

Characterization of Photosynthetic Efficiency and  
Growth of Selected Microalgae in Dense Culture

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Final Contract Report  
Contract Number XK-5-05090-1

August 1986

## INTRODUCTION

An objective of the Aquatic Species Program is the development of large scale culturing systems for the production of fuels from lipid-rich microalgae. A major constraint to any such culturing system is the provision of sufficient light in the most economical manner possible, which has led to the use of shallow outdoor ponds that are illuminated using natural sunlight. The algal cultures in such a production facility will be subject to daily and seasonal temperature and light fluctuations, and thus the ponds must operate under less than ideal temperatures and light conditions. As a result, it is important to determine the response of algal productivity to the changes of temperature and light intensity that will be experienced in such a facility. This information will help to determine: (1) which algal species are best suited for use in a production facility located in a particular area, (2) the ability of these algal species to successfully compete with other species that might invade the pond, and (3) the productivity that might be expected from a given species.

The objectives of our project are to: (1) determine the maximum growth rates of several species of warm-adapted and cold-adapted algae under conditions in which neither nutrients, carbon dioxide, nor light are limiting, and (2) determine the yield and photosynthetic efficiency of various species of algae at different temperatures and light intensities. The results of these studies will provide the Aquatic Species Program with important information on the productivity and light efficiency of the tested species, and the effect of temperature and light intensity on these parameters. This information would be valuable in predicting the potential yield of algae grown in a large scale production facility.

## EXPERIMENTAL APPROACH

The objective of the initial experiments of this project was to determine the maximum growth rate of selected warm-adapted and cold-adapted algae. These experiments were conducted under conditions in which neither nutrients, carbon dioxide, nor light are limiting, and at the recommended optimum temperatures. When necessary, the growth medium was modified to achieve healthy stock cultures and rapid culture growth.

Subsequent experiments were directed towards determining the effect of temperature on biomass productivity and photosynthetic light efficiency. The experimental approach was to establish a continuous culture under conditions of CO<sub>2</sub> saturation at a particular temperature and at a high light intensity. The cell concentration of the culture was systematically varied to determine the cell concentration of maximum productivity for these experimental conditions. At this cell concentration, the temperature of the culture was varied and the response of biomass productivity and efficiency of light utilization to different temperatures was determined.\*

This report covers progress made during the past year towards accomplishing these research objectives. We have determined the maximum growth rate of five warm-adapted algae (Ankistrodesmus, Cyclotella, Chaetoceros, Chlorella, Scenedesmus), and two cold-adapted algae (Navicula and Skeletonema). For most of these organisms it was first necessary to modify standard media to obtain healthy stock cultures of these algae. We have also determined the effect of temperature on the biomass productivity and efficiency of light utilization of a Scenedesmus continuous culture as a function of cell concentration.

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\* The cell concentration of maximum biomass productivity may shift as the temperature changes; thus the actual response of productivity and light efficiency to changes in temperature might be obscured by shifts in the cell concentration of maximum productivity. This necessitated determining the cell concentration of maximum productivity at each temperature in order to obtain reliable data on the temperature response of productivity and light efficiency. Thus, the initial experiment was to determine if the cell concentration of maximum biomass productivity is significantly altered by changes in the temperature.

## MATERIALS AND METHODS

### Organisms and Growth Conditions

Five species of warm-adapted and two species of cold-adapted algae were used in the present work: Ankistrodesmus (S/ANKIS-3), Cyclotella (S/CYCLO-1), Chaetoceros (SS-14), Chlorella (SC), and Scenedesmus obliquus (Gaffron strain D3), Navicula, and Skeletonema costatum. The Ankistrodesmus, Cyclotella, Chaetoceros, Chlorella, and Navicula were supplied by SERI, the Scenedesmus has been used by us in-house for years, and the Skeletonema was acquired from the Culture Collection of Marine Phytoplankton (West Boothbay Harbor, Maine).

Ankistrodesmus was cultured in modified Type II, 25 mmho/cm medium (Barclay, et al., 1985), containing the following components: 3.0 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O, 5.8 g/l Na<sub>2</sub>SO<sub>4</sub>, 0.04 g/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.975 g/l KCl, 8.08 g/l NaCl, 2.0 g/l KNO<sub>3</sub>, 2.3 g/l NaHCO<sub>3</sub>, 0.9 g/l Na<sub>2</sub>CO<sub>3</sub>, 0.5 g/l NaH<sub>2</sub>PO<sub>4</sub> and 1 ml/l of modified Chapman metals solution (0.09g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.60 g H<sub>3</sub>BO<sub>3</sub>, 0.15 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.06 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 10.00 g MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.50 g NaMoO<sub>4</sub>·2H<sub>2</sub>O, 5.00 g FeSO<sub>4</sub>·7H<sub>2</sub>O, and 4.00 g Na<sub>2</sub>EDTA dissolved in one liter of distilled water). NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub> and NaH<sub>2</sub>PO<sub>4</sub> were filter sterilized and added to the autoclaved medium.

Cyclotella was cultured in a medium that is based on a recipe supplied by Dr. Mahasin Tadros (Alabama A & M). This medium contains the following components: 0.25 g/l NaNO<sub>3</sub>, 0.1 g/l Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, 20.0 g/l artificial sea salts (Rila Marine Products), 0.005 g/l NaH<sub>2</sub>PO<sub>4</sub>, 0.02 mg/l thiamine, 0.1 mg/l vitamin B<sub>12</sub>, 0.1 mg/l biotin, and 1.0 ml/l trace metal solution (0.55 g H<sub>3</sub>BO<sub>3</sub>, 0.06 g ZnCl<sub>2</sub>, 0.3 g CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.3 g NaMoO<sub>4</sub>·2H<sub>2</sub>O, 0.04 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 2.5 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.0 g Na<sub>2</sub>EDTA, 0.04 g MnCl<sub>2</sub>·4H<sub>2</sub>O per liter of distilled water). The vitamins and NaH<sub>2</sub>PO<sub>4</sub> were filter sterilized and added to the autoclaved medium.

Chaetoceros was cultured in a growth medium based on the Type II, 25 mmho/cm medium, and contains the following components: 3.0 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O, 5.8 g/l Na<sub>2</sub>SO<sub>4</sub>, 0.04 g/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.975 g/l KCl, 8.08 g/l NaCl, 0.1 g/l Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, and 1 ml/l modified Chapman metals solution (see Ankistrodesmus medium above). After autoclaving this medium, the following components were filter sterilized and added aseptically: 2.3 g/l

NaHCO<sub>3</sub>, 0.9 g/l Na<sub>2</sub>CO<sub>3</sub>, 0.25 g/l NaH<sub>2</sub>PO<sub>4</sub>, 0.2 mg/l thiamine, 0.1 mg/l vitamin B<sub>12</sub>, 0.1 mg/l biotin and 0.6 g/l urea.

Chlorella was cultured in modified Type II, 10 mmho/cm medium (Barclay et al., 1985) containing the following components: 2.0 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O, 2.6 g/l Na<sub>2</sub>SO<sub>4</sub>, 0.04 g/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.45 g/l KCl, 1.5 g/l NaCl, and 1 ml/l modified Chapman metals solution (see Ankistrodesmus above). After autoclaving these medium components, the following chemicals were filter sterilized and added aseptically to a final concentration of: 1.2 g/l NaHCO<sub>3</sub>, 0.2 g/l Na<sub>2</sub>CO<sub>3</sub>, 0.25 g/l NaH<sub>2</sub>PO<sub>4</sub>, and 0.6 g/l urea.

Scenedesmus was cultured in medium containing 1.0 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 g/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.38 g/l K<sub>2</sub>HPO<sub>4</sub>, 0.15 g/l KH<sub>2</sub>PO<sub>4</sub>, 6.0 g/l KNO<sub>3</sub> and 2 ml/l micronutrient solution (5.0 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.0 g Na<sub>2</sub>EDTA, 2.86 g H<sub>3</sub>BO<sub>3</sub>, 1.81 g MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.22 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.39 g Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.08 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.05 g Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O dissolved in one liter of distilled water).

Navicula was cultured in a growth medium based on the Type II, 25 mmho/cm medium, and contains the following components: 3.0 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O, 5.8 g/l Na<sub>2</sub>SO<sub>4</sub>, 0.04 g/l CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.975 g/l KCl, 8.08 g/l NaCl, 2.3 g/l NaHCO<sub>3</sub>, 0.9 g/l Na<sub>2</sub>CO<sub>3</sub>, 0.5 g/l NaH<sub>2</sub>PO<sub>4</sub>, 0.1 g/l Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, 0.2 mg/l thiamine, 0.1 mg/l vitamin B<sub>12</sub>, 0.1 mg/l biotin, 0.6 g/l urea and 1 ml/l of modified trace metals solution (0.06 g ZnCl<sub>2</sub>, 6.84 g H<sub>3</sub>BO<sub>3</sub>, 0.026 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.002 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.86 g MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.005 g Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 5.00 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 6.0 g Na<sub>2</sub>EDTA, and 0.052 g NiSO<sub>4</sub>·6H<sub>2</sub>O dissolved in one liter of distilled water). The NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, NaH<sub>2</sub>PO<sub>4</sub>, urea and vitamins were filter sterilized and added aseptically to the autoclaved medium.

Skeletonema was cultured in a growth medium that is based on a modification of the medium suggested by the Culture Collection of Marine Phytoplankton. This medium contains the following components: 0.75 g/l NaNO<sub>3</sub>, 0.3 g/l Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, 37 g/l artificial sea salt (Instant Ocean), 0.015 g/l NaH<sub>2</sub>PO<sub>4</sub>, 0.6 mg/l thiamine, 0.3 mg/l vitamin B<sub>12</sub>, 0.3 mg/l biotin and 3 ml/l of trace metal solution (0.01 g ZnCl<sub>2</sub>, 0.007 g CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.006 g Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.01 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 3.2 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.0 g Na<sub>2</sub>EDTA, 0.18 g MnCl<sub>2</sub>·4H<sub>2</sub>O per liter of distilled water). The NaH<sub>2</sub>PO<sub>4</sub> and vitamins were filter sterilized and added aseptically to the autoclaved medium.

Stock cultures of these organisms were maintained in 1000 ml Roux bottles (containing 500 ml of culture) kept in an environmental chamber. The cultures were continuously bubbled with a gas mixture of 2% CO<sub>2</sub> in air. Prior to introduction to the cultures, the gas mixture was humidified by bubbling it through a water reservoir maintained at the same

temperature as the cultures. Warm-adapted stock cultures were maintained at 29 +/- 2 °C, and cold-adapted cultures were kept at 19 +/- 1 °C. Illumination was supplied by high output, cool white fluorescent bulbs, and the photon flux for the stock cultures varied between 40-90  $\mu\text{E}/\text{m}^2/\text{sec}$ . In addition to maintaining each organism in liquid culture, we have grown each alga (with the exception of Chaetoceros) on agar plates. We also undertook an effort to free each culture from bacterial contamination; to date we have only been successful in purifying Navicula. All cultures were handled and sampled using aseptic techniques.

### Growth Rate Determinations

Growth rate experiments with each alga were performed using Roux bottles that were maintained in an environmental chamber with continuous illumination and bubbling with 2% CO<sub>2</sub> in air. Each experimental Roux bottle was placed in a black cardboard box that had one side removed. The box was placed directly against the light source (two high output, cool white fluorescent bulbs) with the open side of the box facing the light. Photon flux was measured on the back of the bottle using a LiCor 190SB Quantum Sensor. This arrangement eliminated stray light from the Roux bottle, thereby permitting a very accurate measurement of the photon flux on the culture. The temperature of the experimental bottle was determined by placing a thermometer in a Roux bottle that was maintained under identical conditions, with the exception that it did not contain algae. At time zero, the experimental bottle was inoculated at a very dilute cell density using cells from an exponentially growing stock culture that had been maintained at a temperature and light level similar to the experimental conditions. Periodically (every 8-12 hours) a small sample (2 ml) of the culture was removed, and the cell concentration (cells/ml) determined using a Coulter Counter Model TAU with PCAII population accessory. The temperature and photon flux for each bottle were recorded immediately after each sampling. When the culture reached a density at which the transmitted photon flux was significantly lower than the initial measurements (i.e. when the culture was dense enough to provide a faint trace of color to the bottle), subsequent measurements of the cell concentration were not used in determining the growth rate. The temperatures and photon fluxes used for the growth rate determinations of each alga are noted in the Results section.

## Description of the Continuous Culture Apparatus

The continuous culture apparatus used in this work is similar to the device constructed by Myers and Clark (1944). The apparatus is made of glass and consists of three concentric, cylindrical chambers (Figure 1). The outermost chamber is a temperature regulated water jacket, the middle chamber contains the algal culture, and the inner chamber can house a fluorescent light source. The middle (culture) chamber has a volume of 1200 ml and a path length of 1.25 cm. The vessel is continuously illuminated by eight high output, cool white fluorescent bulbs (four banks of two bulbs each) surrounding the apparatus. A single high output, cool white fluorescent bulb, located in the center light chamber, is completely masked with black tape (so as not to significantly alter the measured photon flux on the algal chamber) except for a small area that is directed at the density detector. Cell density is maintained by monitoring the light transmission from this bulb through the culture using a photocell (Clairex CL604L) whose output is fed directly to a microcomputer (Sym-1, Synertek Corp.). When the output of the photocell exceeds a preset reference value (i.e. when the culture grows and absorbs more light), the microcomputer activates a peristaltic pump for a preset period of time, fresh medium is added to the culture, and a corresponding volume of algal culture leaves the vessel via the overflow tube. After a latent time of 60 sec. to allow for mixing of any newly added medium, the monitoring cycle is reinitiated. A gas mixture of 2% CO<sub>2</sub> in air is admitted through the fritted bottom of the culture chamber at a total gas flow rate of 150 ml/min. This flow rate is sufficient to provide relatively rapid mixing of the culture and to prevent the cells from settling.

## Analytical Methods

Dry weight determinations involved filtering a 10 ml aliquot of culture through a glass fiber filter (approximate retention 2.6  $\mu$ m), rinsing thoroughly with distilled water, drying at 105 °C overnight, and cooling to room temperature in a dessicator. (The filter paper was previously dried at 105 °C before beginning the procedure). The doubling time of a continuous culture was taken as the ratio of the overflow rate (ml/hr) and the total volume of the culture vessel (1200 ml); the biomass productivity

(mg dry weight/hr) was taken as the product of the cell concentration (mg dry weight/ml) and the overflow rate. The temperature of the continuous culture was monitored daily by use of a thermocouple immersed in the culture. The chlorophyll concentration of Scenedesmus was determined by adding an aliquot of culture to a 1:1 mixture of Triton X-100 and 5% KOH in methanol, heating at 63 °C for three minutes, and centrifuging. Optical density was determined at 645 nm.

### Efficiency of Light Utilization

The efficiency of light utilization by a continuous culture is defined as the ratio of the calories of biomass produced per unit time to the calories of light absorbed per unit time. The calories of biomass produced per hour can be calculated from the culture productivity (i.e. for Scenedesmus 1 g biomass = 5500 calories, determined by Galbraith Laboratories, Knoxville, Tennessee; see also Myers, 1957). The amount of light absorbed by a culture was calculated using the Beer-Lambert Law ( $I = I_0 e^{-\alpha c l}$ , where  $I$  is the amount of transmitted light at a particular wavelength,  $I_0$  is the amount of incident light at that wavelength,  $\alpha$  is the extinction coefficient of the culture at that wavelength,  $c$  is the cell concentration, and  $l$  is the light path length of the culture). The difference between  $I_0$  and  $I$  represents the amount of light absorbed by a culture at a particular cell concentration. To determine the efficiency of light utilization by a continuous culture at a particular cell concentration it was necessary to determine: (1) the incident light intensity on the algal culture chamber, and (2) the extinction coefficient as a function of wavelength over the range of 400-700 nm.

The light intensity supplied to the continuous culture apparatus ( $I_0$ ) was determined by removing the center glass portion of the culture vessel and measuring the light that passed through the water jacket and illuminated the outer wall of the culture chamber. Light measurements (LiCor 190SB Quantum Sensor) were made at eight locations around the perimeter of the algal chamber, and at several different heights within the culture vessel. These measurements were averaged to obtain a value for the incident photon flux ( $\mu\text{E}/\text{m}^2/\text{sec}$ ). This value, plus knowledge of the spectrum of the light bulb, permits the conversion of photon flux into light intensity ( $\text{calories}/\text{m}^2/\text{hr}$ ).



The relationship of extinction coefficient to wavelength for a culture of a particular cell concentration was independently calculated by measuring  $I_0$  and  $I$  using a large glass cuvette. The cuvette was illuminated by a high output, cool white fluorescent bulb, and the light intensity crossing the cuvette in the presence and absence of cells was measured. Light intensities were measured over the range of 400–700 nm using an ISCO spectroradiometer.

## RESULTS AND DISCUSSION

### I. GROWTH RATE EXPERIMENTS

The objective of these experiments was to determine the maximum growth rate of selected warm-adapted algae (Scenedesmus, Anksitrodesmus, Cyclotella, Chaetoceros, and Chlorella), and cold-adapted algae (Navicula and Skeletonema). These experiments required measurement of the growth rate under conditions in which neither nutrients, carbon dioxide, nor light were limiting. We found that the medium in which we received the original algal inoculum was, in some cases, not well-suited for growth of the particular organism, or that the basic medium required supplements that were not well documented. Therefore, it was first necessary to define media that would support healthy stock cultures. Although the media listed in the Materials and Methods section are not necessarily optimized in every respect, these media will support good growth of the particular organisms. All cultures (stock and experimental) were bubbled with a gas mixture of 2% CO<sub>2</sub> in air to ensure that the cultures were not carbon dioxide limited. In addition to nutrients and carbon dioxide, it was also necessary that light not limit the growth rates of these organisms. To verify that the algae in these experiments were not significantly light limited, the growth rate of each organism was determined at two different photon fluxes; similar growth rates at each photon flux indicated lack of light limitation.

A. Scenedesmus was cultured at 32 +/- 1 °C, and at a pH of 7.5. Figure 2 shows the results of an experiment in which we determined the doublings per day of a Scenedesmus culture as a function of photon flux. Note that the doublings per day increased in direct response to an increase in photon flux up to approximately 120 μE/m<sup>2</sup>/sec. At higher photon fluxes there was no longer a linear relationship between these two parameters, and at photon fluxes greater than about 180 μE/m<sup>2</sup>/sec Scenedesmus was not significantly light limited. The maximum growth rate of Scenedesmus was approximately 3.3 doublings per day at this temperature.

B. Anksitrodesmus (S/ANKIS-3) cultures were maintained at 30 +/- 1 °C and a pH of 7.9 to 8.2; the photon flux varied between 166 and 190  $\mu\text{E}/\text{m}^2/\text{sec}$ . This organism tends to stick to glass surfaces and to form small clumps, making it difficult to maintain a uniform culture. These problems were reduced by including a small stir bar in the bottom of each Roux bottle. Figure 3 is representative growth curves for this alga. The squares show the results of duplicate experiments carried out at 166  $\mu\text{E}/\text{m}^2/\text{sec}$ . The resulting curve corresponds to a growth rate of 2.67 doublings per day. Experiments at a photon flux of 190  $\mu\text{E}/\text{m}^2/\text{sec}$  (diamonds) gave similar growth rates, indicating that the cultures were not light limited at these photon fluxes and dilute cell concentrations. The last three points of the latter curve deviate from a straight line, reflecting the fact that the culture eventually became light limited. These four experiments gave growth rates of 2.64, 2.70, 2.48, and 2.65 doublings per day, with the average rate being 2.62 doublings per day.

C. Cyclotella (S/CYCLO-1) was maintained at 30.5 +/- 1 °C, at a photon flux of 200-218  $\mu\text{E}/\text{m}^2/\text{sec}$ , and a pH of 6.4. Figure 4 shows the results of several growth rate experiments. At 218  $\mu\text{E}/\text{m}^2/\text{sec}$  the growth rate was 3.73 doublings per day, and at 200  $\mu\text{E}/\text{m}^2/\text{sec}$  the growth rate was 3.95 doublings per day. The results of a total of six experiments indicate an average growth rate of 3.89 doublings per day. Although Cyclotella grew rapidly using the medium listed in the Materials and Methods, we were unable to attain cell concentrations greater than about  $10^6$  cells/ml. We found that this medium limits growth beyond these cell densities, since doubling or tripling the concentrations of all the medium components (except the artificial sea salt) resulted in higher cell concentrations.

D. Chaetoceros (SS-14) was grown at 30.5 +/- 1 °C, at a photon flux of 165-195  $\mu\text{E}/\text{m}^2/\text{sec}$  and a pH of 7.9. Figure 5 shows the results of several growth rate experiments. The diamonds correspond to a growth rate of 4.54 doublings per day, and the squares represent a growth rate of approximately 4.6 doublings per day. Nitrate ( $\text{KNO}_3$ ) can also be used as a nitrogen source for Chaetoceros; however, we found that the growth rate with nitrate is approximately 10% lower than with urea (data not shown).

E. Chlorella (SC) was grown at  $31 \pm 1$  °C, at a photon flux of 195–218  $\mu\text{E}/\text{m}^2/\text{sec}$  and a pH of 7.9. Figure 6 shows the results of several growth rate experiments. The observed growth rate was 4.89 doublings per day at 195  $\mu\text{E}/\text{m}^2/\text{sec}$  and 4.95 doublings per day at 218  $\mu\text{E}/\text{m}^2/\text{sec}$ . Like Chaetoceros, Chlorella can also use nitrate (2 g/l  $\text{KNO}_3$ ) as a nitrogen source; unlike Chaetoceros, nitrate does not adversely affect the growth rate of Chlorella (data not shown).

F. Navicula was cultured at  $18 \pm 1$  °C, at a photon flux of 160–175  $\mu\text{E}/\text{m}^2/\text{sec}$  and a pH of 8.1. Under these conditions Navicula formed small clumps and settled to the bottom of the Roux bottle. The tendency to clump and settle was reduced by stirring the culture with a small stir bar included in the culture bottle. Figure 7 shows the results of growth rate experiments; the observed growth rates were 1.9 doublings per day at 160  $\mu\text{E}/\text{m}^2/\text{sec}$  (diamonds) and 2.0 doublings per day at 175  $\mu\text{E}/\text{m}^2/\text{sec}$  (squares).

G. Skeletonema costatum was cultured at  $19 \pm 1$  °C, at a photon flux of 170–200  $\mu\text{E}/\text{m}^2/\text{sec}$  and a pH of 7.1. Figure 8 shows the results of several growth rate experiments. At a photon flux of 170  $\mu\text{E}/\text{m}^2/\text{sec}$  the growth rate was 2.1 doublings per day (diamonds) and at 200  $\mu\text{E}/\text{m}^2/\text{sec}$  the growth rate was 1.9 doublings per day (squares).

Table 1 summarizes the growth data for each organism. Each alga grew well in the media described and under the experimental conditions stated. Chlorella and Chaetoceros grew very rapidly (4.89 and 4.54 doublings per day, respectively); this plus their ease of handling would make them attractive species for further studies. Cyclotella grew well, but the medium must be optimized to attain higher cell concentrations. The growth rates of Ankistrodesmus and Navicula were among the slowest of the organisms studied. This relatively slow growth, coupled with their tendency to clump, makes these organisms the least attractive for further study. Although Skeletonema grew relatively slowly it posed few problems in culturing.

Table 1

## Summary of Growth Data

| Organism              | Temperature (°C) | Photon Flux ( $\mu\text{E}/\text{m}^2/\text{sec}$ ) | Growth Rate (doublings/day) |
|-----------------------|------------------|---|-----------------------------|
| <u>Ankistrodesmus</u> | 30.0 +/-1        | 166-190   | 2.6                         |
| <u>Chaetoceros</u>    | 30.5 +/-1        | 165-195   | 4.6                         |
| <u>Chlorella</u>      | 31.0 +/-1        | 195-218   | 4.9                         |
| <u>Cyclotella</u>     | 30.5 +/-1        | 200-218   | 3.9                         |
| <u>Navicula</u>       | 18.0 +/-1        | 160-175   | 2.0                         |
| <u>Scenedesmus</u>    | 32.0 +/-1        | >180  | 3.3                         |
| <u>Skeletonema</u>    | 19.0 +/-1        | 170-200   | 2.0                         |

II. THE EFFECT OF TEMPERATURE ON ALGAL YIELD

The objective of these experiments was to determine the effect of temperature on the cell concentration of maximum algal biomass productivity. It is important to determine the extent of this effect, since outdoor cultures will experience daily and seasonal changes in temperature.

For these experiments three continuous cultures of Scenedesmus were established at 15, 25, 35 °C, with incident photon fluxes of 295 +/- 35, 361 +/- 46, 312 +/- 69  $\mu\text{E}/\text{m}^2/\text{sec}$ , respectively. [These temperatures were chosen because previous data (obtained at a non-saturating photon flux of approximately 70  $\mu\text{E}/\text{m}^2/\text{sec}$ ) indicated that Scenedesmus productivity: (1) is maximal at 32 °C, (2) decreases sharply at temperatures above 35 °C, and (3) is approximately half the maximum value at 15 °C.] However, we found that stable, continuous cultures could not be maintained at 15 °C and 35 °C at these high photon fluxes; these two cultures were successfully restarted at 18 °C and 32 °C.

Figure 9 shows the relationship of Scenedesmus productivity to cell concentration at 18, 25, and 32 °C. At both 18 and 32 °C, a cell concentration of approximately 1.5 g/l dry weight yielded the maximum

productivity (48 and 90 mg dry weight/hr, respectively). In contrast, at 25 °C both the cell concentration of maximum productivity (approximately 3.0 g/l dry weight) and the biomass productivity (108 mg dry weight/hr) were higher than at the other two temperatures.

The relationship of light utilization efficiency to cell concentration is shown in Figure 10 for these same three temperatures. Although there is scatter in the data, the efficiency of light utilization generally declined as the cell concentration increased at each of the three temperatures. This decline is the result of the competing processes of photosynthesis and respiration; as the cell concentration increases the photosynthetic capacity of the culture approaches a maximum, but the respiratory load of the culture continues to increase with increasing cell concentration. The net result is that as the cell concentration increases the efficiency with which absorbed light is converted into a net gain of biomass decreases. Extrapolation of the efficiencies back to zero cell concentration indicates that the maximum efficiency of light utilization for these cultures was approximately 8.3%, 8.1%, and 5.2% for 32, 25, and 18 °C, respectively which are well below the theoretical maximum efficiency of 20% (e.g. see Radmer and Kok, 1977). These low values can be attributed to the fact that these experiments were conducted at high light intensities, where the efficiency is reduced due to the rather slow turnover of the photosynthetic dark reactions (Radmer and Kok, 1977).

## REFERENCES

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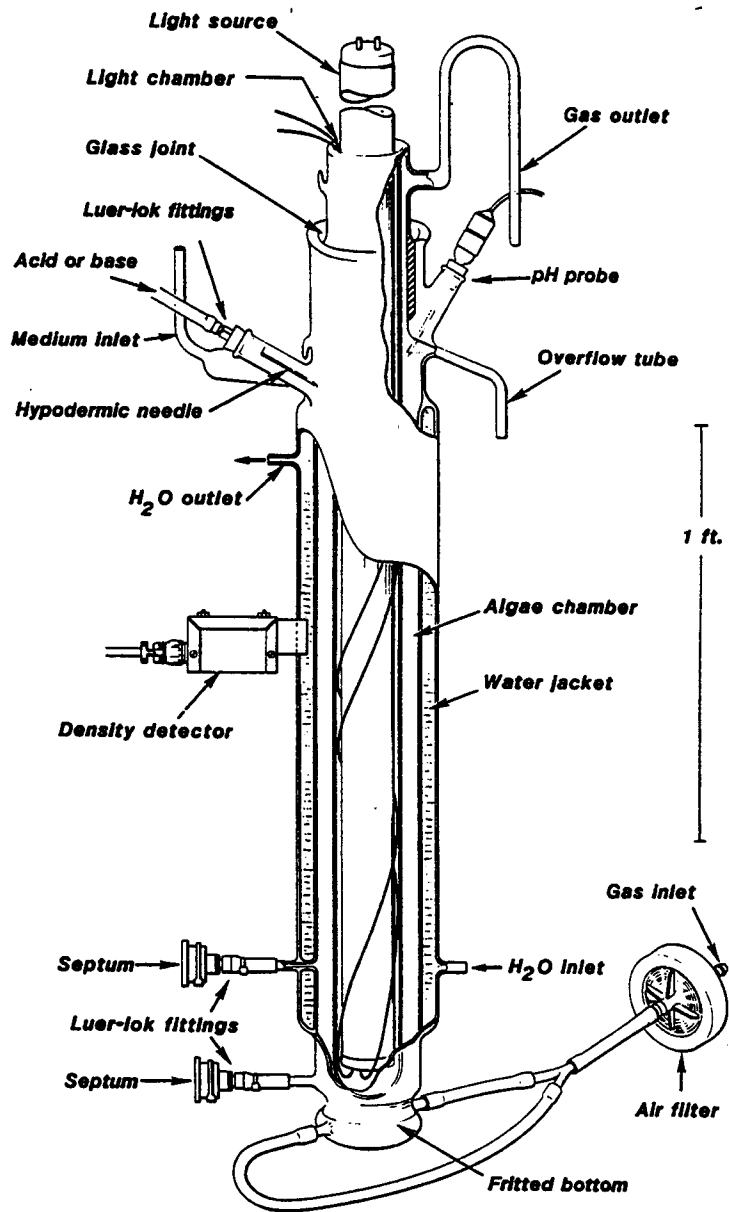


Figure 1. Continuous culture apparatus



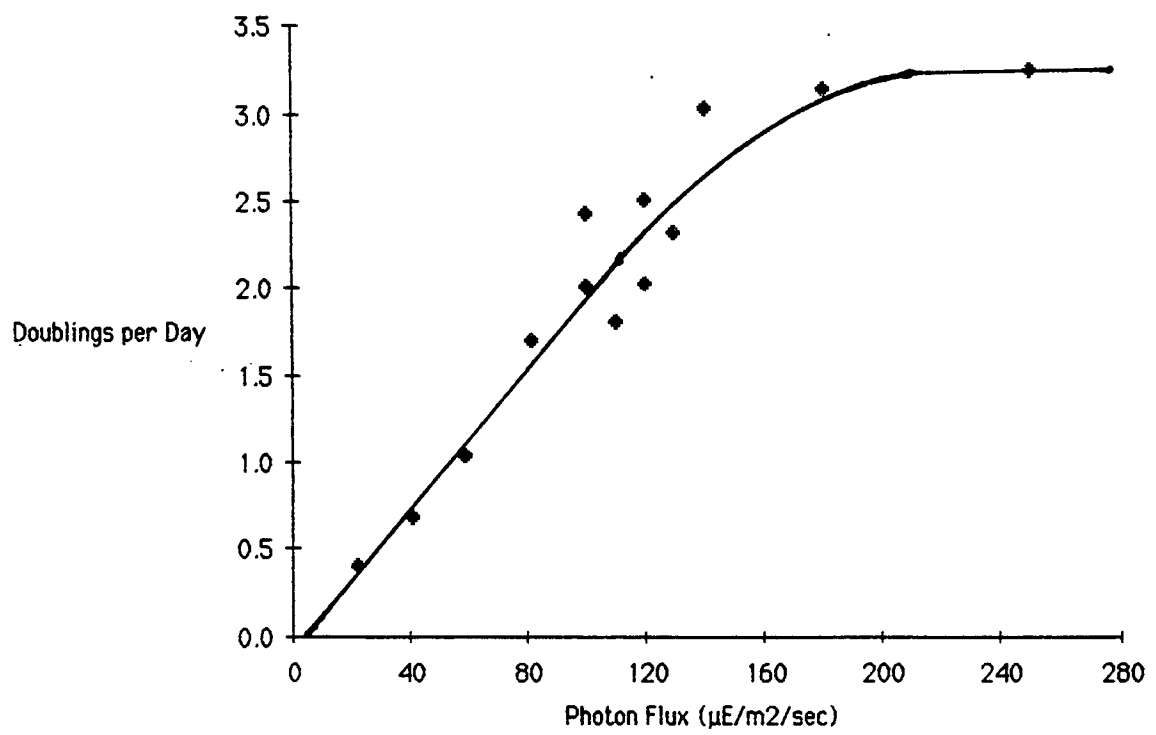


Figure 2. Doublings per day versus photon flux for Scenedesmus.

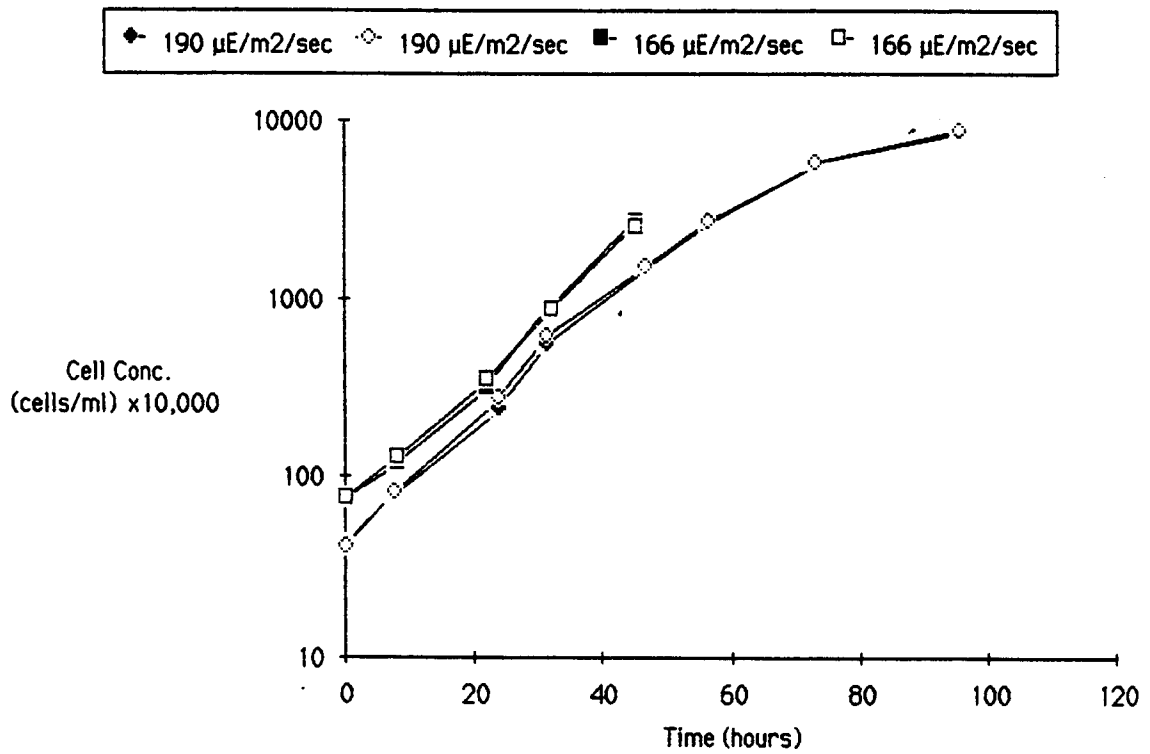


Figure 3. Growth curves for Ankistrodesmus.

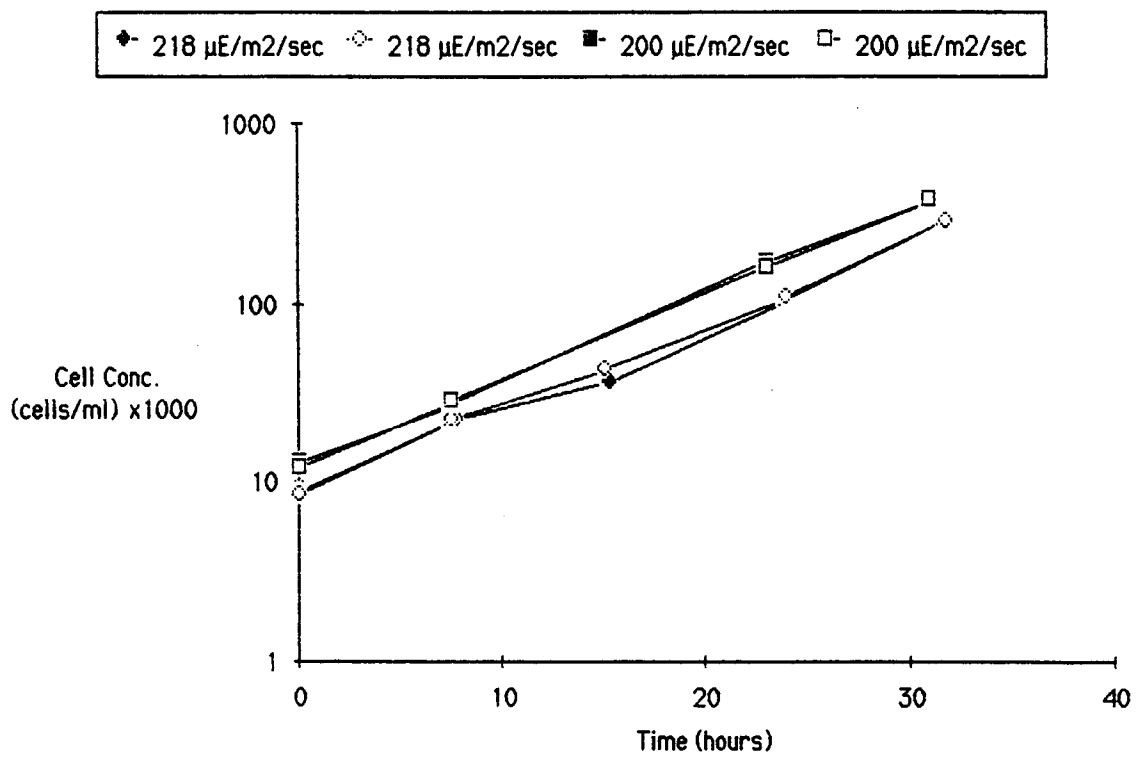


Figure 4. Growth curves for Cyclotella.

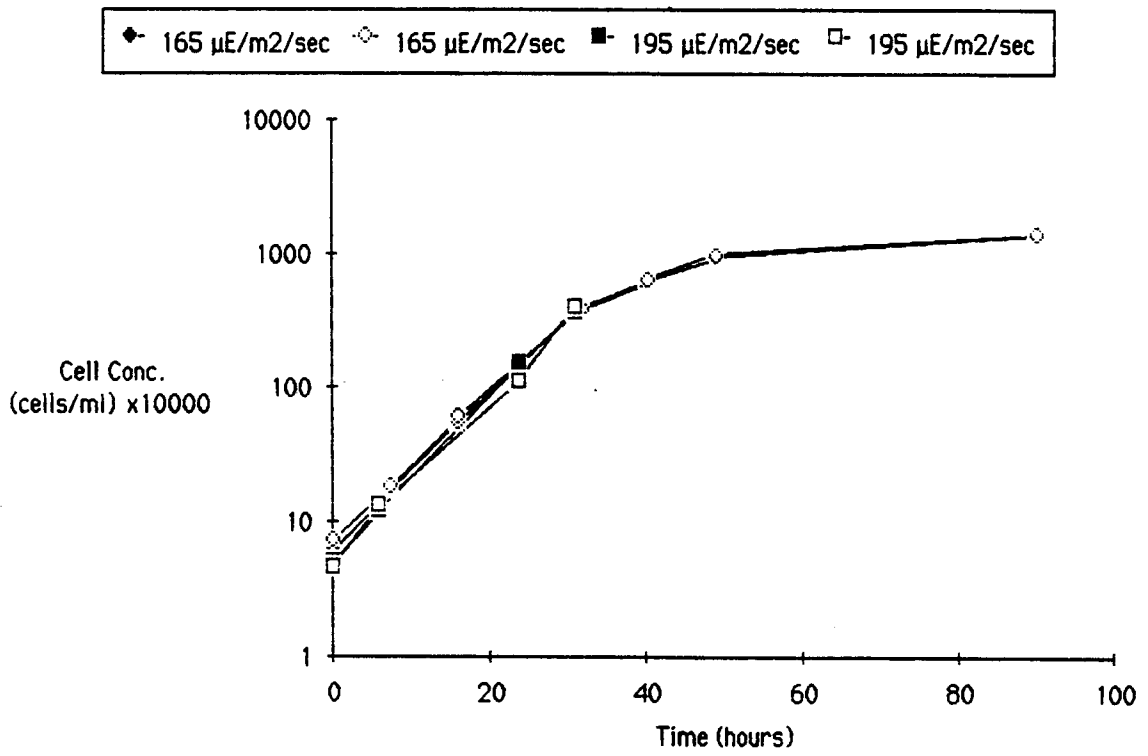


Figure 5. Growth curves for Chaetoceros.

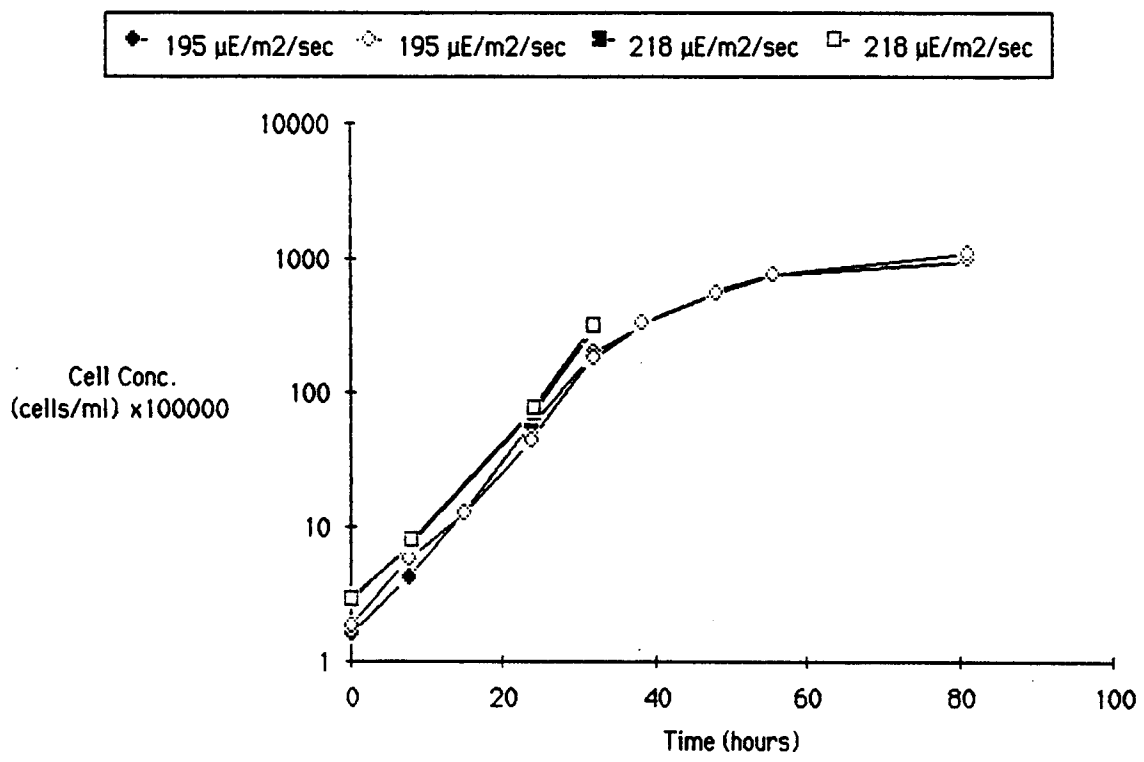


Figure 6. Growth curves for Chlorella.

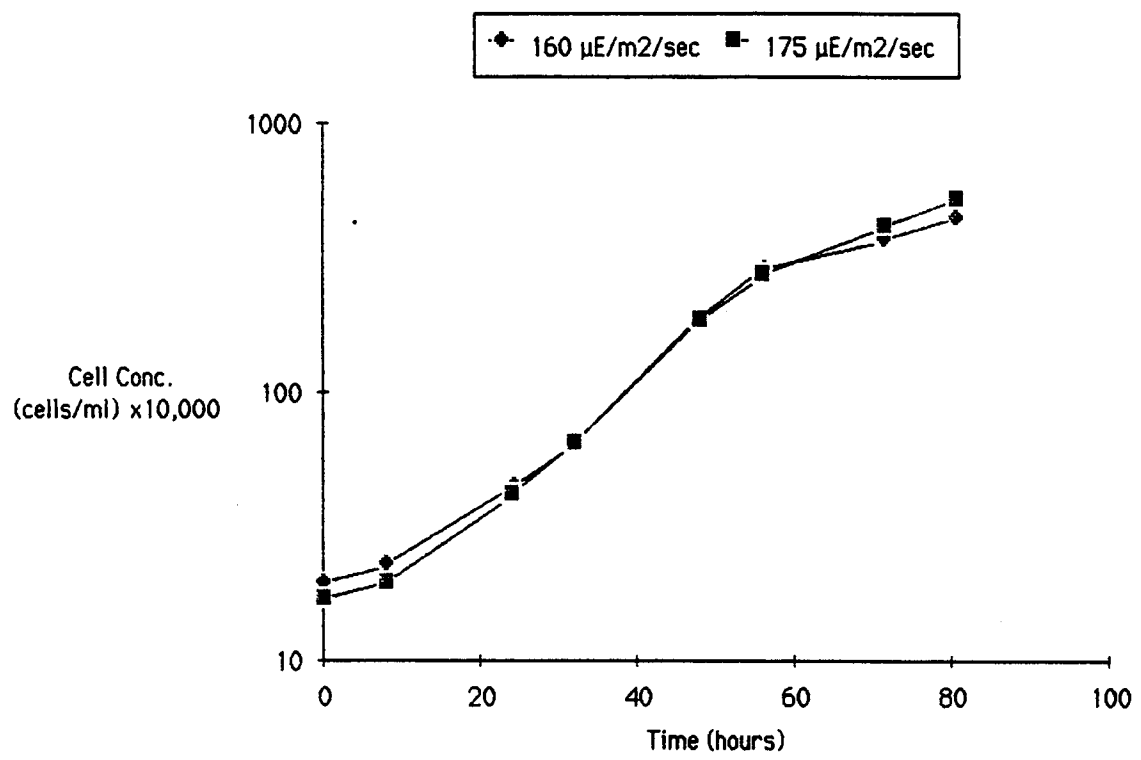


Figure 7. Growth curves for Navicula.

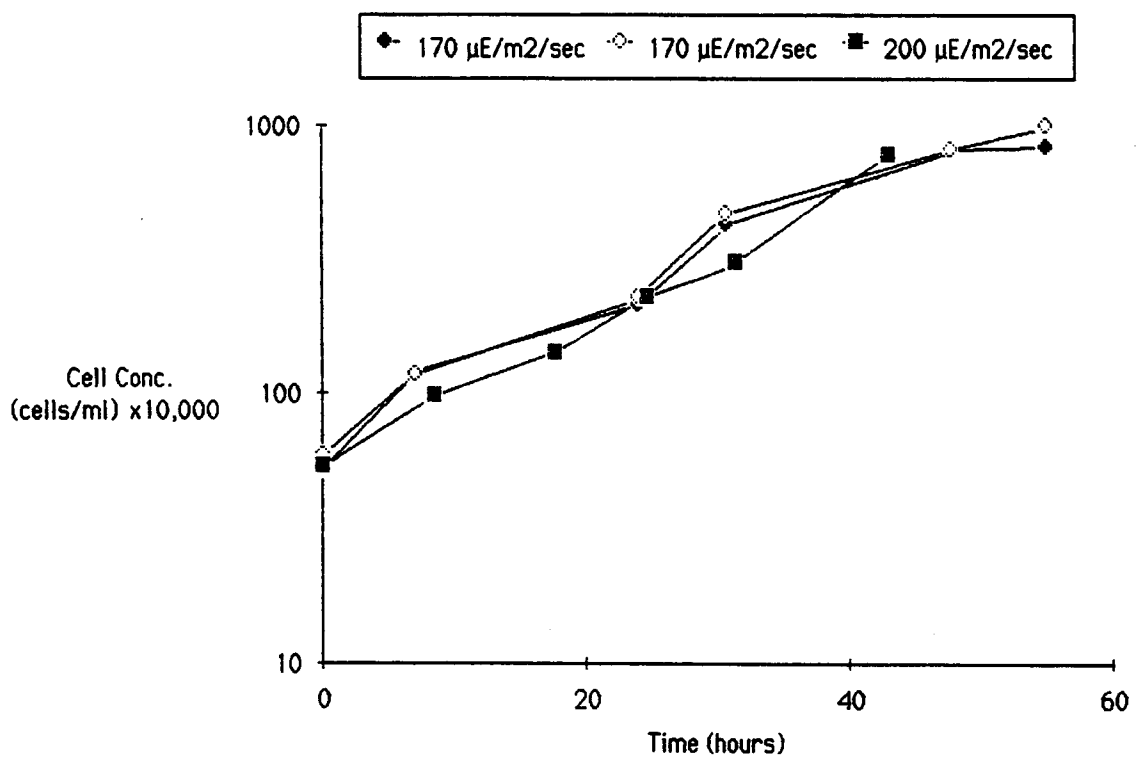


Figure 8. Growth curves for Skeletonema.

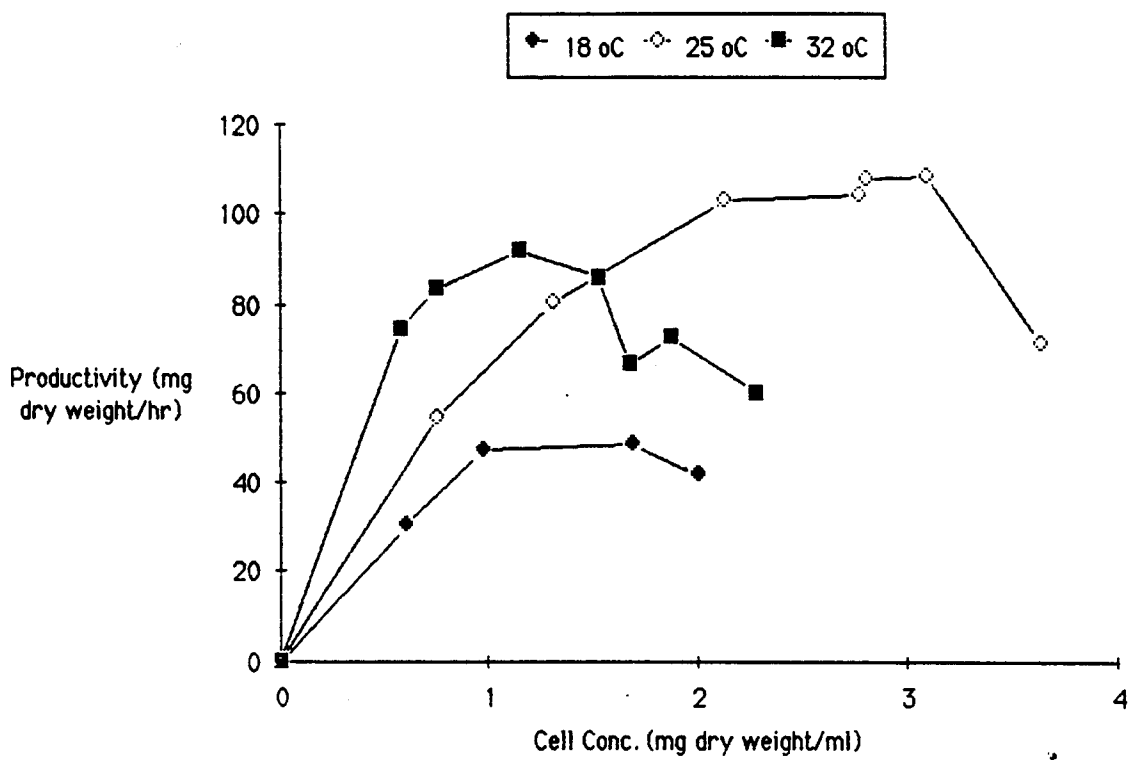


Figure 9. Productivity of a Scenedesmus continuous culture as a function of cell concentration at 18, 25, and 32 °C.



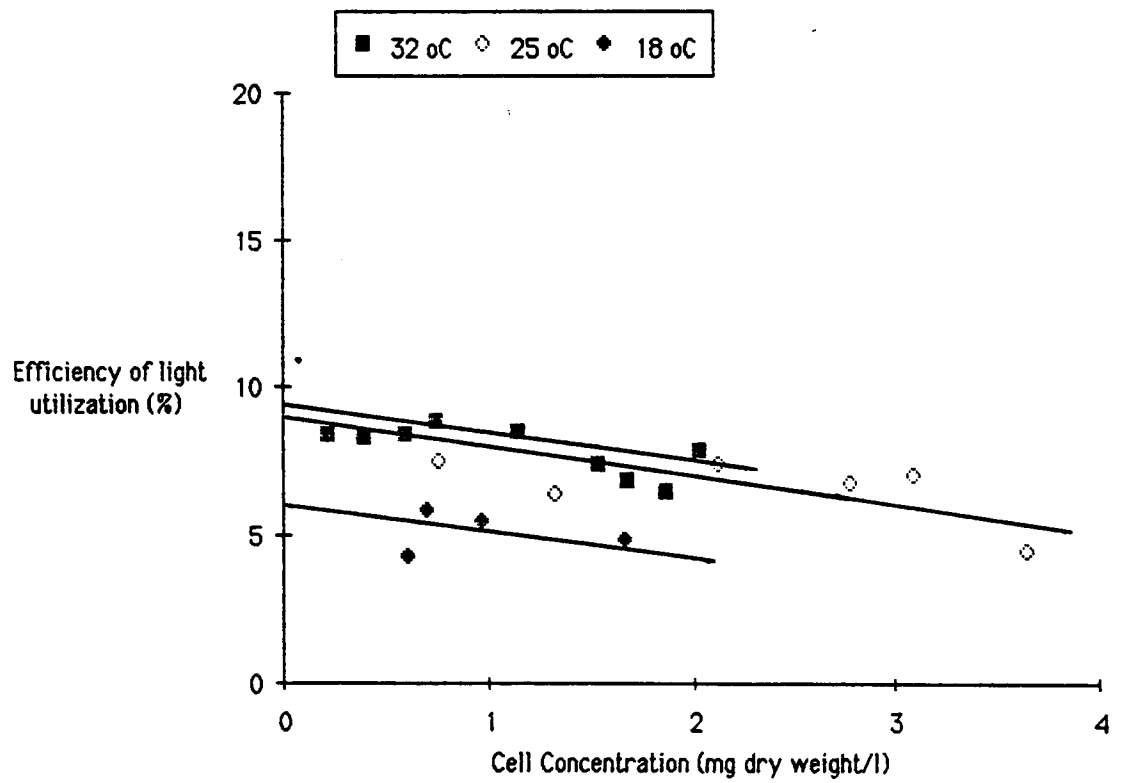


Figure 10. Efficiency of light utilization by a *Scenedesmus* continuous culture as a function of cell concentration at 18, 25, and 32 °C.