3$ - N or $NO_3$ - N frequently depended upon the particular strain of algae. Some strains have been reported to have a preferential utilization of $NO_3$ - N, but the majority of algae tend to utilize the ammonium nitrogen more readily (112). The reason for prefering $NH_3$ - N appears to be because the pathway for utilization of $NO_3$ is through the reduction of $NO_2$ to $NH_3$. Thus, it appears that more energy is required to use $NO_3$ as a source of nitrogen than $NH_3$ - N (134). This was confirmed by Syrett in that he indicated (154) nitrate reduction utilized energy and thus ammonium nitrogen tended to be more efficient in terms of energe requirements. This relationship was more noticeable at low light intensities.

The energy requirements in nitrate reduction use 8 electrons or their hydrogen equivalent to produce ammonium nitrogen. Thus $HNO_3 \longrightarrow HNO_2 \longrightarrow H_2N_2O_2 \longrightarrow NH_2OH \longrightarrow NH_3$. Some of the other forms of nitrogen may have advantages over the most common ones mentioned, nitrate or ammonia nitrogen. Goeluke (69) indicated that urea has definite advantages as a source of nitrogen because phosphate precipitation is prevented and pH problems in media containing ammonium sulphates or nitrates are frequently avoided. Kratz and Myers (43) indicated that blue-green algae utilized nitrate and ammonia nitrogen readily with some showing higher growth rates (A. nidulans) on

ammonia nitrogen rather than nitrate. Urea appeared to be the only source of organic nitrogen readily utilized by blue-green algae although some did not use this nitrogen as readily as others.

In any laboratory growth study at least one of the major forms of nitrogen is used in synthetic media. The utilization of nitrogen involves carbon, but nitrogen is primarily involved in protein synthesis (134). Myers and Johnston (158) studied carbon-nitrogen balances using Chlorella under low light intensities. From these studies they found that 233 mg of carbon was used for every 37.5 mg of nitrogen. This gave a ratio of carbon to nitrogen of 5.75 for low light intensity. There was a slight shift in this ratio to about 5.3, carbon to nitrogen, under high light intensity. An evaluation was made to determine how much of the carbon and nitrogen supplied ended up in the cells, and it was found that there was about 95% to 100% recovery. Krauss (134) indicated that there might be some excretion of various nitrogen containing compounds into the growth media under conditions of nitrogen fixation. In some nitrogen fixation studies (153) it was found that 0.1 mg/l of molybdemum was necessary for utilization of nitrate, but nitrogen fixation generally required actively growing cells.

The possibility of the occurrence of proteinatious compounds in the substrate of growing cultures appeared to be predominately associated with blue-green algae under nitrogen fixation conditions. Myers (159) indicated that in experiments with Chlorella the organism was found to demonstrate 95% or greater conversion of carbon dioxide and nitrate into algal mass. There appeared to be very little in the way of excretory products produced under normal growth conditions. Elemental analysis of Chlorella indicated that the only product produced in photosynthesis

was new cell protoplasm. This conclusion was based on the results of the analyses, ash free weight basis, of carbon, 53.0%; hydrogen, 7.5%; oxygen, 28.5% and nitrogen, 10.8%. The only noted difference was that nitrate nitrogen tended to utilize more water and produced more oxygen in the production of protoplasm as compared with ammonia nitrogen. The assimilatory quotient, $\frac{CO_2}{O_2}$, was determined for a condition using nitrate nitrogen with a resulting value of -0.68. When ammonia nitrogen was used the quotient was -0.94. During the course of the studies it was found that nitrate reduction was affected by light intensities. Low light intensity tended to show a depression in carbon dioxide uptake. However, if carbon dioxide was absent it did not appear that the nitrate was reduced.

Other laboratory studies have indicated that in addition to the interaction of carbon and nitrogen during growth there is a relationship between phosphorus and nitrogen. Abbott (143) performed microcosm studies on samples of Gulf of Mexico estuarine water. In these studies it was found that nitrate dosages did not show any particular interaction with phosphate content on production or respiration during algal growth. The two nutrients appeared to produce independent reactions, and their combined results were additative. This influence of the nitrate level on gross production and the lack of nitrate and phosphate interaction would be applicable to heavily fertilized bodies of water or cultures.

Nitrogen has been shown to have an influence on the resulting pH of a culture as a result of growth. In media where nitrogen is from $NO_3$ the pH always rises. Pratt and Fong (112) used nitrogen media with $KNO_3$ and the pH tended to rise as the $NO_3$ ion tended to remove hydrogen

ions from the solution. Krauss (107) indicated that if nitrate nitrogen is used as the source of N the algal growth will produce an increase in pH. Kratz and Myers (43) in experiments with several blue-green algae indicated that when nitrate was assimulated the pH tended to increase from 7.5 to 7.7. Utilization of urea did not produce any change in pH with the particular organisms and culture conditions.

Ammonium nitrogen utilization generally produces a decrease in pH. Pratt and Fong (122) showed that ammonium nitrogen, from $(NH_4)_2SO_4$ utilization decreased the pH, and it was thought that this was the result of hydroxll ions being used with the ammonium ion and thus $H_2SO_4$ was produced. This tendency to reduce the pH was utilized by Mayer, et al. (110), in mass culture studies. In these studies the algal growth medium consisted of 3 g/l of $(NH_4)_2SO_4$, 0.15 g/l KOH and other nutrients common to algal media. This combination maintained a pH between 6.0 and 6.5 for months at a time. Krauss (107) indicated that if ammonium compounds are used (not ammonium nitrate) algal growth will produce a reduction in pH. However, if two sources of nitrogen, an ammonium compound and a nitrate compound, are balanced in the medium they will tend to maintain a relatively constant pH. Adjustment can be obtained by adding nitric acid or ammonium hydroxide.

Other Nutrients

The major nutrients compose the margest percentage of the algal structure and thus are utilized to the greatest extent or are most often limiting under many cultural conditions. However, there are many inorganic nutrient materials which have been found to be necessary in culturing media and are necessary parts of the protoplasm even though they may be

very small in total quantity. Some of the trace elements identified in algae are copper, zinc, calcium, boron, antimory, arscnic, beryllium, germanridm, molybdenum, manganese, iodine, lead, nickel, rubidium, silver, strontium, tin, titanium, tungsten, uranium, vanadium, and yttrium (144).

Myers (94) indicated that potassium and sulfate are commonly required in algal media. Potassium deficiency has been shown to induce lower growth rates and reduced photosynthetic capability with an increasing respiration requirement. There have been indications that the potassium requirement could be replaced by rubidium but not by lithium, sodium and cesium. Krauss (107) indicated that a deficiency in a macro or one of the micro nutrients tended to produce two types of response. The first, an immediate response, when there was a deficiency of a particular metabolite, was the complete cessation of growth. The other response was modified growth or photosynthesis. Growth continued but at a much lower rate. Thus, observed reduction of growth in algal cultures or in algal blooms may at times be the result of changes in the availability of some of the micro nutrient materials.

One of the most important trace materials is iron (160). The lack of iron tends to produce reduction of growth. The amount required varies with the species and with the type of solution. It has been found that required concentrations are x - $10^{-8}$ to 1 x $10^{-10}$M (160). Reduced growth rate in iron deficient media is usually the result of reduction in photosynthesis. Dark reactions and the utilization of nitrate in the light do not appear to be affected by iron deficiency. Myers (95) reported that in some of his experiments on Chlorella iron in some solutions formed colloidal complexes which destroyed the biologic availability, and it was indicated that it would be very difficult to reverse the complex or that

the reversal might be very slow. Additions of iron to samples of natural water have shown improved growth characteristics (21). Deficiencies in laboratory culturing situations may be the result of iron being precipitated from solution with calcium phosphate (160).

Manganese concentrations of $10^{-7}$ to $10^{-10}$M have been shown to be necessary for the utilization of nitrogen as either nitrate or ammonia nitrogen. A deficiency of manganese will tend to produce a reduction in the photosynthetic capability under most light conditions. However, respiration does not appear to be affected. Manganese additions to some natural waters showed improved growth characteristics (21).

Molybdenum appeared to be necessary for nitrogen metabolism in many algae and especially in nitrogen fixing blue-green algae. It appeared that higher concentrations were desirable in the nitrogen fixation process (160). Goldman and Carter (21) have indicated that in some surface waters molybdenum deficiencies have been demonstrated. Fogg (93) indicated that molybdenum was necessary for the growth of blue-green algae and desirable concentrations were 0.1 to 0.2 mg/1. The lower concentration tended to be satisfactory for some organisms if they utilized nitrate nitrogen but the higher concentrations were necessary for nitrogen fixing.

Cobalt has been related to vitamin $B_{12}$ and has been shown to be necessary for the growth of blue-green algae (160). Greenfield (128) indicated that studies of inhibiting action of cobalt sulfate, $CoSO_4$, tended to produce slight inhibition in concentrations from $10^{-6}$ to $10^{-2}$M with extreme toxcity being demonstrated at concentrations higher than $10^{-2}$M. Cobalt was reported to be necessary for the growth of several blue-green algae (93).

Sodium has been found to be necessary for growth of some algae and in particular some of the blue-green algae (160). In some instances potassium and sodium have been shown to be interchangeable but not in all situations. Kratz and Myers (43) indicated that blue-green algae showed maximum growth with a sodium concentration of 40 mg/1, and a concentration of 4 mg/1 did not support maximum growth. Fogg (93) indicated that sodium may be necessary in the growth of blue-green algae if a high photosynthetic rate is to be maintained. Omission of the element, however, did not affect respiration rates. Sodium was necessary for the growth of A. cylindrica at a minimum concentration of 5 mg/1. This organism would not use potassium, lithium, rubidium, or cesium as replacement elements.

Zinc may be necessary at times for algal growth, but there has not been much evidence of its specific function or requirement (160). Greenfield (128), in inhibiting action studies, indicated that zinc sulfate ($ZnSO_4$) showed only moderate inhibiting effect in concentrations of 0.01 - 0.1 M. Beyond 0.1 M concentration there was rapid increase in inhibition. Natural waters have shown an improvement in algal growth characteristics when zinc has been added to the environment (21).

Copper has appeared to be a rather unusual compound in that it has been necessary in Chlorella cultures in $10^{-7}$M concentration, but also it has had toxic effects on some strains of Chlorella in this range of concentration (160). This element appeared to be involved in the photosynthetic process. The toxicity studies of Greenfield (128) indicated that $CuSO_4$ showed little toxicity at a concentration of $10^{-8}$M, slight toxicity at $10^{-7}$M, and extreme toxicity at a concentration of $10^{-4}$M. Goldman and Carter (21) did not find that copper had any effect

on stimulating photosynthetic activity by being added to samples of Lake Tahoe water.

Many other trace elements have been considered and evaluated for growth influence. Vanadium has shown little effect on growth, but also it has not been extensively investigated (160). Boron may be required at times in that it has been found beneficial to have concentrations of 0.1 mg/l (160). Toxicity studies (128) using boron as $H_3BO_3$ showed little if any inhibition of growth in concentrations of 0.01 - 0.1 M, but there was increasing evidence of inhibition beyond 0.1 M. Fogg (93) indicated that boron was probably necessary for the growth of some blue-green algae.

Potassium deficiency produced lower photosynthetic rates and increased respiration (95). Kratz and Myers (43) indicated that Anacystis nidulans required 5 mg/l of potassium to support maximum growth and that a concentration of 0.5 would not support maximum growth. Toxicity studies (128) using potassium in various forms gave varying effects on algal growth. Potassium as KI produced only moderate inhibition in 0.01 - 0.1 M concentrations with progressively pronounced inhibition at concentrations higher than 0.1 M. KCl showed little, if any, inhibition in this concentration range, but there was increasing inhibition effect beyond 0.1 M. When using $KNO_3$, inhibition was found to appear at a concentration of 0.4 M. It has been indicated that sometimes potassium and sodium may be interchangeable. However, Fogg (93) found that A. cylindrica could not utilize potassium as a replacement for sodium in its growth. Krauss (144) indicated that algal growth may require manganese, strontium, yttrium and uranium. Algae will tend to concentrate these elements for no explainable reason. This could be of

significance because many of these materials are frequently radioactive.

Chelating agents have been found to be very useful and quite necessary at times in utilizing many of the trace elements, and in particular it has been very useful in holding iron in suspension. The most common agents have been citric acid and EDTA. EDTA has some advantages in that it will not support bacterial growth, but also if it is used to keep iron or other inorganic materials in suspension it may be necessary to use higher concentrations in a medium because the metal may not be as readily available. When it has been considered for use in most media it has been assumed that EDTA is not utilized by algae. However, there have been indications that Scenedesmus has used EDTA biologically. Also, there has been some indication that if EDTA is used it may be desirable to use zinc in the medium. Myers (133), in his studies on mass culturing of algae, reported that EDTA used with inorganic nutrients increased the maximum population density for any given medium. This provided evidence that this chelating agent prevented some micro elements from becoming limiting as soon as they did in a medium without EDTA. Davis and Dedrick (102) reported that growth of Chlorella was 68% greater utilizing EDTA as a complexing agent than in the regular control medium without EDTA. Jacobson (161) reported that utilization of EDTA to maintain iron in a plant culture medium was effective in a molar ratio of 1:1. Kratz and Myers (43) indicated a growth rate of Anacystis nidulans cultures 2 - 3 times those without EDTA. Kraus (107) indicated that EDTA was the useful material in maintaining availability of micro nutrients. He reported that experiments on EDTA comparison studies with a control medium using Ca, Nn, Cu, Zn, Co and Fe showed more complete uptake of the elements in the cellular structure when EDTA was used. This uptake appeared to

be expecially noticeable in older cells of Scenedesmus obliquus as compared to younger cells.

Other trace materials have been found to be necessary for growth. Cramer and Myers (97), in studies utilizing Euglena gracilis, found that this organism required vitamins $B_{12}$ and $B_1$ (thiamin) for satisfactory growth. A deficiency of either one of these vitamins in a growing culture produced deficiency and retardation characteristics, and replenishing additions immediately showed growth response. However, Kratz and Myers (43) could not find advantages in their growth factor studies on Anacystis nidulans when subjected to vitamins, soil extract, yeast, and liver extracts. Gusev (108) indicated that many blue-green algae will tend to synthesize vitamin $B_{12}$, and it has been reported that Anabaena cylindrica has been found to contain 1.0 mg or more of $B_{12}$/g of algal dry weight.

Desired concentration levels of other inorganic elements may be partially evaluated from Greenfield's (128) studies on inhibiting action of many common inorganic compounds found in water and algal media. Moderate inhibition was demonstrated by $NiSO_4$ and $(NH_4)_2SO_4$ in concentrations ranging from 0.01 - 0.10 M, but beyond the 0.1 M concentration there was a rapid increase in inhibition. No inhibiting action was noticed in $MnSO_4$ and $MgSO_4$ solutions until a concentration of 0.4 M was reached and then progressive inhibition was evident. Sucrose was the only organic nutrient material investigated, and the inhibition range started to appear in the range of 0.40 - 0.60M depending upon whether the solution was buffered or not buffered.

If ions are necessary for growth it should be possible to show that they are removed during the growth process. Bush, et al. (101),

indicated that some of the common inorganic ions are removed in ponds treating sewage treatment plant effluent. The indications were that the amount utilized was a function of various factors. Calcium was removed up to about 38% under a pH condition of 10.9 and a detention time of 29 hours. For a shorter detention time of 7 hours there was only 1.6% removal and the pH was 8.7. Magnesium showed similar characteristics with 44% removal at a 29 hour detention time with only 7% removal at a 7 hour detention time, pH of 8.7. Potassium showed rather variable removal from a high of 17% to indications of negative removal. Sulfate varied from 7% to 40% removal and bicarbonate removal was in the order of 50% to 90% even when carbon dioxide was provided.

# APPENDIX III

## pH

pH

The pH change may influence the type of growth and such change may be the result of the type of media. Davis, et al. (102), grew Chlorella in a pH range of 6.0 to 6.5. This was selected because it appeared to be halfway between favorable values for bacteria and for mold. The pH of their culture medium showed considerable change when nitrate or ammonium ion was used as the source of nitrogen. Frequently such changes may be undesirable and thus glycine and urea were used as nitrogen sources in some of their growth studies in comparison with $KNO_3$. All their studies started at a pH of 5.0 and in 5 days the $KNO_3$ medium reached a pH of 8.3, the urea medium had a pH of about 6.5 and the glycine medium showed a slight drop to a pH of 4.9. They concluded that urea was the most desirable source of nitrogen for pH control because it had less of a tendency to support other growths which tended to contaminate the culture and at the same time it did not show excessive pH variation. Dyer and Gafford (92) also compared the effect of several types of media and they reported most favorable growth conditions for Synechococcus lividus at a pH of 7.5 in a nitrate medium. This same organism had demonstrated that its most favorable pH was 7.0 in urea medium.

Benson, et al. (107), indicated similar pH relationships in their studies of Chlorella pyrenoidosa and Scenedesmus in that the pH of their medium started at 4.5 and increased with growth to values of 6.5 to 7.3. Chlorella did not grow in a medium prepared by replacing $KNO_3$ with $(NH_4)_2SO_4$ and replacing $Ca(NO_3)_2$ with $CaCl_2$. Growth was stimulated under these conditions with an adjustment of the pH to 9.0 by means of an

addition of NaOH. The growth which resulted was similar to the growth for the previous nitrate medium at a pH of 4.5. However, in using ammonium medium the pH tended to decrease during growth. After 24 hours of growth there was a decrease in pH to about 6.5 - 7.0 and in 48 hours the pH tended to drop to 3.0. During all of these studies the pH had to be adjusted by adding NaOH in order to maintain values in the range of 7.0 - 9.0.

Pratt and Fong (112) showed that Chlorella vulgaris, in growing under varying nitrate and ammonium nitrogen conditions, produced pH shifts in the growth medium. When there was only $NO_3$ available and initial pH was 3.8 - 5.9, the pH tended at all times to rise during growth to about 7.0 - 7.1. When the ammonium ($NH_4$) ion was available, from $(NH_4)_2SO_4$ the pH remained low, at about 3.8, until all of the ammonium ion was used and then the pH tended to rise rapidly as the organism utilized $NO_3$. If a sufficient quantity of $NH_4$ ion was available the pH remained constant or in some cases droped to a value of about 3.1.

The effect of pH has been shown to be of concern with respect to what happens in the general physical and chemical conditions of a medium. Myers (117) found that high pH values, higher than 6.7, tended to produce conditions which precipitated iron from solution at a very slow rate, using a modified Knop's medium. It was also indicated that culture pH values below 5.0 tended to produce flowing and accumulation of cells on the walls of the culture chamber.

Droop (114) indicated similar importance of pH on the media under variable pH conditions. The variation of pH may affect the nutrient variables. Weak acids, bases and other materials may be inhibited with

respect to nutrient potential or they may even become toxic as the degree of ionization changes during pH change. Chelating materials may also have their effectiveness varied. Thus he concluded that the effectiveness of synthetic media may be limited by the pH range. This availability of nutrients was further demonstrated by Osterline (115) in his experiments with inorganic carbon sources with Scenedesmus quadricauda. Carbon dioxide and pH concentration appeared to have a close relationship in defining the necessary conditions for bicarbonate assimulation. At pH values less than 7.0 the algae could be activated if they were aerated with a 2% carbon dioxide mixture. At a pH of 6.9 to 7.7 illumination activated both old and young cultures. However, in the range of pH of 3.3 to 8.5 light only activated young cells and the old cells appeared to lose their activation capability. It was thought that the longer light period was required to produce activation. In lower concentrations of $CO_2$ (0.03% $CO_2$) the algae appeared to be active at all pH values, 4.5 to 8.9 if the cultures were not too old. Old cultures and a high pH of 11.1 produced conditions whereby it was not possible to activate the cells under any condition. The algae showed satisfactory activity in the pH range of 8.6 to 9.2, if the bicarbonate concentration was high and $CO_2$ concentration was kept low.

Many others report the effects of pH on algal growth in laboratory media (103,97) as well as the effect of $KNO_3$ and $NH_4Cl$, the latter showing a pH change from 5.9 to 3.5 in old cultures with Chlorella (119).

Gates and Borchardt (111) indicated that Scenedesmus showed complete stoppage of growth at a pH of 5.5 and a pH of about 11.0 but tended to grow well in the pH range between these two extremes.

Light conditions have been shown to be influenced by the pH of the environment (59,73,51,42,116). Nielsen (59) indicated that in studies on Chlorella in high light intensities a phosphate buffer was effective in maintaining a pH of 6.5. Maintaining this constant pH appeared to have limited significance because in other studies on Chlorella pyrenoidosa he found (116) that the pH had very little influence on the photosynthetic rate under conditions of light saturation. The only condition noted was that at a low pH of about 4.7, the photosynthetic capability tended to increase with an increase of $CO_2$, which was probably the result of more carbon being available. However, Bogan (42) tended to attach some significance to the light relationship with pH in his studies on growth in a sewage medium. The utilization of $CO_2$ produced an adjustment in the carbonate-bicarbonate ion balance. Thus, light intensity was the principal factor affecting the rate of pH change up to a mean light intensity of about 200 ft.c. Additions of $HCO_3$ to the sewage treatment plant effluent, up to 400 mg/l, at 18°C - 20°C and a light intensity of 1000 ft.c. produced a steady increase of pH from 7.5 to about 9.0 in about 300 minutes. During the period of time from 300 to 1100 minutes the pH tended to remain constant. There was another rapid rise in pH to about 10.2 at 1300 minutes. The pH tended to level off at this value. The high concentration of $HCO_3$ ion, 400 mg/l, tended to produce more of a buffering action and the pH did not rise much above 9.4 in about 1500 minutes.

Light stimulated pH effects have also been reported by Oswald (51) in studies on the efficiency of algal light conversion in sewage. The typical sewage medium tended to have a pH of about 9.0 - 9.3. During the studies there were prolonged dark and light periods. In the

dark period the pH remained about 8.0 and on exposure to light the pH rose. Duration of a pH value in excess of 8.0 for longer than two hours produced a marked decrease in efficiency, to about 6%, with continuing decrease to about 1.5% at the end of eight hours. In these experiments long periods of high light intensity produced pH values as high as 11.2 which decreased to 8.0 during the dark period.

The pH of a growth medium may be buffered by including appropriate nutrients in the medium. The most frequent ingredients have generally been phosphates, nitrates, ammonia nitrogen sources, bicarbonate and hydroxides. Krauss (107), in discussions concerning the nutrient requirements for commercial algal culturing, indicated that frequently the optimum characteristic of a particular medium formulation has been advantageous because of its capability to maintain pH or carbon dioxide concentrations rather than many of the other influencing growth factors. He indicated that ammonium salts, other than ammonium nitrate, generate a reduction in pH during growth and nitrate nitrogen generated a rise in pH during growth. If these two sources of nutrients are balanced, growth tended to maintain a buffering effect and thus maintain the pH at a fairly constant level. Slight variation from the desired pH level was corrected by applying an acid or a base. He also (107) indicated that the pH of the medium has significance with respect to maintaining iron concentrations. An increasing pH, above 7.0 - 8.0, tended to precipitate many iron compounds from solution. If higher pH values are desired in a growth system EDTA has been shown to be of material assistance. In the higher ranges of pH, above 7, the diol of EDTA NaFe did not show tendencies to precipitate, up to at least 10 mg/l concentration. Mayer, et al. (110), in studies concerning deep mass culture systems,

maintained the pH of the system between 6.0 and 6.5 for long periods of time using $(NH_4)_2SO_4$ as the nitrogen source along with KOH.

Temperature and pH tend to be related for some organisms. The optimum or maximum growth temperature may vary with pH. Fogg (93) studied and compared growth characteristics of blue-green algae and indicated that pH may be related to the temperature inhibiting growth effect. Mastigocladus laminosus was found to have an upper growth limiting temperature of 40°C at a pH of 5.5 and pH of 7.3. At a pH of 6.5 the upper limit of growth with respect to temperature showed a considerable change to a value of 54°C. This value was somewhat low for blue-green algae as in general most algae of this type favor more alkaline environments. However, he did indicate that Schizothrix SPT. has been found to grow at a pH of 5.0 in sphagnum swamps.

It has been indicated (93) that there are broad but definite preferences of pH for various classifications of algae. Kratz and Myers (68) compared blue-green algae and green algae with respect to optimum growth conditions under variable pH. The blue-green algae Anabaena variabilis, Anacystis nidulens and Nostoc muscorum were grown in medium starting at a pH of 7.3 to 7.5. After growing in the medium the substrate pH rose to about 7.7 to 8.0. A detailed study was made of the growth characteristics of Anabaena variabilis with respect to pH and it was found that there was a rather broad range of favorable pH for this organism, 7 - 10, with a sharp decrease in photosynthetic rate on either side of the range. The results for the blue-green alga were compared with Chlorella pyrenoidosa. This blue-green alga demonstrated fairly constant photosynthetic capability over the pH range of 6.0 - 7.5.

McLachlan and Gorham (163) found that Microcystis aeruginosa showed good growth in the pH range 6.5 - 10.0 if it had proper nutrient composition. Optimum growth was reported to be at a pH of about 10.0 but this was not well defined as there was relatively satisfactory growth from pH 7 to 10. High pH favoring blue-green algae was confirmed by Gerloff, et al. (118), in their studies of this group. There results indicated poor growth at pH 6 with marked improvement up to pH 9. The most favorable pH was 10 and this showed a very pronounced and narrow range as there was a very sharp decrease in growth at a pH of 11. This indicated a pronounced preference for alkaline conditions for this blue-green alga.

Natural environmental systems have shown similar response with respect to pH. Anderson (104) reported that in studies of Lake Washington the pH was about 7 most of the time, except for the middle of the summer, when it tended to rise above 8. This rise was the result of $CO_2$ being removed by photosynthesis. The bicarbonate concentration fluctuated between 16 - 32 mg/1 of $HCO_3$.

Goldman and Carter (21) in their eutrophication studies of Lake Tahoe indicated that the pH ranges were about 8.0 to 7.4 in the upper waters (surface) from May to December, 7.9 to 7.3 in May to July, and then up to 7.8 from July to December. The highest pH values corresponded with the highest photosynthetic levels. Reid (16) reported similar characteristics in natural bodies of water with the pH rising near the surface from 7.0 to 7.5 and even up to 9.0. This indicated increased photosynthetic activity of the surface water. Similar changes did not take place in the deeper portions. This characteristic was demonstrated for Lake Maraeti in New Zealand during times of prolonged lake stratification.

Sewage ponds are another natural environmental condition where pH variation may be of great significance, either because of its action or because of the need for control. Bush, et al. (113), in reporting on removal of dissolved solids from organic waste water by means of algal ponds, indicated there were variations of pH from 7.0 to 11.0 under natural conditions because of the high demand for carbon dioxide during daytime. At night the effluent pH tended to fall below the influent pH, which had a relatively constant pH of 7.6 - 7.9. Additional carbon dioxide had to be added to keep the pH about 7.0 when growth was taking place. This pH variation under controlled but natural conditions could be of considerable significance. Fruh (12) indicated that in many natural bodies of water the phosphorus removal reported during high photosynthetic activity has been the result of high pH conditions induced by the pH shift. This removal has thus been the result of precipitation rather than biologic growth.

Moyer (106) in discussions of tertiary waste treatment facilities for Lake Tahoe indicated that high pH values might be advantageous for ammonia stripping by forced draft aeration. He indicated that phosphate removal could be accomplished at pH values of 11.5 to 12.0. Thus, it appeared that it might be possible to accomplish some of this desired pH rise by controlled algal photosynthesis. This would perhaps contribute to reduced chemical requirements.

Parker (109), in his discussion of both anerobic and aerobic sewage stabilization ponds, indicated that in either case the pH tended to rise to a maximum pH level of about 8.0. This appeared to be the result of the system being a mixed culture of algae and bacteria.

The oxygen content and pH of a medium have shown interdependent relationships for some organisms. Allen (79), in studies on anaerobiosis with respect to photosynthetic rates, during continuous illumination, indicated that Scenedesmus, at pH 8.2, showed no change in photosynthetic capability after 5 to 7 hours of anerobisity prior to being subjected to illumination. When maintained at pH 5,5, under anerobic conditions, there was progressive decrease in photosynthetic capability up to about 10 hours and then only a slight decrease from 10 to 20 hours. Chlorella showed severe reduction within 2 hours under anerobic conditions and pH values of 5.0 or 8.2.

# APPENDIX IV

## CHEMICAL TESTS--PROCEDURES AND REAGENTS

(All procedures and reagents according to Standard Methods except as noted or modified.)

## AMMONIA - NITROGEN

Procedure: (164), pg. 173.

1. Measure 10.0 ml (or other appropriate volume) of sample and dilute to 100 ml in a volumetric flask, using $NH_3$ free distilled water.
    a. Mix well.
2. Measure 10.0 ml of this diluted sample and place in a beaker. Add reagents to the 10 ml portion in the beaker.
    a. Add 3 drops Rochelle salts and mix well.
    b. Mix again.
    c. Add 1.0 ml NaOH.
    d. Add 1.0 ml Nessler reagent.
    e. Mix and cover.
3. Run blank at same time.
    a. Use 10.0 ml $NH_3$ free distilled water and all reagents.
4. Time for 10 minutes and then determine percent T at 410 mμ.

Reagents: (164), pg. 173, Method B.

1. Rochelle salts.
2. NaOH - 160 g/1.
3. Nessler reagent - modified - 100 g $HgI_2$ and 70 g KI/1 - heat to dissolve.

## NITRITE - NITROGEN

Procedure: (164), pg. 180.

1. Use 10 ml sample (or aliquot).
    a. Adjust pH to about 7.
    b. Use dilute HCl or NaOH.

2. Place adjusted sample in Nessler tubes.

    a. Dilute to about 25 ml.

3. Add 1.0 ml sulfanilic acid reagent.

    a. Mix.

    b. Allow to stand 4 minutes.

4. Add 1.0 ml 1-naphthylamine hydrochloride reagent, followed by 1.0 ml sodium acetate buffer solution.

    a. Dilute to 50 ml.

    b. Mix well.

5. Blank run at same time.

    a. Distilled water and reagents.

6. Time for color development, 20 minutes. Measure color density at 525 mμ.

Reagents: (164), pg. 180.

1. Dilute HCl and NaOh - about 0.005 N.
2. Sulfanilic acid reagent.
3. 1-napthylamine hydrochloride reagent.
4. Sodium acetate buffer solution.

## NITRATE - NITROGEN

Procedure: (164), pg. 175 - Method A.

1. Use 5 - 25 ml sample (to give about 0.03 - 0.04 mg $NO_3$ - N).

    a. Adjust pH of sample to about 7.0 (use dilute HCl or NaOH).

    b. Place in evaporating dish and evaporate to dryness over steam.

2. Add 2.0 ml phenoldisulfonic acid to evaporating dish.

a. Rub residue with stirring rod to dissolve solids.

3. Transfer to Nessler tube.

a. Rinse evaporating dish 2 to 3 times and add to tube.

b. Dilute to about 35 ml.

4. Add 7.0 ml KOH solution.

a. Dilute to 50 ml with distilled water.

b. Mix.

5. Blank run at same time.

a. Distilled water and reagents.

6. Measure 5% T at 410 mμ.

Reagents: (164), pg. 175 - Method A.

1. Dilute HCl and NaOH - about 0.005 N.
2. Phenoldisulfonic acid reagent.
3. KOH solution - 12 N.

## ORTHO - PHOSPHATE

Procedure: (164), pg. 702 - Method B, using Hach Chemical Co. modification.

1. Place a measured sample (to give about 0.06 - 0.08 mg $PO_4$) in a Nessler tube.

a. Dilute to 50 ml with distilled water.

2. Add 2.5 ml of ammonium molybdate solution to the Nessler tube.

a. Mix well.

3. Add 5 scoops (0.5 g) of "Stanna-Vor" powder.
4. Blank run at same time.

a. Distilled water and all reagents.

5. Time for 10 minutes and then determine percent T at 700 mμ.

Reagents: (164), pg. 202 - Method B, using Hach Chemical Co. modification.

1. Ammonium molybdate reagent - strong acid.
2. "Stanna-Vor" powder (166), Cat. No. 293.

## TOTAL $CO_2$

Procedure: (165), pg. 227.

1. Place 200 ml of sample in evolution flash and close.
2. Add 0.2 ml phenolphthalein to absorption flash with a mixing bar.
    a. Add 50.0 ml of $Ba(OH)_2$ - (0.0315 N).
3. Acidify sample - 15 ml $H_2SO_4$ (.25 N) - clamp.
4. Start cooling $H_2O$.
5. Heat to boiling.
    a. While heating, start and stop pump a few times. Reduce heat when boiling.
6. Start circulation pump.
7. Circulate 10 minutes.
8. Titrate $Ba(OH)_2$ without stopping heat or circulation.
9. Open relief valve slowly.

Reagents: (165)

1. Phenolphthalein indicator solution.
2. $Ba(OH)_2$ - 0.0315 N.
3. $H_2SO_4$ - 0.25 N.
4. HCl - 0.0383 N.

C.O.D.

Procedure: (166), pg. 44 - titration method.

1. 5.0 ml of 0.250 N potassium dichromate in digestion flask.
2. Add 0.2 gm mercuric sulfate.
3. 4 glass beads and mix.
4. 15.0 ml conc. $H_2SO_4$ (containing silver sulfate 4.95 g/500 ml). Swirl to mix, and cool with cold $H_2O$.
5. 10.0 ml sample.
6. Place digestion flash on previously heated electric hot plate and attach to operating reflux condenser. Heat to gentle boil and simmer for 2 hours.
7. Turn off heat and cool.
8. After cool - rinse with slight amount distilled $H_2O$.
9. Remove flask, add 5 drops ferroin indicator and titrate with standard ferrous sulfate solution. Titrate to red-brown end point.
10. Calculation: C.O.D. = (5-ml $FeSO_4$)200 = mg/1.

Reagents: (166) - by Hatch Chemical Co.

1. Potassium dichromate - 0.250 N - Cat. No. 1809.
2. Mercuric sulfate - Cat. No. 1915.
3. Conc. $H_2SO_4$ reagent with 4.95 g silver sulfate/500 ml.
4. Ferroin indicator solution - Cat. No. 1812.
5. Ferrous sulfate solution - 0.250 N - Cat. No. 1811.

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