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# Yields, Photosynthetic Efficiencies, and Proximate Chemical Composition of Dense Cultures of Marine Microalgae

## A Subcontract Report

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## PREFACE

As supplies of nonrenewable fuels (oil, coal, gas, uranium) become depleted, it will be desirable to utilize renewable energy sources. Such sources include the microalgae. These plants capture solar energy through photosynthesis and are generally much more efficient in such utilization than higher plants.

This report describes laboratory research on cultures of marine microalgae with regard to yields, photosynthetic efficiencies, and cellular proximate composition, especially lipid content. The latter components are important because they are high-energy compounds that might be converted to petroleum substitutes. Marine microalgae were investigated because they had not received the attention for mass culture applications that freshwater algae had, despite the fact that seawater and its salts are an abundant resource for microalgal growth.

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## SUMMARY

The yields, photosynthetic efficiencies, and proximate composition of several miroalgae were compared in dense cultures grown at light intensities up to 70% sunlight. Yields ranged from 3.4 to 21.7 g dry weight $m^{-2}$ $day^{-1}$. The highest yield was obtained with Phaeodactylum; the lowest occurred in Botryococcus cultures. The same two species had the highest and lowest efficiencies of utilization of photosynthetically active radiation: 12.2% and 1.1%, respectively. In nitrogen-sufficient cells of all but one species, most of the dry weight consisted of protein: up to 64% in Dunaliella and Tetraselmis. Lipid content of all species ranged from 20% to 29% in healthy cells, and carbohydrate content ranged from 11% to 23%. Lipid content increased somewhat in N-deficient Phaeodactylum and Isochrysis cells, but decreased in deficient Monallanthus cells. Because the overall dry weight yield was reduced by deficiency, lipid yields did not increase. However, since the carbohydrate content increased to about 65% in N-deficient Dunaliella and Tetraselmis cells, the yield of this component was increased, even though the dry weight yield decreased. In Phaeodactylum the optimum light intensity was about 40% of full sunlight in La Jolla. Yields were reduced below this at higher light intensities, probably because of photoinhibition. Most experiments with this alga included a $CuSO_4$ filter to decrease infrared irradiance. When this filter was removed, the yield increased because more red light in the photosynthetically active spectral range was included. These results should prove useful to workers attempting to maximize yields and efficiencies, but additional studies are needed to investigate the effects of $CO_2$ supply, culture thickness, cell density, and turbulence in the proper management of mass cultures.

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# SECTION 1.0

# INTRODUCTION

## 1.1 CONCEPT

As supplies of fossil fuels become scarce, we are turning our attention to other surces of energy. Various sources of biomass have been suggested as renewable resources that photosynthetically capture solar energy. Among these are the microalgae (phytoplankton), which have been shown to be photosynthetically more efficient than land plants. This comparison was originally made by Wassinck et al. (1) and Shifrin (2). The latter data show the microalgae are generally 2-5 times more efficient than higher plants. Most of the work on algal yields and efficiencies was done in the 1950's and 1960's with freshwater algae, notably Chlorella.

Marine microalgae are a promising source of biomass because seawater is an abundant resource and contains ample supplies of some of the major nutrients, such as K, Mg, Ca, S, and, of course, water, required by algae. However, there has been little work on growing marine microalgae at maximum densities and on their photosynthetic efficiencies, although some studies have been made of their chemical characteristics.

Eventually it may be possible and desirable to set up outdoor culture systems (ponds) in which microalge are grown using natural sunlight and harvested for the energy content of their biomass. In addition, protein and perhaps other useful cellular components, would be by-products of a large-scale culturing endeavor.

## 1.2 PREVIOUS WORK - MICROALGAL MASS CULTURES, YIELDS, AND EFFICIENCIES

Much work was done in the 1950's and 1960's on the mass culture of Chlorella, a freshwater green alga. This work resulted in a monograph published by the Carnegie Institution (3) and was also reviewed by Tamiya (4). Mass culture investigations have continued sporadically since then, especially in Germany, Israel, Thailand, Mexico, Peru, and India (5). Outdoor mass culturing was the subject of a recent symposium held in Israel (6).

For algae and land plants, yields are typically reported as dry weight $m^{-2}$ $day^{-1}$. These values can be converted to tons $acre^{-1}$ $year^{-1}$ by multiplying by 1.4 or to tons $ha^{-1}$ $year^{-1}$ by multiplying by 3.7 (2). Efficiencies are generally reported as the percentage of light energy supplied in the photosynthetically active region (PAR) of the spectrum (400-700 nm) that is fixed as cellular energy (calories).

According to the review by Tamiya (4), dense Chlorella cultures grown under laboratory conditions can yield as much as 43 g $m^{-2}$ $day^{-1}$ with efficiencies of up to 20%. Recently Myers (7) has summarized his elegant experiments on the maximum efficiency of laboratory-grown Chlorella cultures. HIs value was 18% for nitrate-grown cells, which compares favorably with the 20% value very carefully determined earlier by Kok (8).

In Tokyo, bottle cultures of Chlorella incubated outdoors in natural light gave yields of 4-6 g $m^{-2}$ $day^{-1}$ (3%-5% efficiency) in the winter and 16 g $m^{-2}$ $day^{-1}$ (12% efficiency) during the most favorable months of October and November (9). In outdoor cultures grown with sewage as a nutrient source, Oswald and Goluke (10) reported an average efficiency of 6.2% (range 2%-10%). Shelef et al. (11) report an average yield of sewage-grown freshwater algae of 20 g $m^{-2}$ $day^{-1}$ in outdoor ponds (average efficiency 5.3%).

Goldman (12-14) has reviewed the history of outdoor mass cultures in terms of yields and efficiencies. He notes that yields of 30-40 g $m^{-2}$ $day^{-1}$ have been regularly achieved, although the average yield over long periods was less, and that 30-40 g $m^{-2}$ $day^{-1}$ is close to the maximum to be expected on the basis of the quantum efficiency of photosynthesis. He notes that the maximum theoretical efficiency is about 20%, a value achieved by Myers (7) and Kok (8). The highest yield value reported was 60 g $m^{-2}$ $day^{-1}$, but this included nonalgal solids from wastewater (15).

More recent values for yields are reported in the Israel symposium volume edited by Shelef and Soeder (6). The values reported by any of the authors in that volume do not exceed 30 g $m^{-2}$ $day^{-1}$ and average around 20 g $m^{-2}$ $day^{-1}$.

Much higher values in an outdoor Chlorella culture were recently reported by Mauldin et al. (16). Their average value was 71 g $m^{-2}$ $day^{-1}$. They only report data from four determinations made in December 1980 in New Mexico. The pH in the culture was very closely controlled by a computerized system and the culture was aerated with 100% carbon dioxide. These authors attribute the high yields to saturation of the ribulose diphosphate carboxylase system with carbon dioxide and inhibition of the photorespiration system by removal of oxygen from the culture. Their data are sparse and the work should be repeated by others to see if increased carbon dioxide supplies and oxygen removal will increase yields.

The above discussion concerns papers on freshwater microalgae; less work has been done on growing dense cultures of marine microalgae and measuring their yields and efficiencies. Ansell et al. (17) cultured the marine diatom, Phaeodactylum, in outdoor tanks and reported yields of up to 8 g $m^{-2}$ $day^{-1}$ with efficiencies of about 4%. Raymond (18) has also grown outdoor cultures of this alga in shallow troughs (raceways) in Hawaii. He reports a yield of 41 g $m^{-2}$ $day^{-1}$ and an efficiency of 13%. Goldman et al. (19) and D'Elia et al. (20) give yields of 10-25 g $m^{-2}$ $day^{-1}$ for marine diatoms in outdoor tanks.

## 1.3 PREVIOUS WORK - PROXIMATE CHEMICAL COMPOSITION OF MICROALGAE

The proximate chemical composition of algae can be expressed by values for total protein (often calculated as 6.25 X %N), total carbohydrate, total lipid, and ash as percentages of the dry weight. Such values are discussed in the following paragraphs with special emphasis on the effects of nitrogen deficiency on the composition. Compositions of both freshwater and marine microalgae are included.

Spoehr and Milner (21) were the first to study the chemical composition of Chlorella as a function of environmental conditions. They showed that extremely N-deficient cells (illuminated cultures that had stopped growing for two weeks) contained about 86% lipid as compared to a value of 4.5% in normal cells. Ketchum and Redfield (22) made similar determinations for Chlorella grown in mass cultures in the laboratory. Normal cells contained 48% protein, 27% carbohydrate and 25% lipid; N-deficient cells contained 16% protein, 43% carbohydrate and 40% lipid on an ash-free dry weight basis. Milner (23) generalized that for a number of algae, protein ranged from 8.7% to 88.2%, carbohydrate from 5.7% to 69%, and lipid from a trace (in seaweeds) to 85.6% (N-deficient Chlorella). Fogg (24) and Healey (25) among others have reviewed the N nutrition of algae. They distinguish two phases of N deficiency. In the early phase (incipient deficiency) protein content is reduced and carbohydrate is accumulated. In a later phase (extreme deficiency) lipid is accumulated.

Parsons et al. (26) grew eleven species of marine microalgae in several classes and measured their chemical composition during the exponential phase of growth (healthy cells). Among the species, protein varied from 17.6% to 57% of the dry weight, carbohydrate from 4.1% to 37%, lipid from 2.9% to 18.0%, and ash from 7.6% to 57%. In Phaeodactylum there was 33% protein, 24% carbohydrate, 6.6% lipid, and 7.6% ash. These latter protein and lipid contents seem low as compared with other algae.

In contrast to the paper by Parsons et al., most studies of algal chemical composition have been done on diatoms. Fogg (27) noted that N-deficient cells of Navicula pelliculosa contained up to 70% lipid. Opute (28) showed that lipids accumulated in the diatom Nitzschia palea under N-deficient conditions, and Handa (29) found an accumulation of both carbohydrate and lipid in Skeletonema costatum under such conditions. Incipiently deficient cells of Cyclotella cryptica produced carbohydrate, while extremely deficient cells produced lipid (30). Other workers showed an accumulation of carbohydrate in N-depleted cells of several species of diatoms (31,32).

Several investigators other than Parsons et al. studied the composition of the diatom Phaeodactylum tricornutum. Lewin et al. (33) found that protein ranged from 34% to 41%, carbohydrate from 2% to 19%, lipid from 24% to 34%, and ash from 12% to 13%. Culture conditions were not given. Similar values were found by Ansell et al. (17). Holdsworth and Colbeck (34) obtained a lipid content of 34%, but Morres et al. (35) showed that most of the photosynthetically incorporated carbon (carbon-14) was in the protein fraction of this species.

There have been a few studies of the effects of N deficiency in algae other than diatoms. Richardson et al. (36) found no accumulation of lipids by Chlorella sorokiniana and Oocystis polymorpha in N-limited batch cultures. This may be attributed to differences in the degree of N deficiency (incipient vs. extreme). Sakshaug et al. (37) noted that protein and carbohydrate declined in N-limited cells of the dinoflagellate, Amphidinium carterae. In N-deficient cells of two other dinoflagellates there was an increase in carbohydrates and lipids (38). Shifrin and Chisholm (39,40) investigated the effects of N deficiency on lipid content of many microalgal species. In nearly all cases there was an increase in lipid content under N-deficient con-

ditions as compared with normal cells in the exponential phase of growth. The ratio between the lipid content of deficient cells to that of normal cells ranged from 0.4 to 3.2, and was almost always >1.5. Considerable differences were found among the species. The highest ratio was found in Chlorella vulgaris and the lowest in Biddulphia aurita.

Other recent investigations of the proximate composition of various (mostly freshwater) algae are found in the Israel symposium volume (6). Protein makes up as much as 71% of the dry weight of Spirulina, a blue-green algae which is being promoted as a food source (41).

It is not our intent to review the literature on the detailed composition of the protein, carbohydrate, and lipid fractions. However, the reader is referred to the following papers: on amino acid composition, Low (42), Parsons et al. (26), Holdsworth and Colbeck (34); on fatty acids, Holten et al. (43), Opute (44), Kates and Volcani (45); on sterols, Orcutt and Patterson (46); on sugars, Parsons et al. (26), Holdsworth and Colbeck (34), Ford and Percival (47); and on hydrocarbons, Blumer et al. (48,49), Gelpi et al. (50) and Knights et al. (51). The detailed chemical composition of diatoms has been reviewed by Darley (52) and various authors have reviewed the chemical composition of algae in general - see Lewin (53) and Stewart (54).

## 1.4 AIMS OF THE PRESENT STUDIES

The above review covers the proximate composition of microalgae and indicates that it can vary greatly with N nutrition. Few investigators have measured the yield of protein, carbohydrate, and lipid; that is, the composition times the growth rate. To maximize the yield of lipid (a high-energy fraction) it may be best to grow incipiently deficient cells which may contain a moderate amount of lipid, but still grow at a reduced but reasonable rate. Extremely deficient cells may have a high lipid content, but not be growing, in which case the overall yield of lipid would be low.

Our aims were to investigate this hypothesis in several marine microalgae and to study the yields and efficiencies of dense cultures of algae growing at high rates. Some data are presented on yields in preliminary cultures of some additional species, although it was not possible to measure efficiencies accurately in such cultures. Additional experiments on the effects of light intensity on Phaeodactylum yields and efficiencies are also described. Our work was carried out under controlled conditions in the laboratory and our basic data may be useful to others who are studying yields in outdoor systems.

# SECTION 2.0

# MATERIALS AND METHODS

## 2.1 ALGAL SPECIES

Microalgal cultures were generally obtained from our extensive culture collections of marine algae. The origins of the six species cultured are as follows: Phaeodactylum tricornutum, from the University of California Marine laboratory at Bodega Bay; Dunaliella primolecta, culture no. 81 from the Plymouth, England, culture collections; Monallanthus salina (GSB Sticho), from the Woods Hole culture collection (Dr. R. R. L. Guillard); Tetraselmis sueica, from Dr. M. Diran, Brest, France; Isochrysis sp., Tahitian strain from the Woods Hole culture collection (Dr. R. R. L. Guillard); and Botryococcus braunii, from Dr. S. Lien of SERI.

## 2.2 CULTURE MEDIA

Marine species were grown in steile filtered Scripps Pier seawater enriched with 15 mg-at $L^{-1}$ N, 1.25 mg-at $L^{-1}$ P, and Fe and other trace metals used in medium "f" (55). Ammonium was generally used as an N source, but nitrate was used in culturing Isochrysis. Botryococcus was grown in an artificial medium (modified Chu No. 10 enriched as for marine cultures).

## 2.3 PRELIMINARY CULTURES

The above algal species plus some others from our culture collection were precultured in 9-L serum bottles in a lighted water bath using continuous fluorescent illumination at about 0.056 cal $cm^{-2}$ $min^{-1}$ in the photosynthetically active spectral range (PAR) and at a temperature of 21°C. This intensity is about 10% of that of noon sunlight on a clear day at La Jolla. The cultures were aerated with 1% carbon dioxide-in-air at 2000 mL $min^{-1}$. These preliminary cultures were set up to see what densities and rough yields could be achieved for a variety of species. Efficiencies could not be estimated in these round bottles because the light received by the cultures came from several directions. For accurate estimation of efficiencies, yields, and proximate composition, the more promising species were cultured in our main culture apparatus (see Figure 2-1 and below). Growth in these bottles was estimated from daily aliquots of the cell suspensions as described below. The pH dropped as ammonium was taken up by the cells and was controlled by periodic additions of 0.1 M KOH. Preliminary cultures of the six primary species (origins described above) were used as precultures for inoculation into the main culture apparatus described below.

## 2.4 MAIN CULTURES

The primary experimental methods for studying yields, efficiencies, and proximate cellular composition are described in this section.

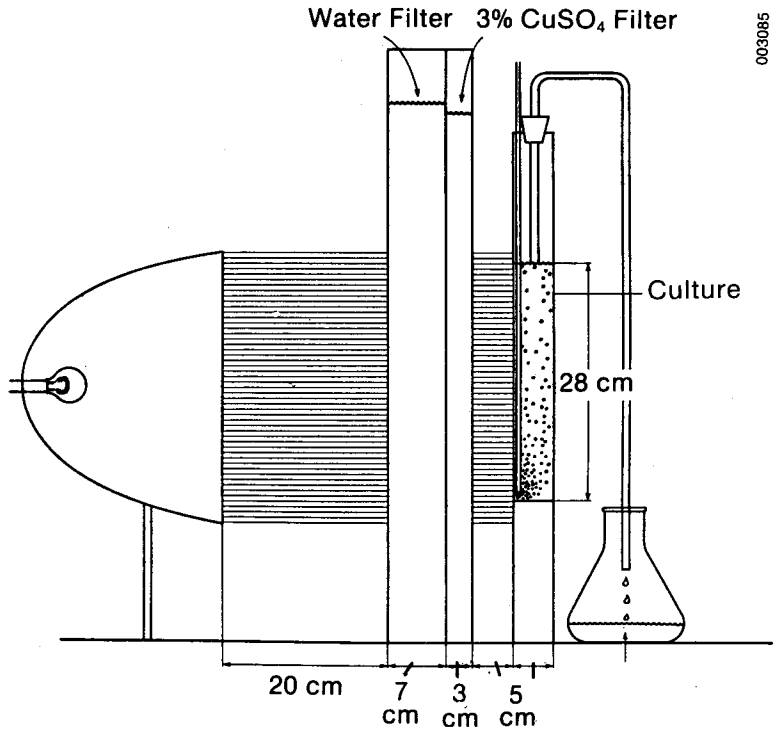


**Figure 2-1. Main Culture Apparatus**

### 2.4.1 Culture Apparatus

Figure 2-1 shows the side configuration of the main culture container, which was constructed of clear plexiglass. It was 5 cm thick, 39 cm deep and 24 cm wide. The bottom was curved for ease of mixing. The container was filled to a depth of 28 cm with 3.4 L of preculture (see above) after sterilization of the container with 70% ethanol followed by a rinse with sterile deionized water. Two aeration (1% carbon dioxide-in-air at 2000 mL $min^{-1}$) tubes reached the bottom of the culture and aeration mixed the culture vigorously. The gas mixture was washed by bubbling through sterile deionized water. An overflow and pressure-release tube extended to the culture surface and regulated the culture volume at 3.4 L. A sampling tube extended into the center of the culture. Sampling and harvesting were carried out by clamping off the overflow tube so that air pressure forced the sample out into a collection container. Another tube did not reach into the culture and was for addition of medium via peristaltic pumping from a 20-L glass reservoir. Medium was continuously mixed by aeration. The culture and medium were in contact only with plexiglass plastic, Pyrex glass, and silicone rubber. The culture could be operated as a continuous culture by continuous pumping at given flow rates, as a semi-continuous culture by withdrawing a given volume and pumping rapidly up to the 3.4 liter mark, or as a batch culture by eliminating dilution. While temperature-controlled water was circulated through a cooling jacket surrounding the sides of the culture container, this was not adequate to control culture temperature; this was controlled by placing the entire apparatus, including the light source, in a cold room. Culture temperature in most experiments was 22 $\pm 1^{o}C$, but Isochrysis was grown at 25 $\pm 1^{o}C$. We did not vary temperature diurnally.

### 2.4.2 Light Source

The light source (see Figure 2-1) was a 200-watt tungsten-halide lamp (3200 K) mounted in a 50-cm-diameter parabolic reflector such as is generally used for stage lighting. The lamp was operated diurnally (12:12 h L/D cycle). When the lamp was turned on, voltage was applied gradually over a one minute period and was controlled by an automatic dimmer connected to a timing circuit. This prolonged lamp life beyond the rated 500 h to >1500 h so that one bulb was used throughout each experiment. To remove infrared radiation, the light was filtered through a 7-cm thickness of flowing tapwater and, in some experiments, through a 3-cm thickness of 3% copper sulfate solution. Figure 2-2 shows spectral distributions (350-800 mm) of light energy (three intensities) with and without the copper sulfate filter. The latter removed red and far-red irradiance very effectively. These spectral distributions were measured with an Optronics 741-V spectroradiometer. Light intensity was not uniform over the whole culture surface. For example, in one set of measurements it varied from 0.095 to 0.247 cal $cm^{-2}$ $min^{-1}$ in the PAR as measured at 143 points over the culture surface area (705.3 $cm^2$) to obtain values for total calories received by the culture per 12-h light period. Depending on the experiment, the daily PAR supplied to these cultures ranged from 40% to 69% of the maximum total daily PAR measured by Strickland (56) at La Jolla from April to September 1967.

### 2.4.3 Sampling, Harvesting, and Growth Measurements

Daily sampling for routine growth measurements was done by withdrawing <100 mL of cell suspension. Periodically larger amounts (300-700 mL) were withdrawn

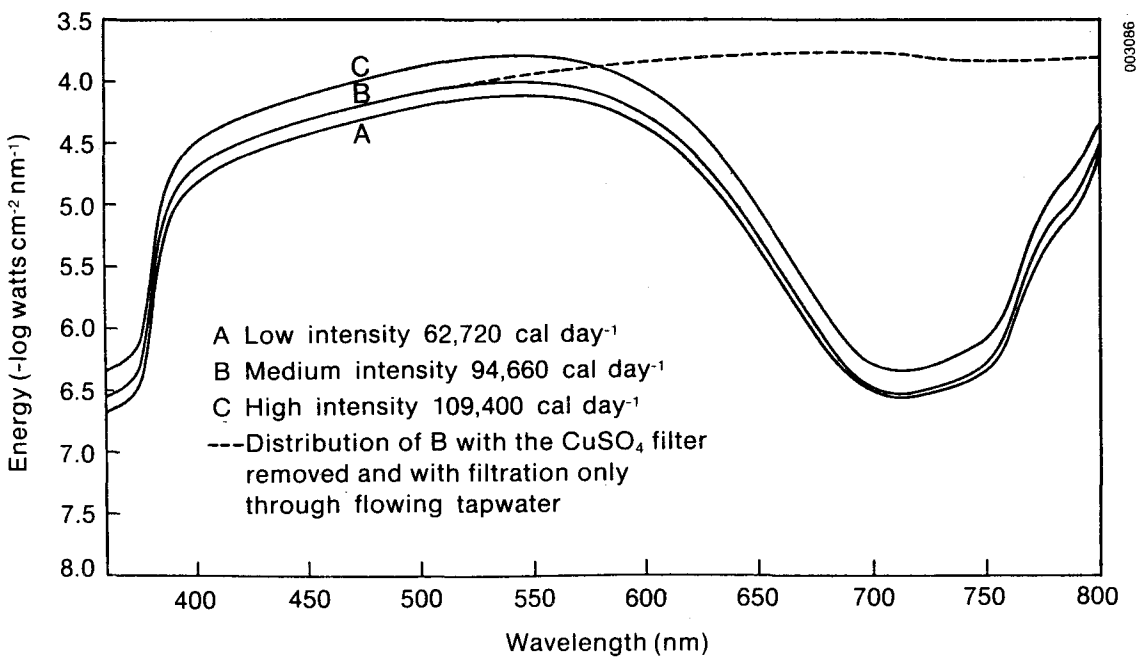


**Figure 2-2. Spectral Distribution of Filtered 3200 K Light Supplied to Main Culture**

as harvests for proximate and caloric analyses. Portions of each harvest were centrifuged and the cells were washed once with either deionized water or isotonic ammonium formate. They were then dried at 60°C. Other portions of the harvest were filtered on glass-fiber filters for proximate analysis and dry weight determinations. Dry weight samples were dried at 60°C and other samples were frozen for analysis.

Growth was measured by cell numbers, optical density, in vivo fluorescence and dry weight. In most cases cells were counted in diluted (filtered seawater) suspensions with a Model B Coulter Counter. Monallanthus suspensions were counted microscopically and Botryococcus cell numbers were not determined because the cells occurred in colonies. Optical density of diluted suspensions was measured at 750 nm in a 1-cm spectrophotometer cell. For dry weights, filters were weighed on a Cahn micro-balance. The weights were corrected for absorbed salts by weighing blank filters through which filtered seawater had been run. In vivo chlorophyll fluorescence was measured on diluted cell suspensions with a Turner Designs fluorometer. Measurements of optical density within the main culture were also taken by measuring light (I) (Li-Cor Sensor) transmitted through the 5-cm culture thickness and light incident (Io) to the culture; log I/Io (optical density) correlated very well with dry weight.

This latter finding has allowed us to begin setting up a microcomputer plus light sensors to measure growth at half-hour intervals and to display and plot daily growth curves as required.

### 2.4.4 Analytical Methods

Total cellular carbon and nitrogen analyses were performed with a Hewlett-Packard Model 185 CHN analyzer (57). Cellular protein was calculated from the %N value X 6.25. Cellular carbohydrate was analyzed by the phenol-sulfuric acid spectrophotometric method of Myklestad and Haug (32). To ensure that polysaccharide was hydrolyzed completely by the 80% sulfuric acid, the samples were heated for one hour at 100°C and then allowed to stand at room temperature for 24 hours. Lipids were extracted by a monophasic mixture of 1:2:0.8 of chloroform, methanol, and water (58). The 9-mL extracts were then incubated with 40 μL of concentrated hydrochloric acid (59) at 50°C for a few hours (60) and at room temperature overnight. Chloroform and water were then added to change the ratio to 2:2:1.8. The system then became biphasic and formed a chloroform phase containing the lipids and a water-methanol phase containing the polar molecules (58). Aliquots of the chloroform phase were then dried at 40°C in teflon cups and weighed on a Cahn microbalance. Without the water wash, the lipid values would have been much higher. After their initial weighing, dry-weight filters were heated at 450°C overnight and reweighed to determine ash content. Ash was also determined by weighing the residue from combustion of caloric samples and by combustion of 5- to 10-mg pellets of cells at 450°C. Caloric values were determined with a Phillipson microbomb calorimeter on dried, centrifuged cells. The calorimeter was connected to a recorder and millivoltmeter and the apparatus was standardized by combusting known weights of benzoic acid. Some cell samples were filtered and frozen for pigment analyses (61), but this was not done in all experiments.

In one experiment the development of N deficiency was followed by measuring the decrease in ammonium in the cell suspension using a Lazar ammonium electrode.

## 2.5 CALCULATION OF YIELDS AND EFFICIENCIES

Yields were calculated from increases in dry weight per day in the whole 3.4-L culture. Since such increases occurred over a 705.3-$cm^2$ area, they could be extrapolated to those that might occur over a 1-$m^2$ (10,000 $cm^2$) area to give values in g dry weight $m^{-2}$ $day^{-1}$.

Increases in cellular calories in the whole culture per day were determined by multiplying dry weight increases by the measured caloric content of the cells. Efficiencies are expressed as the ratio of cellular calories produced by the whole culture per day to the integrated light supplied to the culture in calories per day. Generally the amount of light absorbed by the culture was >99% of that supplied.

# SECTION 3.0

# RESULTS

## 3.1 YIELDS IN PRELIMINARY CULTURES

Yields in the cultures are expressed as linear increases in in vivo chlorophyll fluorescence, optical density, and cell numbers. No dry weight data were taken. Table 3-1 presents comparative rate data for several species, and Table 3-2 shows the maximum values of each parameter that were achieved in these preliminary cultures.

**Table 3-1. Linear Daily Increases in Chlorophyll (in vivo fluorescence), Optical Density, and Cell Number Increases for Preliminary Batch Cultures**

| Species | Mg Chlorophyll $L^{-1}$ $day^{-1}$ | Optical Density Increase $day^{-1}$ | $10^6$ cells $mL^{-1}$ $day^{-1}$ |
|---|---|---|---|
| *Phaeodactylum tricornutum* (Thomas Strain) 34 0/00 Salinity | 5.63 | 0.58 | 4.90 |
| *P. tricornutum* (Woods Hole Strain) 34 0/00 Salinty | 3.37 | -- | 2.06 |
| *P. tricornutum* (Thomas Strain), 46.8 0/00 Salinity | 3.76 | 0.55 | 4.46 |
| *Tetraselmis sueica*, ammonium | 0.74 | 0.41 | 0.72 |
| *T. sueica*, nitrate | 0.40 | 0.27 | 0.73 |
| *Porphyridium cruentum*, ammonium | 0.31 | 0.38 | -- |
| *P. cruentum*, nitrate | 0.58 | 0.65 | -- |
| *Isochrysis* sp. (Tahitian Strain), nitrate | 1.54 | 0.75 | 1.53 |
| *Chaetoceros gracilis*, ammonium | 0.52 | 0.43 | 1.58 |
| *Dunaliella sp.*, nitrate | 0.42 | 0.41 | 2.35 |
| *D. primolecta*, nitrate | 1.9 | 0.86 | 3.28 |
| *Monallanthus salina*, ammonium | 4.61 | 0.68 | -- |
| *M. salina*, nitrate | 2.67 | 0.98 | -- |

**Table 3-2. Maximum Values of Chlorophyll (in vivo fluorescence), Optical Density, and Cell Numbers for Several Species of Marine Microalgae Attained in Preliminary Batch Culture**

| Species | Mg Chlorophyll $L^{-1}$ | Optical Density | $10^6$ cells $mL^{-1}$ $day^{-1}$ |
|---|---|---|---|
| Phaeodactylum tricornutum (Thomas Strain) 34 0/00 Salinity | 102 | 13.7 | 123 |
| P. tricornutum (Woods Hole Strain) 34 0/00 Salinty | 31.1 | -- | 21.5 |
| P. tricornutum (Thomas Strain), 46.8 0/00 Salinity | 80 | 23.9 | 145 |
| Tetraselmis sueica, ammonium | 7.7 | 5.19 | 11.1 |
| T. sueica, nitrate | 27.0 | 4.11 | -- |
| P. cruentum, nitrate | 2.7 | 4.74 | -- |
| Isochrysis, sp. (Tahitian Strain), nitrate | 10.8 | 6.28 | 30.1 |
| Chaetoceros gracilis, ammonium | 2.2 | 1.86 | 12.9 |
| C. gracilis, nitrate | 7.5 | 3.97 | 31.1 |
| Dunaliella sp., nitrate (San Diego Bay Strain) | 10.4 | 5.74 | 47.6 |
| Monallanthus salina, ammonium (G. S. B. Sticho.) | 22.8 | 7.04 | -- |
| M. salina, nitrate (G. S. B. Sticho.) | 19.7 | 8.22 | -- |

Comparing the yields and maximum values for one species with those of another can only be done in a rough manner. For instance, the fluorometer is well calibrated against extracted chlorophyll for Phaeodactylum but in vivo fluorescence values for Dunaliella primolecta did not agree well with extracted chlorophyll values. Moreover, because of differences in cell size and possibly in cellular optical scattering characteristics, cell number and optical density values are not necessarily quantitatively comparable among the species.

With regard to chlorophyll increases (Table 3-1), Phaeodactylum outgrew most other species but Monallanthus grew nearly as well with ammonium and an N source. Differences in the rates of increase of optical density were not so apparent among the species. Cell number increases of Tetraselmis, Isochrysis, and Chaetoceros were generally lower than those for Phaeodactylum or Dunaliella. Maximum values for each of the parameters (Table 3-2) were greater for Phaeodactylum than for any other species.

All of these species grew quite well in our simple media, and could be "pushed" to achieve very dense cultures in which most of the light was absorbed. The cultures were quite green or brown. Any one of these species was suitable for further study in the main culture apparatus or would be useful in outdoor cultures.

## 3.2 MAIN CULTURE EXPERIMENTS

Each experiment for each species is described as follows: 1) A narrative of the experiment is presented. This includes the manipulation of the culture, difficulties encountered, and time-series figures showing changes in dry weight, optical density, and cell numbers. 2) Yields and efficiencies during the several parts of the experiment are presented. 3) Proximate analyses (protein, carbohydrate, lipid, and ash) plus cellular caloric values are given for both healthy (N-sufficient) and N-deficient cells.

### 3.2.1 Phaeodactylum Main Culture Experiment 1

This alga was chosen because many studies had been done with it (17,18,26,33,34,35) and the alga was also being grown in an outdoor raceway in Hawaii by another SERI subcontractor, Dr. Edward Laws (62). Out studies on this alga (see also Section 3.2.2) were much more extensive than those on other algae.

#### 3.2.1.1 Narrative

This experiment was carried out for 94 days during December 1980 and January, February and March 1981. The incubation temperature was $22^{o}$-$23^{o}$C and during most of the experiment the light energy (with the copper sulfate filter) supplied to the whole culture was 94,660 cal $day^{-1}$ until days 80-84 when it was reduced to 62,714 cal $day^{-1}$ from days 84-94. Figures 3-1 thru 3-3 show time series changes in dry weight, optical density, and cell numbers for the whole experiment. The culture was operated in the batch mode (no pumping of fresh medium) for the first seven days and then fresh medium was pumped into culture continuously at various rates to attempt to stabilize the growth parameters. This was finally achieved on day 10 and the culture was operated continuously from days 10-28 with pumping of fresh nutrient-enriched medium at 0.4-0.5 volume per day. The growth parameters were reasonably stable during this period. Several harvests for proximate analyses of the cells were taken during this period. Pumping of nitrogen-deficient medium at 0.5 volume $day^{-1}$ was carried out from days 28-34. The ammonium concentration in the medium decreased and was undetectable on day 32. Growth parameters also decreased as the culture became N deficient and the cells were washed out of the vessel. The pump was turned off on day 34 and the culture was allowed to become extremely N deficient. The crop was stable during days 34-40 and extreme deficiency was achieved on day 40. Continuous pumping with N-sufficient medium was then started so that recovery could occur. However, recovery was slow and cells were washed out at a pumping rate of 0.5 volume $day^{-1}$ (to day 44). The crop grew when the pumping rate was reduced to 0.1 volume $day^{-1}$ but not as fast as with batch growth. Therefore the pump was turned off on day 46

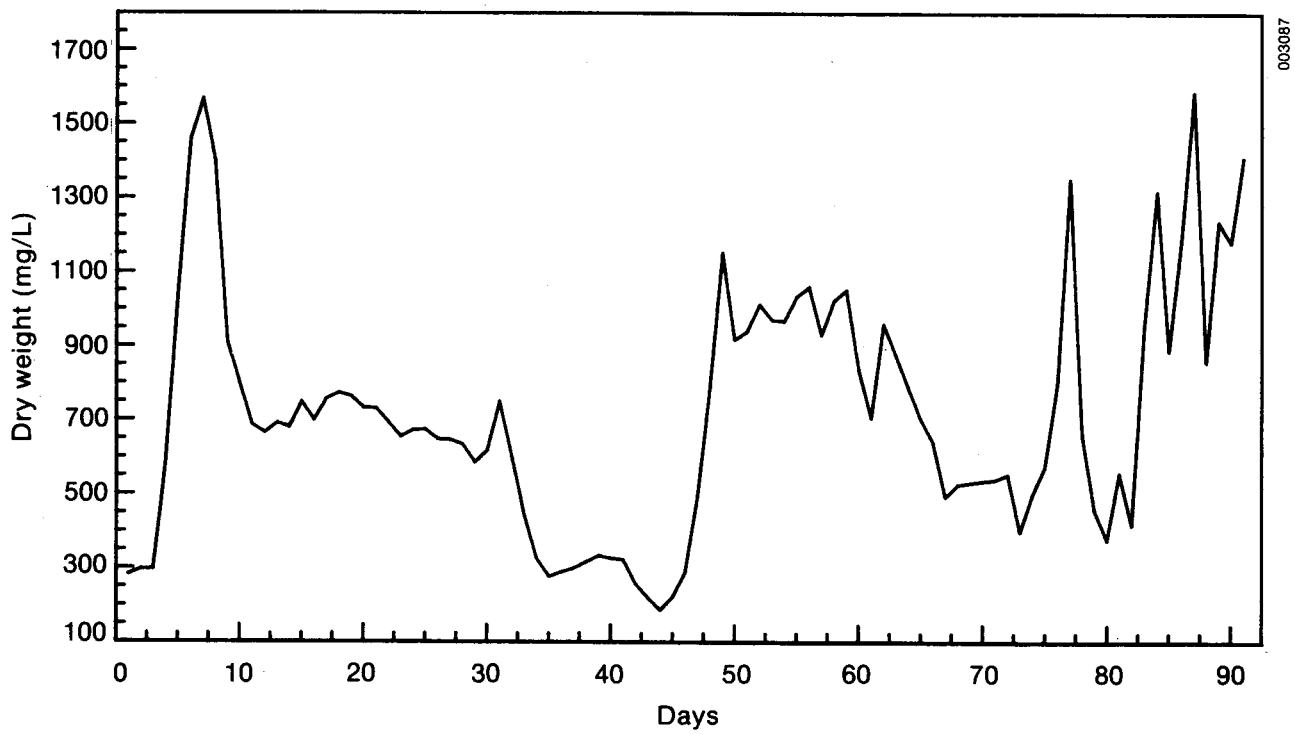


**Figure 3-1. Dry Weight Changes - Phaeodactylum Main Culture Experiment 1**

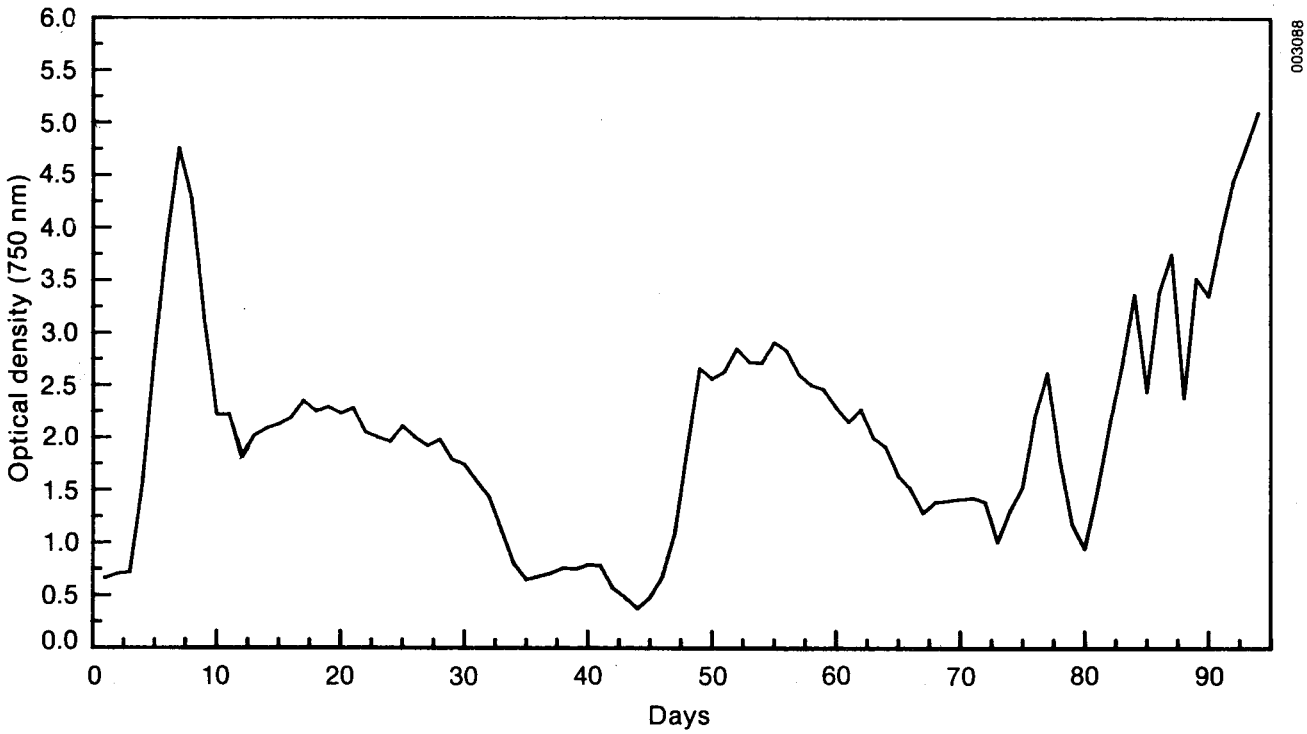


**Figure 3-2. Optical Density Changes - Phaeodactylum Main Culture Experiment 1**

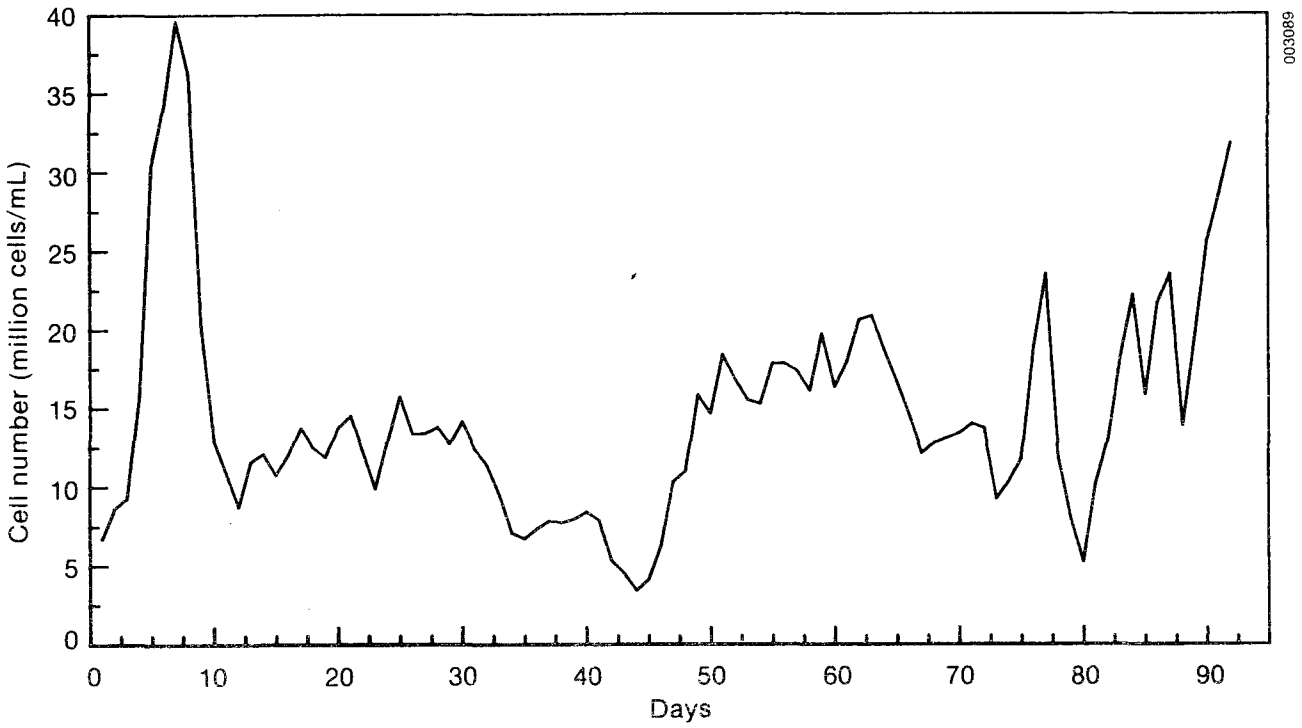


**Figure 3-3. Cell Number Changes – Phaeocadtylum Experiment 1**

and the culture recovered strongly from deficiency during this period (days 46-49) of batch growth. From days 49-59 the culture was operated continuously with pumping of nutrient-sufficient medium at a rate of 0.25 volume day$^{-1}$. Growth parameters were reasonably stable at this rate and were about 20% higher than during the time (days 28-34) that the culture pumped at 0.5 volume day$^{-1}$ with N-sufficient medium. Another cycle of N deficiency was carried out by pumping N-dificient medium at 0.25 volume day$^{-1}$ from days 59-67. At this rate the cells did not run out of N as fast as before. Crop parameters remained stable until day 63 and then decreased. The pump was turned off on day 67 to achieve extreme N deficiency (day 72). The culture was diluted with N-sufficient medium at that time and operated in the batch mode so that recovery would occur. Rapid increases in growth parameters indicated that recovery was complete on day 77. Then the culture was pumped continuously with N-sufficient medium until day 80 at a rate of 0.75 volume day$^{-1}$. This rate was too fast and growth parameters decreased greatly. Batch growth was measured at a lower light intensity (62,715 cal day$^{-1}$) from days 80-84 and at a higher light intensity (109,400 cal day$^{-1}$) from days 84-94 when the experiment was terminated.

### 3.2.1.2 Yields and Efficiencies

As can been seen from the above narrative, many different manipulations of the culture were carried out during this experiment. The results of these manipulaions on yields and efficiencies are shown in Table 3-3. These can be separated into a) effects of light intensity on batch growth; b) continuous culture at three dilution rates with N-sufficient medium; and c) continuous culture with N-deficient medium.

**Table 3-3. Yields and Efficiencies during the First *Phaeodactylum* Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 1-7 | Batch growth; 94,660 cal $day^{-1}$ (+N) | 21.2 | 8.1 |
| 80-84 | Batch growth; 62,715 cal $day^{-1}$ (+N) | 21.7 | 12.2 |
| 84-94 | Batch growth; 109,400 cal $day^{-1}$ (+N) | 17.1 | 5.4 |
| 46-49 | Batch growth; 94,660 cal $day^{-1}$ (+N, recovery) | 13.9 | 5.3 |
| 72-77 | Batch growth; 94,660 cal $day^{-1}$ (+N, recovery) | 18.8 | 7.1 |
| 10-28 | Continuous culture; 94,660 cal $day^{-1}$ (+N 0.5 volume $day^{-1}$) | 16.2 | 6.2 |
| 49-59 | Continuous culture; 94,660 cal $day^{-1}$ (+N, 0.25 volume $day^{-1}$) | 11.6 | 4.2 |
| 28-34 | Continuous culture; 94,660 cal $day^{-1}$ (-N, 0.5 volume $day^{-1}$) | 7.7-10.4 | 4.1-4.9 |
| 59-67 | Continuous culture; 94,660 cal $day^{-1}$ (-N, 0.25 volume $day^{-1}$) | 7.1 | 3.2 |

Highest yields were obtained at light intensities of 62,715 and 94,660 cal $day^{-1}$. These correspond to about 40% and 60% of the maximum sunlight recorded at La Jolla for the summer of 1967 by Strickland (56). The yields were about the same at the two light intensities, but the efficiency was greater at the lower light intensity; that is, it took less light to produce an equivalent amount of cell calories. Yield and efficiency were reduced at a higher light intensity (109,400 cal $day^{-1}$ - equivalent to 70% of La Jolla sunlight). These yields and efficiencies were measured for batch growth at dry weight levels ranging from 300-1900 mg $L^{-1}$ and can be compared with those for different crop levels at three light intensities in the second *Phaeodactylum* experiment (see Section 3.2.2).

Yields and efficiencies during batch growth recovery from N deficiency (days 46-49 and days 72-77) were somewhat less than those found at the same moderate light intensity for normal batch growth (days 1-7). Yields and efficiencies during continuous culture at either 0.5 or 0.25 volume $day^{-1}$ (days 10-28 and 49-59) were also less than those achieved during normal batch growth. Continuous culture at 0.75 volume $day^{-1}$ (days 77-80) resulted in decreasing crop levels since cell growth could not keep up with this dilution rate.

Continuous pumping of N-deficient medium at either 0.5 or 0.25 volume $day^{-1}$ (days 28-34 and 59-67) resulted in greatly reduced overall yields and efficiencies as protein content was reduced.

### 3.2.1.3 Proximate Cellular Composition

The proximate composition of the cells at the three light intensities is shown in Table 3-4. At the lowest light intensity the lipid content increased slightly but otherwise the cellular composition was nearly the same at all intensities. The caloric content did not change significantly with light intensity.

During the first induction of N deficiency (days 28-34; pumping of N-deficient medium at 0.5 volume $day^{-1}$), the ammonium level in the culture decreased from 11.0 mg at N $L^{-1}$ to an undetectable amount (<0.0001 mg at N $L^{-1}$) on the fourth day of deficiency. Changes in the percentages of cellular protein, carbohydrate, lipid, and ash as deficiency developed are shown in Figure 3-4. Protein decreased greatly after the $NH_4^+$ was used up (day four of deficiency). Cellular carbohydrate increased on day 6 of deficiency and then decreased. Cellular lipid increased on days 6 and 7 of deficiency. However, extreme deficiency (day 12 of deficiency) did not result in a maintenance of

**Table 3-4. Proximate Mean Cellular Composition of *Phaeodactylum* at Three Light Intensities under N-Sufficient Conditions - *Phaeodactylum* Main Culture Experiment 1**

| Culture Conditions | % Dry Weight | | | | Cal $mg^{-1}$ dry wt | Ash-Free cal $mg^{-1}$ |
|---|---|---|---|---|---|---|
| | % Protein | % Carbohydrate | % Lipid | % Ash | | |
| 62,715 cal $day^{-1}$ light intensity | 59.7 | 13.0 | 25.9 | 16.9 | 4.97 | 5.81 |
| 94,660 cal $day^{-1}$ light intensity | 57.1 | 10.5 | 19.4 | 10.6 | 5.09 | 5.63 |
| 109,400 cal $day^{-1}$ light intensity | 58.8 | 11.3 | 18.8 | 15.5 | 4.93 | 5.69 |

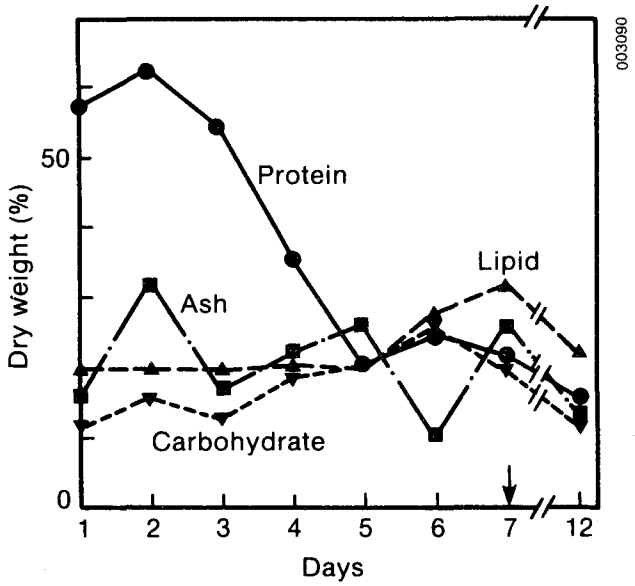


**Figure 3-4. Changes in Proximate Composition, Nitrogen Deficiency - *Phaeodactylum* Experiment 1**

the high lipid content, but the caloric content of the crop remained at higher levels (5.74 cal $mg^{-1}$) than in N-sufficient cells (4.81 cal $mg^{-1}$). However, this caloric increase was not as dramatic as the decrease in yield and in protein content.

The mean yield of lipid in N-sufficient cells (continuous culture; 94,660 cal $day^{-1}$) was 2.95 g $m^{-2}$ $day^{-1}$; that in N-deficient cells (days 5-7 of deficiency) was 2.69 g $m^{-2}$ $day^{-1}$. Thus, even though the lipid content increased with N deficiency (Figure 3-4), the yield of lipid was about the same as in N-sufficient cells because the overall cell yield was decreased. However, mean protein yield decreased greatly; it was 7.59 g $m^{-2}$ $day^{-1}$ in N-sufficient cells and 2.34 g $m^{-2}$ $day^{-1}$ in deficient cells.

In healthy cells, the cytoplasm was dispersed throughout the cell while in N-deficient cells the cytoplasm was clumped in the center of the cell. Cell size and dry weight per cell did not change with deficiency. the latter was 56.6 ± 8.4 picogram $cell^{-1}$ in sufficient cells and under deficient conditions it was 50.5 ± 9.2 picograms $cell^{-1}$. In all of our microscopic observations cells were single and fusiform; we never saw triradiate cells.

Nitrogen deficiency was also induced by pumping in N-deficient medium at 0.25 volume $day^{-1}$ (days 59-67). Deficiency developed more slowly and the effects of deficiency were not as marked as before. That is, lipid increased from 19.7% of the dry weight to only 23.2% and caloric values increased from 4.9 cal $mg^{-1}$ to 5.3 cal $mg^{-1}$. Protein, of course, decreased greatly from 58.3% to 19.7%, but other constituents did not change appreciably. The proximate analysis data for each harvest day are shown in Table 3-5 for both N-sufficent and N-deficient cells. Figure 3-5 summarizes the proximate composition of N-sufficient cells at all light intensities. Protein, carbohydrate, lipid and ash made up nearly 100% of the dry weight. In contrast, in deficient cells (Figure 3-6) an unanalyzed "other" fraction made up 25% of the dry weight. This may have consisted of polar "lipid-like" compounds that were removed by water washing of the lipid extracts. Protein (fraction A in the figures) was very much reduced by deficiency. At times (days 34 and 39) during the final stages of deficiency, the caloric content (particularly the ash-free caloric level) increased greatly. This seems to reflect the general increase in lipid content on those days.

**Table 3-5. Proximate Cellular Composition of *Phaeodactylum* under N-Sufficient and N-Deficient Conditions; 94,660 cal day$^{-1}$ Light Intensity - *Phaeodactylum* Main Culture Experiment 1**

| Culture Day | Culture Conditions | % Dry Weight: % Protein | % Carbohydrate | % Lipid | % Ash | Cal mg$^{-1}$ dry wt | Ash-free cal mg$^{-1}$ |
|---|---|---|---|---|---|---|---|
| 28 | +N | 57.1 | 11.2 | 19.9 | 15.5 | 4.92 | 5.68 |
| 29 | +N | 62.1 | 15.6 | 19.9 | -- | -- | -- |
| 30 | +N | 54.1 | 12.7 | 19.5 | 16.8 | 4.39 | 5.13 |
| 49 | +N | 53.8 | 9.9 | 18.3 | 12.7 | -- | -- |
| 56 | +N | 49.6 | 9.3 | 20.4 | 12.2 | -- | -- |
| 58 | +N | 43.7 | 9.9 | 18.6 | 11.3 | 4.65 | 5.18 |
| 59 | +N | 55.0 | 6.4 | 16.4 | 14.5 | 4.93 | 5.64 |
| 60 | +N | 58.0 | 5.5 | 22.0 | 11.6 | -- | -- |
| 61 | +N | 61.8 | 14.0 | 24.9 | 7.8 | 5.17 | 5.57 |
| | Mean (+N) | 55.0±5.5 | 10.5±3.1 | 19.8±2.3 | 11.6±4.4 | 4.81±0.27 | 5.44±0.24 |
| 31 | -N | 35.8 | 18.3 | 20.3 | 22.0 | 4.25 | 5.18 |
| 32 | -N | 20.3 | 20.0 | 20.0 | 26.1 | 3.69 | 4.65 |
| 33 | -N | 24.1 | 25.8 | 27.7 | 10.0 | 5.53 | 6.08 |
| 34 | -N | 21.6 | 19.4 | 30.8 | 26.3 | 5.48 | 6.92 |
| 39 | -N* | 15.7 | 11.3 | 21.6 | 13.1 | 5.74 | 6.49 |
| 42 | -N | 33.9 | 8.6 | 23.9 | 17.9 | -- | -- |
| 62 | -N | 32.4 | 9.5 | 16.6 | 18.4 | 5.04 | 5.97 |
| 63 | -N | 27.3 | 15.3 | 19.1 | 9.9 | 5.58 | 6.13 |
| 64 | -N | 22.3 | 13.4 | 23.2 | 15.3 | -- | -- |
| 65 | -N | 22.0 | 15.2 | 24.2 | 8.8 | 5.17 | 5.62 |
| 66 | -N | 19.7 | 14.3 | 21.1 | 12.3 | 5.19 | 5.83 |
| 72 | -N* | 13.6 | 10.6 | 24.6 | 10.8 | 5.33 | 5.91 |
| 77 | -N | 36.1 | 14.6 | 15.8 | 15.2 | 4.59 | 5.29 |
| | Mean (-N) | 25.0±7.2 | 15.1±4.6 | 22.2±4.0 | 15.8±5.7 | 5.05±0.6 | 5.82±0.6 |

*Extreme N deficiency.

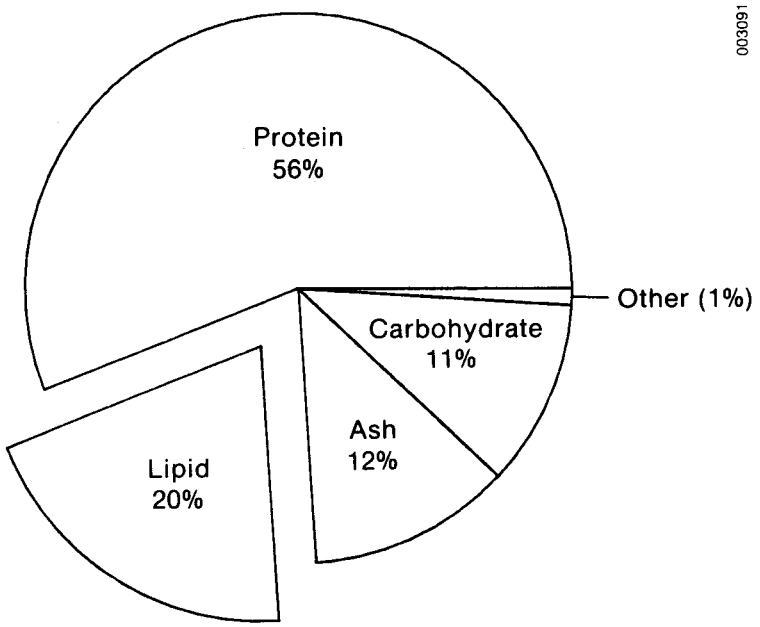


**Figure 3-5. Proximate Composition of Nitrogen-Sufficient Cells - *Phaeodactylum* Experiment 1**

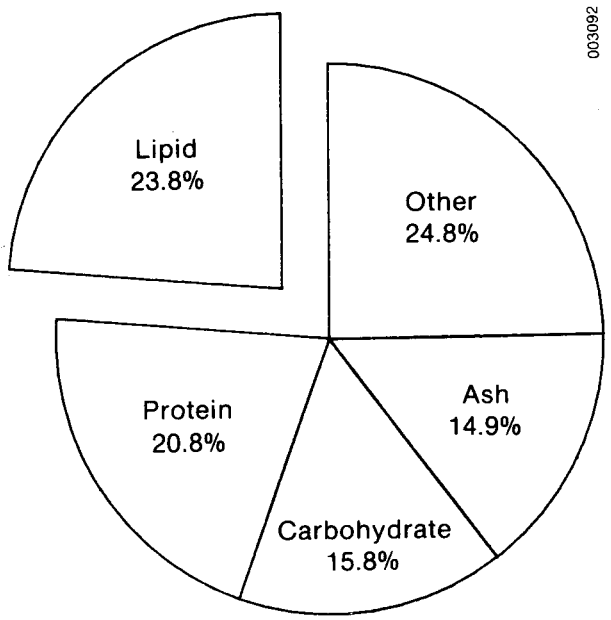


**Figure 3-6. Proximate Composition of Nitrogen-Deficient Cells - *Phaeodactylum* Experiment 1**

Pigment analyses of frozen cell samples were completed for this experiment. These parameters are given in Table 3-6. The chlorophyll content (1% of dry weight) decreased greatly during N deficiency as did the chlorophyll/carotenoid ratio. The carbon/chlorophyll ratio increased during deficiency. These results are further evidence of the shunting of metabolism from protein to lipid production.

### 3.2.2 Phaeodactylum Main Culture Experiment 2

An additional experiment with this alga was performed to study further the effects of light intensity at a higher biomass level in the culture. Near the end of the experiment the $CuSO_4$ light filter was removed; also yields and efficiencies during semicontinuous culturing were measured. Nitrogen deficiency was not investigated.

#### 3.2.2.1 Narrative

This experiment was carried out for 36 days during July and August 1981. Figures 3-7 - 3-9 show changes in dry weight, optical density, and cell numbers for the whole experiment. For the first seven days the culture was operated in the batch mode at a light intensity of 65,580 cal $day^{-1}$. The dry weight in the culture increased from 822 to 2512 mg $L^{-1}$. Then the culture was harvested and diluted with fresh N-sufficient medium. Batch growth was then carried out from days 8-13 at a higher light intensity of 82,910 cal $day^{-1}$. The dry weight increased from 1510 to 2807 mg $L^{-1}$. The culture was again harvested and diluted and was operated from days 14-18 at an even higher light intensity - 105,230 cal $day^{-1}$. The dry weight increased from 1450 to 2516 mg $L^{-1}$. The effects of a low light intensity - 50,960 cal $day^{-1}$ - were then investigated from days 20-24. The dry weight during this batch growth period increased from 1377 to 2004 mg $L^{-1}$. From days 24-28 the culture was operated in the batch mode at a moderate light intensity, 77,160 cal $day^{-1}$, with the $CuSO_4$ filter removed to see if additional red light would affect

**Table 3-6. Mean Pigment Parameters during Phaeodactylum Experiment 1**

| | Nitrogen Status | | |
|---|---|---|---|
| | Sufficiency | Incipient Deficiency | Extreme Deficiency |
| Chlorophyll % of dry weight | 1.70 ± 0.32 | 0.59 ± 0.15 | 0.23 ± 0.04 |
| Carbon/chlorophyll ratio | 2.81 ± 5.5 | 78.8 ± 31.1 | 225.4 ± 69.0 |
| Chlorophyll/ carotenoid ratio | 1.20 ± 0.22 | 0.87 ± 0.09 | 0.77 ± 0.10 |

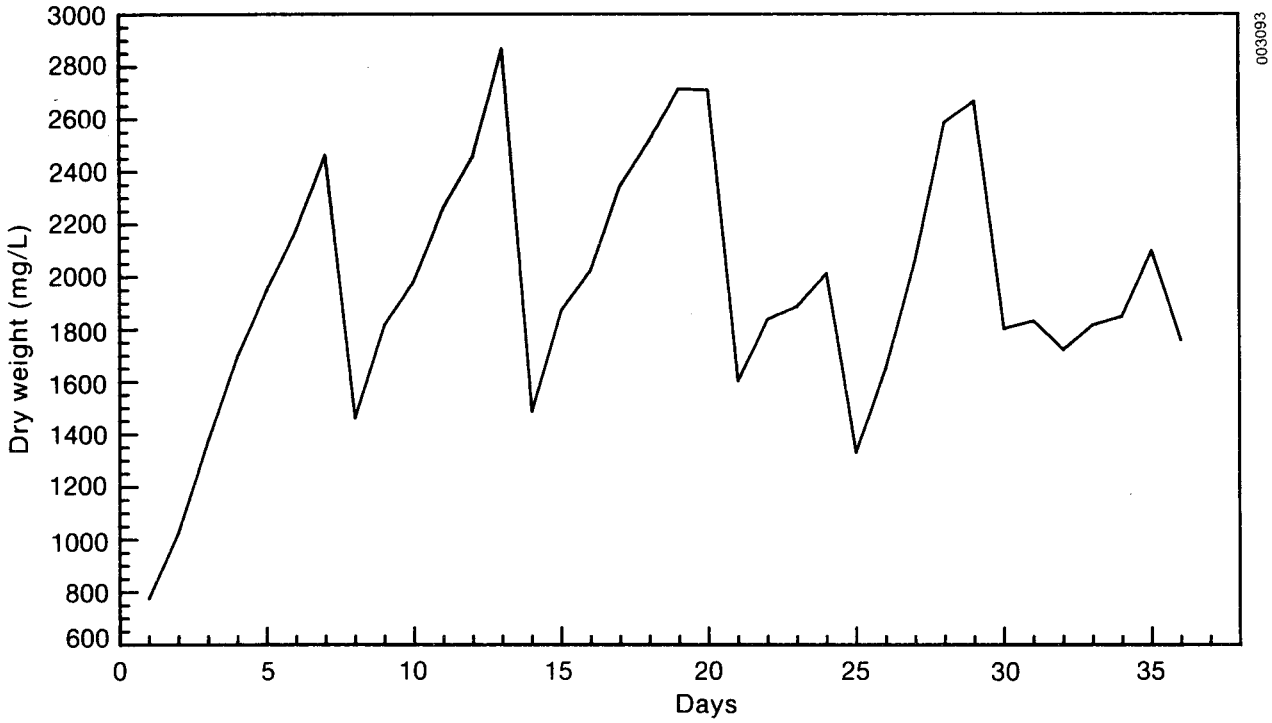


**Figure 3-7. Dry Weight Changes - Phaeodactylum Experiment 2**

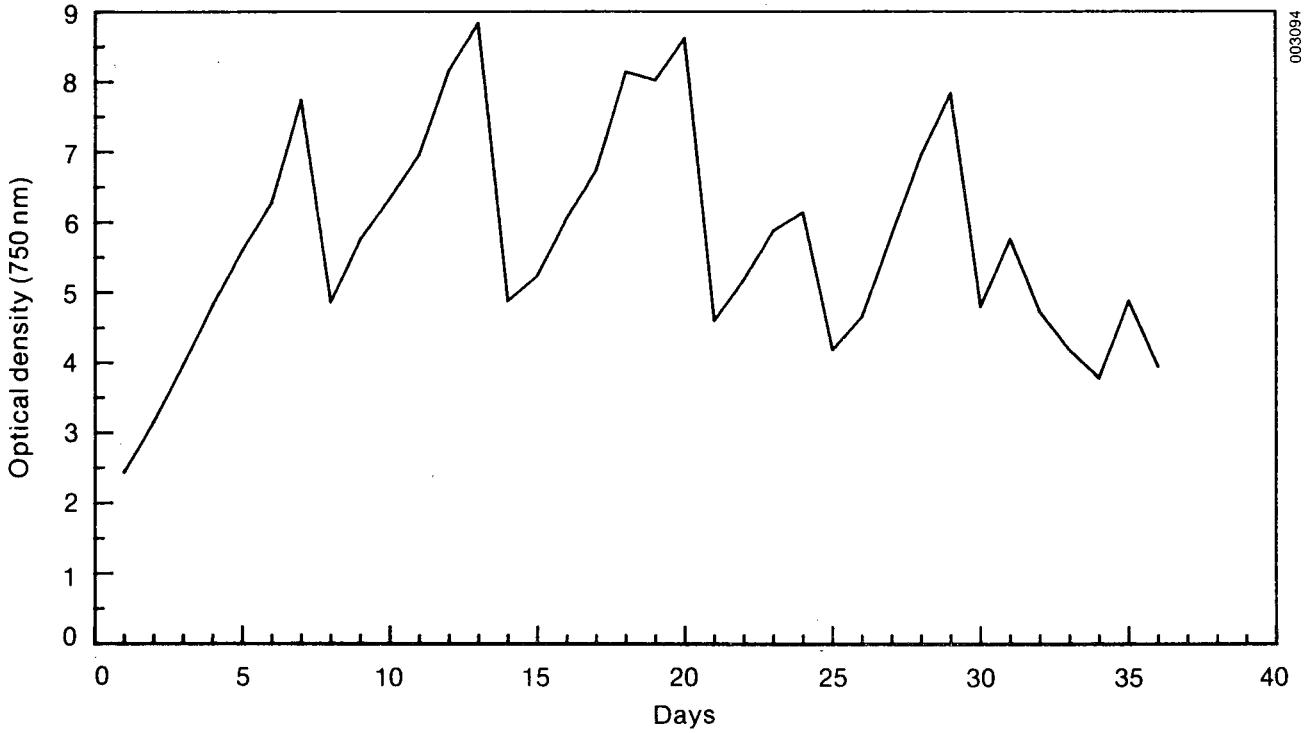


**Figure 3-8. Optical Density Changes - Phaeodactylum Experiment 2**

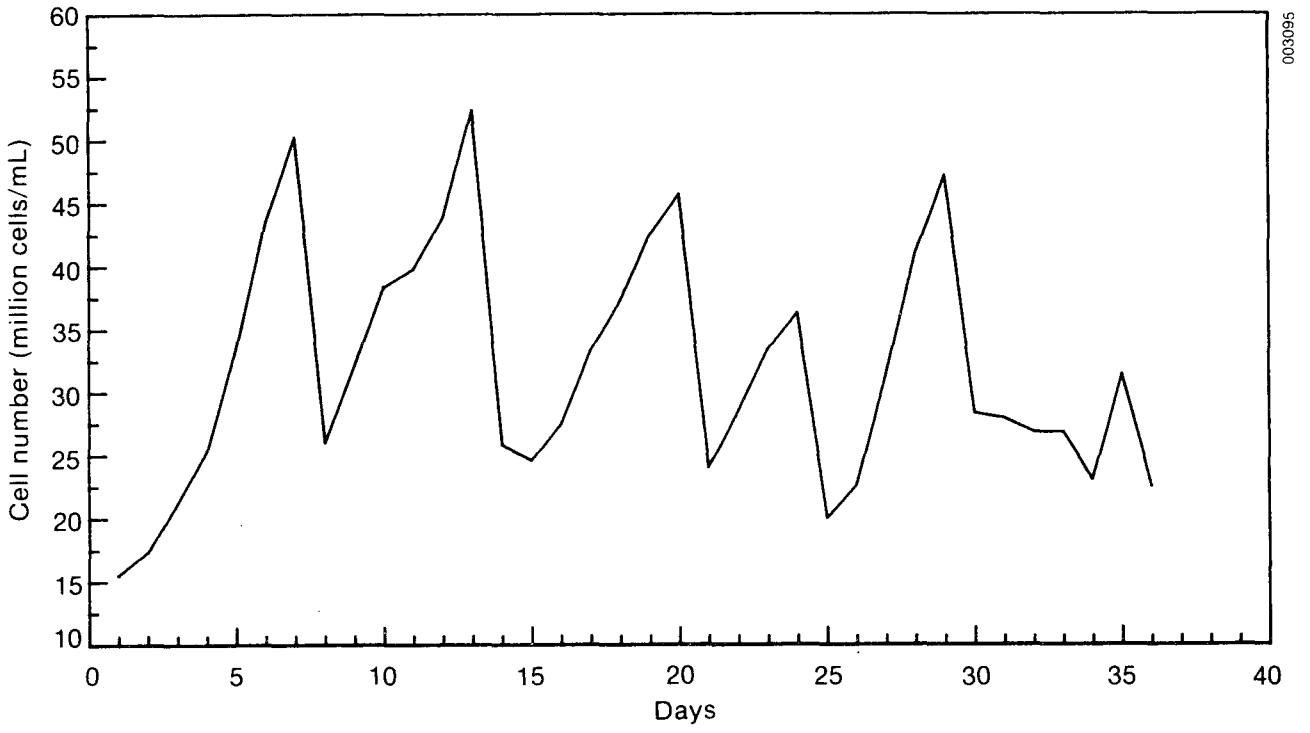


**Figure 3-9. Cell Number Changes - Phaeodactylum Experiment 2**

growth. During this period the dry weight increased from 1401 to 2572 mg $L^{-1}$. From the growth curve for this period we calculated that about 20%-25% of the culture could be removed each day to operate it in a semi-continuous mode and still maintain a steady crop. From days 30-34 the culture was thus operated and the crop before each day's harvest remained steady at from 1803 to 1850 mg dry weight $L^{-1}$. The light intensity during this period was 77,160 cal $day^{-1}$ with the $CuSO_4$ filter removed. Contamination of the culture with an unidentified monad was observed thereafter and the experiment was terminated on day 36.

### 3.2.2.2 Yields and Efficiencies

The results of the above manipulations of the culture are shown in Table 3-7. With the $CuSO_4$ filter, the highest yield and efficiency were obtained at a light intensity of about 66,000 cal $day^{-1}$. Yields and efficiencies were reduced at light levels above and below this. However, these highest yields and efficiency values were only about 60% of those obtained at 62,700 cal $day^{-1}$ in the previous experiment. This is probably due to the fact that crop levels in the previous experiment ranged from 300-1900 mg dry weight $L^{-1}$ while in this experiment they ranged from about 800-2500 mg $L^{-1}$. Thus the mean light intensity seen by each cell may have been less in the second experiment. However, increasing the light intensity in the second experiment did not increase yields and efficiencies.

When the $CuSO_4$ filter was removed, yields and efficiencies were 30%-50% higher than those obtained at nearly an equivalent light intensity with the filter in place. The yield and efficiency obtained during semicontinuous culture

**Table 3-7. Yields and Efficiencies during the Second *Phaeodactylum* Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 1-7 | Batch growth; 65,580 cal $day^{-1}$ | 13.7 | 6.9 |
| 8-13 | Batch growth; 82,910 cal $day^{-1}$ | 12.8 | 5.8 |
| 14-18 | Batch growth 105,230 cal $day^{-1}$ | 11.7 | 3.9 |
| 20-24 | Batch growth; 50,960 cal $day^{-1}$ | 8.2 | 5.6 |
| 24-28 | Batch growth; 77,160 cal $day^{-1}$ $CuSO_4$ filter removed | 20.5 | 9.0 |
| 30-34 | Semicontinuous culture; 20%-25% of culture volume harvested $day^{-1}$; 77,160 cal $day^{-1}$; $CuSO_4$ filter removed | 19.7 | 8.8 |

(days 30-34) were about equal to that obtained during batch culture (days 24-28).

### 3.2.2.3 Proximate Cellular Composition

The proximate composition of cells grown at the different light intensities and with the $CuSO_4$ filter did not change. Protein ranged from 41% to 54% of the dry weight; carbohydrate from 4.4% to 5.8%; lipid from 17.9% to 23.2%; and ash from 8.0% to 11.5%. Caloric values ranged from 4.70 to 5.33 cal $mg^{-1}$ dry weight (5.11 to 5.82 cal $mg^{-1}$ ash-free dry weight) and did not change with light intensity. These compositions were similar to those found in the previous *Phaeodactylum* experiment in N-sufficient cells (see Figure 3-5) except that carbohydrate levels were lower.

### 3.2.3 Dunaliella Main Culture Experiment

This alga was investigated because it is easy to grow and a considerable amount of culture work has been done with it, particularly in studies of glycerol production (63). Furthermore, this alga has been grown earlier in mass cultures in our laboratory, and we are very familiar with its cultural requirements (64).

### 3.2.3.1 Narrative

The duration of this experiment was 49 days during April and May 1981. Figures 3-10 - 3-12 show changes in dry weight, optical density, and cell numbers for the whole experiment. The light intensity used was generally 94,660 cal day$^{-1}$ over the whole culture surface except that on day 46 the $CuSO_4$ filter was removed. The distance between the lamp and the culture was not changed at that time so the light intensity increased to 189,000 cal day$^{-1}$. This intensity was 120% of the maximum solar radiation at La Jolla reported by Strickland (56) and would be comparable to that received at more southern latitudes in the summer. The temperature of the culture was 20°-23°C. For the first seven days of the experiment the culture was operated in the batch mode (no pumping of fresh medium) and the dry weight increased from 459 to 1515 mg L$^{-1}$. Then the culture was harvested and diluted with fresh N-sufficient medium. The culture was then operated with continuous pumping of fresh medium at a rate of 0.33 volume day$^{-1}$ from day 8 to day 20. Crop parameters remained reasonably steady during this period: dry weight ranged from 492-842 mg L$^{-1}$; optical density from 2.76 to 3.26; and cell numbers from 11.8 to 15.8 × $10^6$ cells mL$^{-1}$. From days 20-27, N-deficient medium was pumped into the culture to develop an N-deficient crop. Crop parameters remained steady for a few days and then decreased so that by day 28 the dry weight was reduced to about 400 mg L$^{-1}$, optical density to 0.85, and cell numbers to 3.7 × $10^6$ cells mL$^{-1}$. Pumping ceased from that day to day 31 to allow the cells to become extremely N-deficient; then the culture was harvested and diluted to full volume with N-sufficient medium to allow recovery from N-deficiency. By day 36 cell parameters had increased to a dry weight of 1288 mg L$^{-1}$, optical density of 2.95, and cell numbers of 13.1 × $10^6$ cells mL$^{-1}$. Thereafter, the culture was operated semicontinuously by taking out

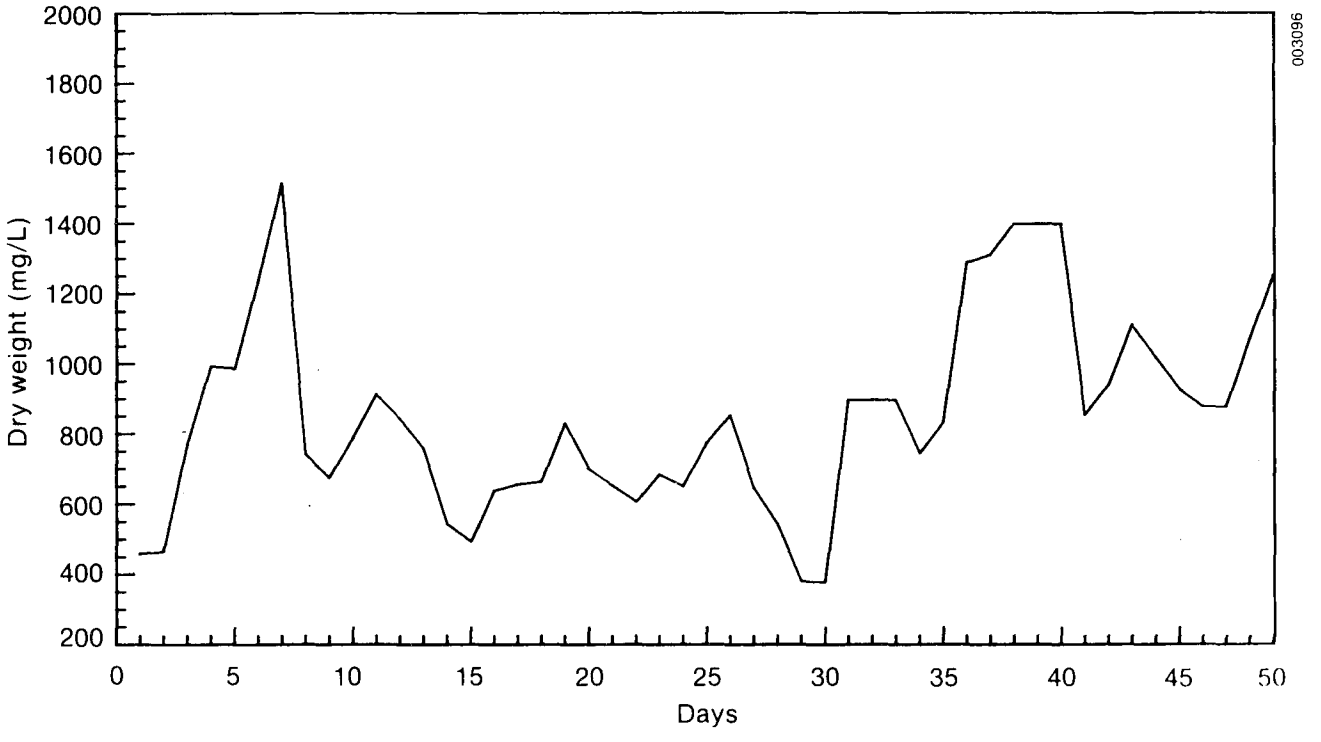


**Figure 3-10. Dry Weight Changes - Dunaliella**

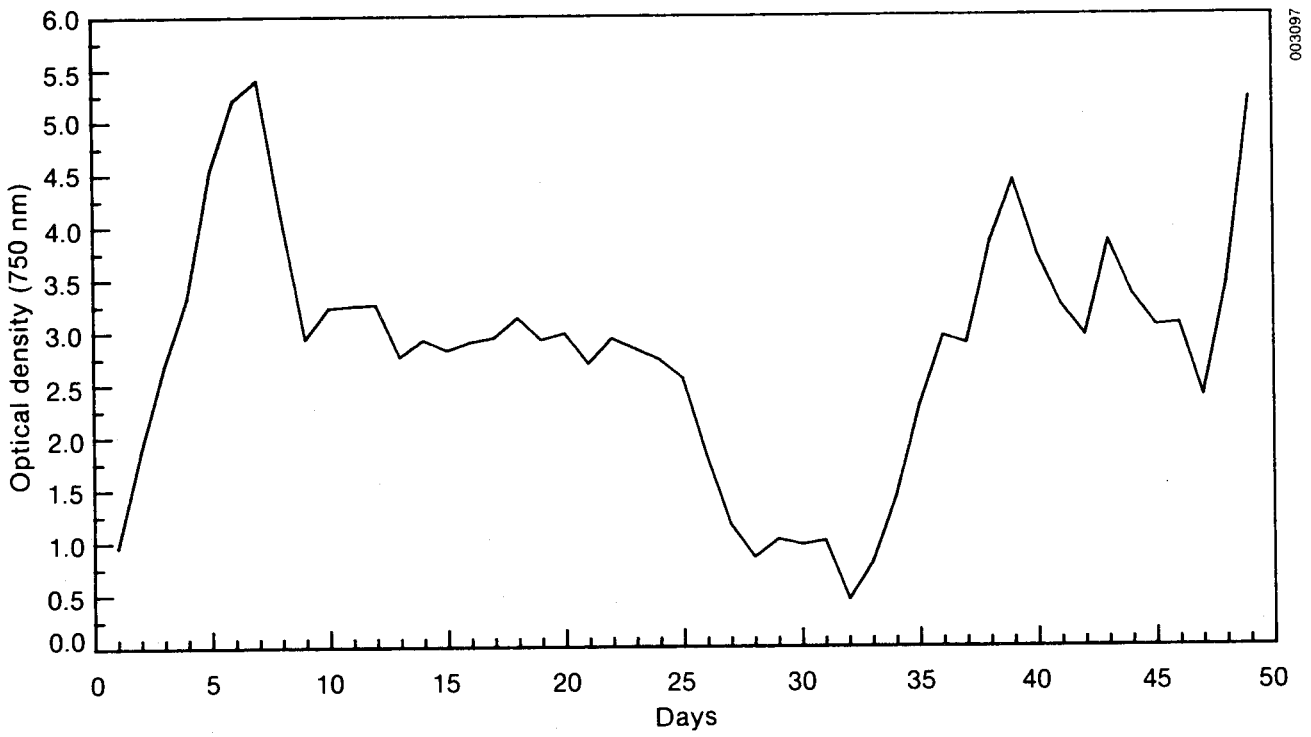


**Figure 3-11. Optical Density Changes - Dunaliella**

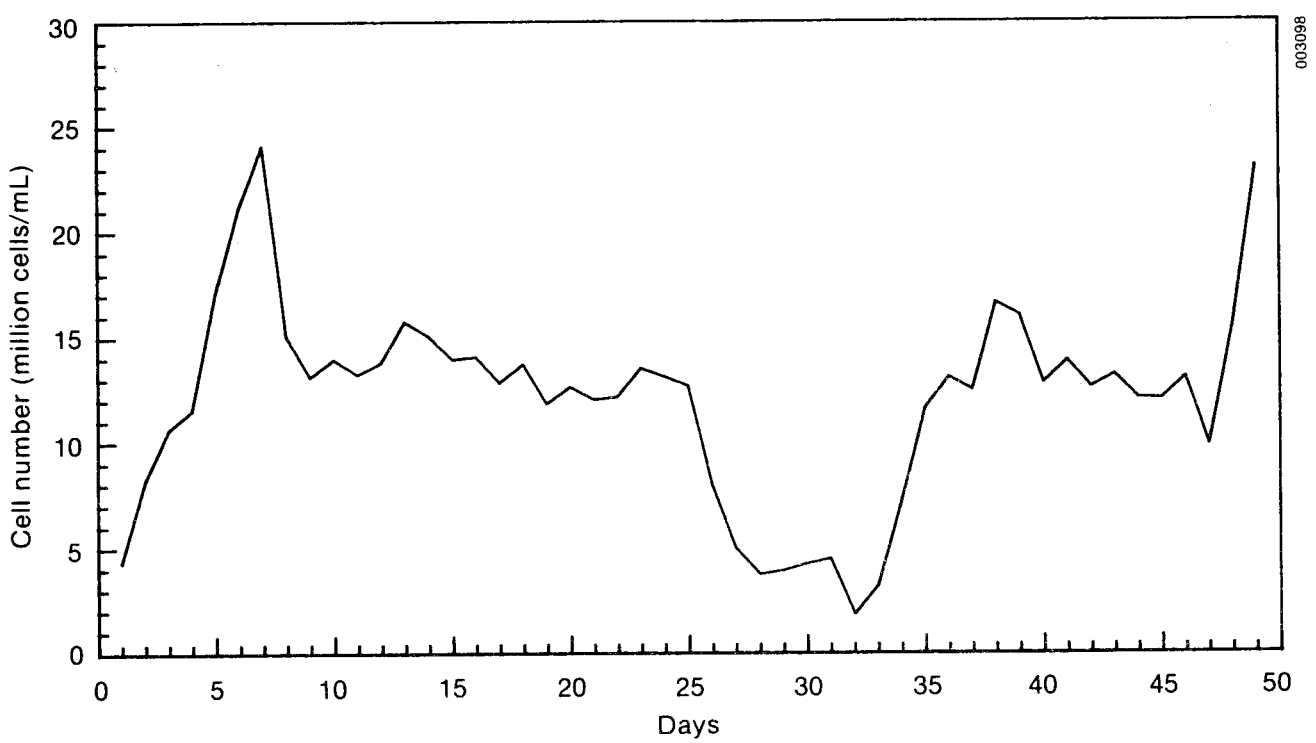


**Figure 3-12. Cell Number Changes - Dunaliella**

800 mL each day (0.24 volume), bringing the culture up to volume with fresh N-sufficient medium, and allowing growth to occur to the initial level. Initial crop parameters during this period ranged from 852 to 1110 mg dry weight $L^{-1}$, 2.95 to 4.43 optical density, and 12.04 to 13.88 × $10^6$ cells $mL^{-1}$. On day 46 the $CuSO_4$ filter was removed and since the lamp remained in the same position, the light intensity was doubled. Pumping was discontinued and batch growth occurred. Dry weight increased to 876 to 1250 mg $L^{-1}$, optical density from 3.04 to 5.20, and cell numbers from 12.0 to 23.1 × $10^6$ cells $mL^{-1}$. The culture temperature increased to 27°C, but vigorous growth occurred with only the flowing tapwater filter to remove infrared radiation.

### 3.2.3.2 Yields and Efficiencies

The results of the above culture manipulations are shown in Table 3-8. Yields and efficiencies during batch growth at 94,660 cal $day^{-1}$ were lower than those during continuous or semicontinuous culture and removing the $CuSO_4$ filter greatly decreased the yield and efficiency. This was probably due to inhibiting light levels since the overall intensity was doubled. Incipient nitrogen deficiency reduced the yield and efficiency somewhat, but growth did not completely cease until the culture became extremely deficient.

**Table 3-8. Yields and Efficiencies during the *Dunaliella* Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 1-7 | Batch growth; 94,660 cal $day^{-1}$ N sufficiency | 9.1 | 3.2 |
| 8-20 | Continuous culture 0.33 vol. $day^{-1}$ 94,660 cal $day^{-1}$ N sufficiency | 12.0 | 4.3 |
| 27 | Continuous culture 0.33 vol. $day^{-1}$ 94,660 cal $day^{-1}$ Incipient N deficiency | 10.2 | 2.8 |
| 39-46 | Semicontinuous culture 0.24 vol. $day^{-1}$ 94,660 cal $day^{-1}$ N sufficiency | 10.8 | 3.8 |
| 46-49 | Batch growth; $CuSO_4$ filter removed 189,000 cal $day^{-1}$ N sufficiency | 6.1 | 1.1 |

### 3.2.3.3 Proximate Cellular Composition

The proximate composition of N-sufficient and N-deficient Dunaliella cells is shown in Table 3-9. Changes in the proximate cellular components during the development of deficiency are shown in Figure 3-13. Deficiency resulted in a great decrease in the protein content, a slight decrease in lipid content, and not much change in ash levels. The most striking effect of deficiency was the general fourfold increase in carbohydrate content. At one point (day 26) the carbohydrate level reached 65% of the dry weight. Thus metabolism in this species was shifted by N deficiency from protein production to carbohydrate production. Pie diagrams of the mean percentages of protein, carbohydrate, lipid, and ash are not presented because these added up to slightly >100%.

In N-sufficient cells (continuous culture) the protein, carbohydrate and lipid yields were about 7.7 and 2.7 g $m^{-2}$ $day^{-1}$, respectively. With deficiency these figures changed to 2.7, 5.0 and 1.5 g $m^{-2}$ $day^{-1}$, respectively, for protein, carbohydrate, and lipid yield. Thus the carbohydrate yield was greatly increased by incipient N deficiency.

In N-sufficient cells the mean cellular caloric value was 4.72 cal $mg^{-1}$ dry weight. This also reflects the shunting of metabolism from protein (higher energy compounds) to carbohydrate (lower energy compounds).

**Table 3-9. Proximate Cellular Composition of Dunaliella under N-Sufficient and N-Deficient Conditions**

| | | % Dry Weight | | | | | |
|---|---|---|---|---|---|---|---|
| Culture Day | Culture Conditions | % Protein | % Carbohydrate | % Lipid | % Ash | Cal $mg^{-1}$ dry wt | Ash-free cal $mg^{-1}$ |
| 20 | +N | 45.1 | 13.9 | 17.2 | 13.6 | 3.96 | 4.50 |
| 21 | +N | 62.5 | 9.8 | 18.3 | 10.7 | 5.93 | 6.56 |
| 22 | +N | 62.0 | 10.9 | 18.5 | 11.1 | 4.99 | 5.54 |
| 23 | +N | 48.9 | 15.8 | 23.1 | 11.8 | 4.25 | 4.75 |
| 36 | +N | 66.6 | 8.5 | 36.8 | 10.7 | 4.68 | 5.18 |
| 46 | +N | 80.0 | 14.5 | 20.5 | 11.4 | 4.44 | 4.95 |
| 49 | +N | 84.6 | 15.1 | 27.0 | 11.0 | 4.79 | 5.32 |
| | Mean (+N) | 64.2±13.5 | 12.6±2.7 | 23.1±6.4 | 11.7±0.9 | 4.7±0.59 | 5.26±0.62 |
| 24 | -N | 34.1 | 32.0 | 21.0 | 13.0 | 4.01 | 4.53 |
| 25 | -N | 31.6 | 45.2 | 21.4 | 12.9 | -- | -- |
| 26 | -N | 24.3 | 65.0 | 16.4 | 12.1 | 3.74 | 4.19 |
| 27 | -N | 26.8 | 59.7 | 13.7 | 11.8 | 3.69 | 4.13 |
| 31 | -N* | 17.8 | 50.8 | 10.5 | 8.5 | -- | -- |
| | Mean (-N) | 26.9±5.7 | 50.5±11.5 | 16.6±4.2 | 11.5±1.6 | 3.81±0.14 | 4.28±0.18 |

*Extreme N deficiency.

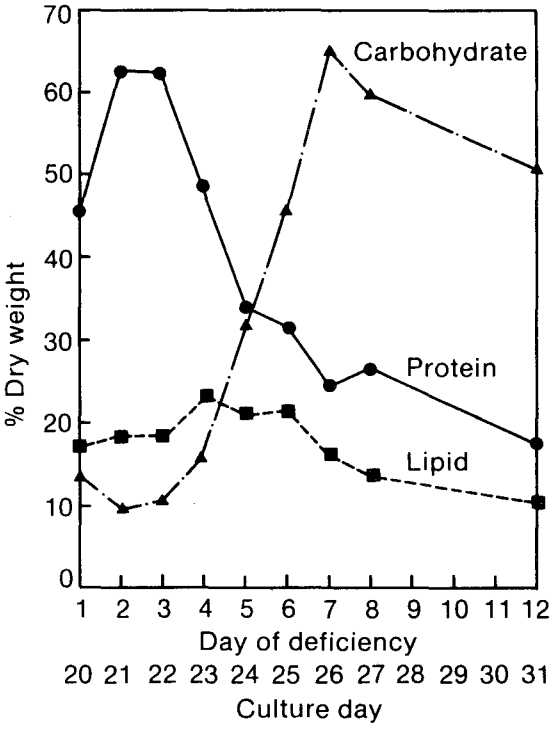


**Figure 3-13. Changes in Cellular Proximate Composition during the Development of Nitrogen Deficiency — Dunaliella**

### 3.2.4 Monallanthus Main Culture Experiment

This alga was chosen because it was reported to have an especially high lipid content when it was N-deficient (39,40).

#### 3.2.4.1 Narrative

The Monallanthus experiment was carried out for 61 days during September, October, and November 1981. The incubation temperature was 22°-25°C and the initial light energy (with the copper sulfate filter) supplied to the whole culture was 68,650 cal day$^{-1}$. On day 19 the filter developed a leak and was removed; after this time the light intensity was 74,400 cal day$^{-1}$. Figures 3-14 - 3-16 show time-series changes in dry weight, optical density and cell number in the batch mode (no pumping of fresh media) for the

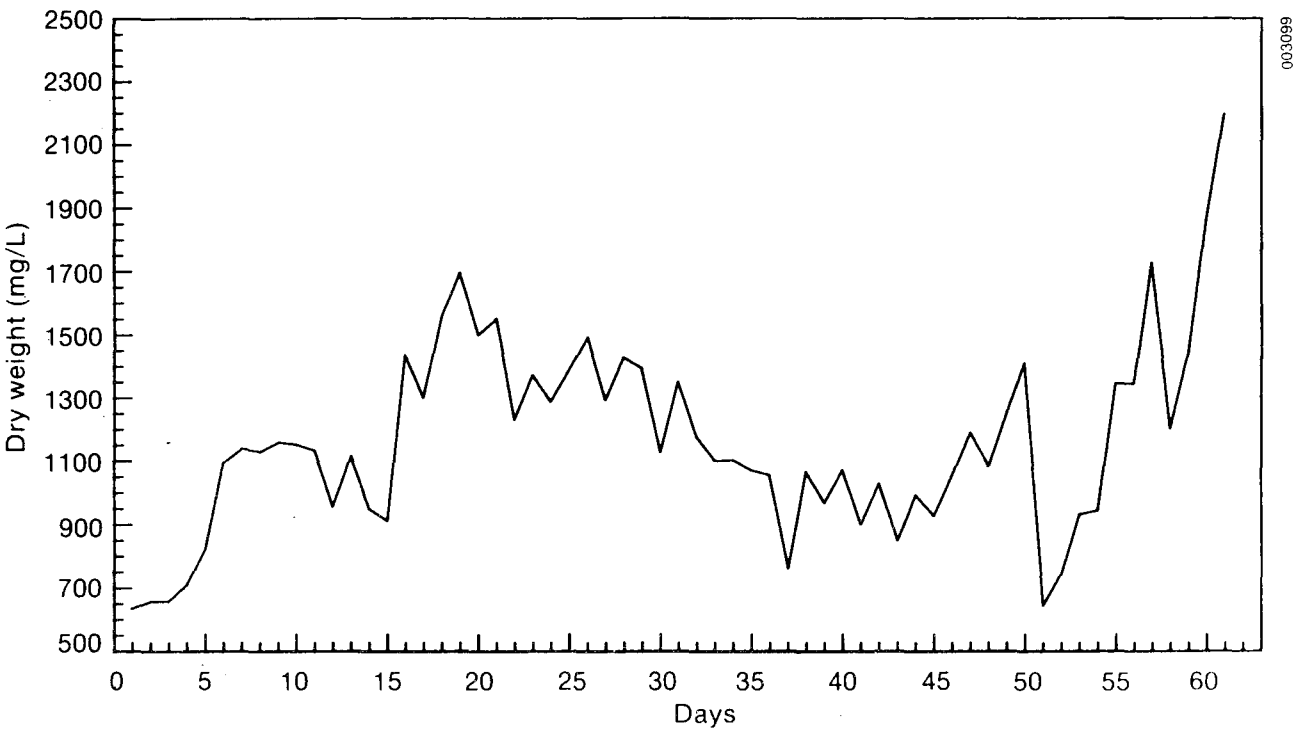


**Figure 3-14. Dry Weight Changes - Monallanthus**

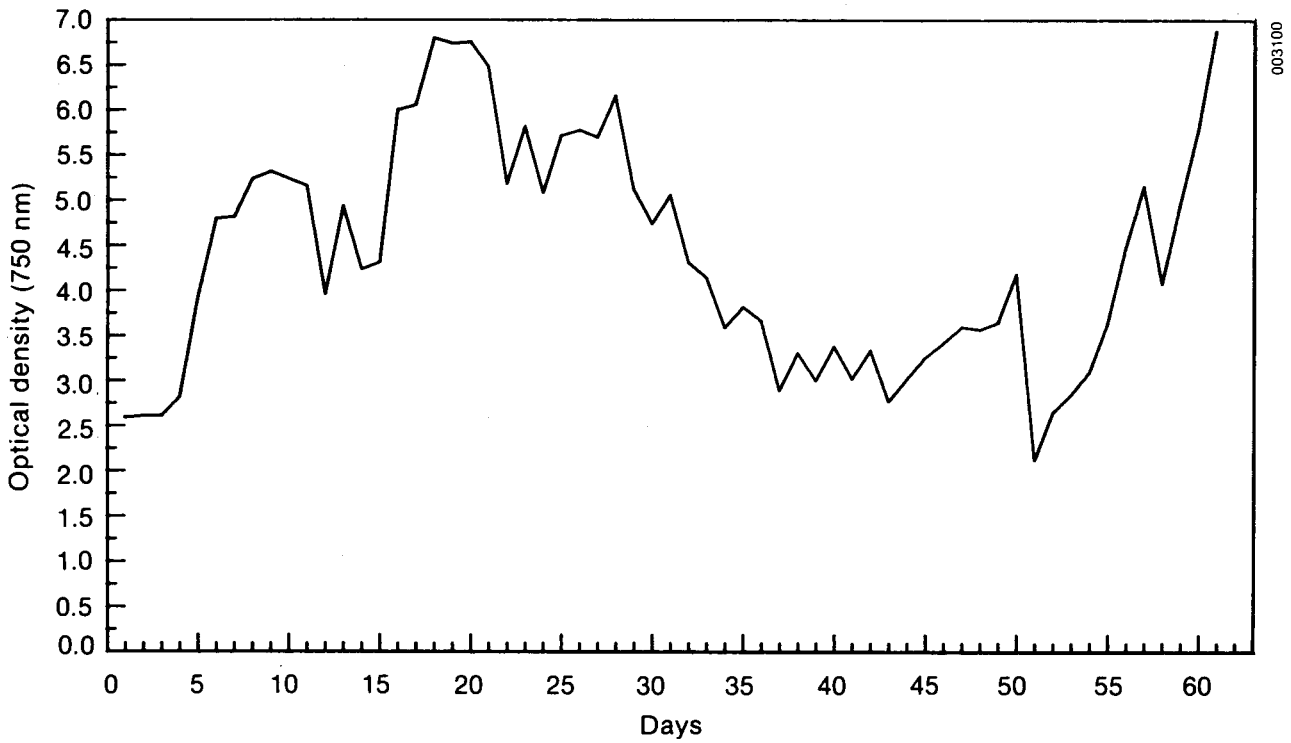


**Figure 3-15. Optical Density Changes - Monallanthus**

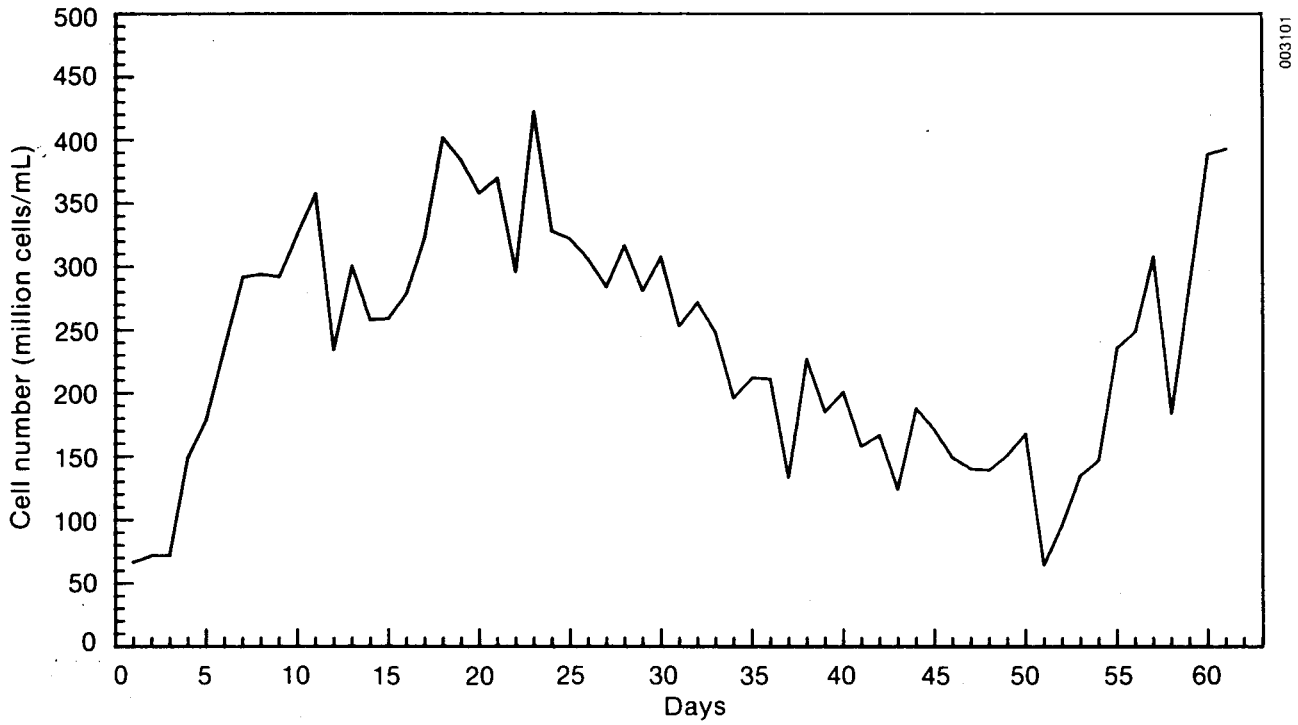


**Figure 3-16. Cell Number Changes - Monallanthus**

first 12 days. The culture was then sampled for proximate analyses. After dilution it was operated batchwise from day 13 to day 18. The batch changes in the various growth parameters suggested that 15%-20% of the culture could be harvested each day and replaced with fresh medium to run the culture semi-continuously. This was attempted from day 19 to day 27, but two growth parameters declined: optical density and cell numbers. Dry weight was more steady at about 1400 mg $L^{-1}$. The N-deficiency cycle was initiated on day 27 with harvesting of 15% of the culture per day and replacement with N-free medium. Population remained fairly steady during this period (days 27-40). Medium replacement was discontinued on day 40 and the culture was allowed to become extremely N-deficient until day 50. At that time about half of the culture was harvested and diluted to full volume with +N medium. Recovery from deficiency was rapid and two periods of rapid batch growth (days 51-57 and days 57-61) ensued.

### 3.2.4.2 Yields and Efficiencies

Yields and efficiencies for this alga during several parts of the experiment are given in Table 3-10. Batch growth yields ranged from 7.5 to

**Table 3-10. Yields and Efficiencies during the *Monallanthus* Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 4-7 | Batch growth 68,650 cal $day^{-1}$ with $CuSO_4$ filter (+N) | 7.5 | 4.0 |
| 15-19 | Batch growth 68,650 cal $day^{-1}$ with $CuSO_4$ filter (+N) | 8.2 | 4.3 |
| 19-27 | Semicontinuous growth 15-20% culture harvested per day; 74,400 cal $day^{-1}$ without $CuSO_4$ filter (+N) | 12.0 | 5.8 |
| 51-57 | Batch growth; +N recovery 74,400 cal $day^{-1}$ without $CuSO_4$ filter | 8.2 | 3.7 |
| 57-61 | Batch growth; +N recovery 74,400 cal $day^{-1}$ without $CuSO_4$ filter | 13.9 | 6.4 |
| 42 | Semicontinuous growth; -N cycle; 15% culture harvested per day; 74,400 cal $day^{-1}$ without $CuSO_4$ filter | 7.5 | 3.7 |

13.9 g $m^{-2}$ $day^{-1}$. The latter value was attained during a strong recovery from N deficiency and was slightly greater than the yield during semicontinuous culture. Removal of the $CuSO_4$ filter and an increased light intensity possibly increased yields, but the values during batch growth from days 51-57 were not especially high. Efficiencies of light utilization ranged from 3.7% to 6.4%.

During the N-deficiency cycle (at day 42), when replacement of the culture was still at 15% per day, the yield was still 7.5 g $m^{-2}$ $day^{-1}$ and the efficiency was 3.7%. Thus N deficiency decreased the yield from about 12 g $m^{-2}$ $day^{-1}$ (days 19-27) but growth did not completely cease.

### 3.2.4.3 Proximate Cellular Composition

The proximate composition of N-deficient cells is given in Table 3-11. Deficiency resulted in a great decrease in the protein content, a slight increase in carbohydrate and ash content, and little or no change in the percentage of lipid. Unidentified other material increased greatly and this fraction may have consisted of polar lipids that were not analyzed by our methods.

**Table 3-11. Proximate Cellular Composition of Monallanthus under N-Sufficient and N-Deficient Conditions**

| | | % Dry Weight | | | | | |
|---|---|---|---|---|---|---|---|
| Culture Day | Culture Conditions | % Protein | % Carbohydrate | % Lipid | % Ash | Cal $mg^{-1}$ dry wt | Ash-free cal $mg^{-1}$ |
| 27 | +N | 39.1 | 13.2 | 22.0 | 8.7 | 4.72 | 5.17 |
| 28 | +N | 40.4 | 13.9 | 21.7 | 8.0 | 5.19 | 5.64 |
| 29 | +N | 45.2 | 15.1 | 24.0 | 7.0 | 5.16 | 5.55 |
| 30 | +N | 43.8 | 15.4 | 23.4 | 8.4 | 5.37 | 5.86 |
| 31 | +N | 43.6 | 15.4 | 20.6 | 7.5 | 5.30 | 5.73 |
| 32 | +N | 45.4 | 14.4 | 18.5 | 8.7 | 5.06 | 5.54 |
| 33 | +N | 34.7 | 14.1 | 20.7 | 8.6 | 5.07 | 5.55 |
| 34 | +N | 41.2 | 14.7 | 21.8 | 8.5 | 5.34 | 5.84 |
| 35 | +N | 42.9 | 14.5 | 22.9 | 8.7 | 5.25 | 5.75 |
| 36 | +N | 40.2 | 15.5 | 22.3 | 8.3 | 4.98 | 5.43 |
| 57 | +N | 37.3 | 9.8 | 13.5 | 9.5 | 4.76 | 5.26 |
| 61 | +N | 25.6 | 10.1 | 16.8 | 7.7 | 4.91 | 5.32 |
| | Mean (+N) | 41.3±3.2 | 13.9±1.9 | 20.7±2.9 | 83.±0.6 | 5.12±0.20 | 5.55±0.21 |
| 38 | -N | 31.5 | 18.3 | 21.5 | 9.9 | 4.90 | 5.44 |
| 40 | -N | 24.3 | 16.6 | 19.2 | 11.3 | 5.20 | 5.86 |
| 42 | -N | 17.6 | 17.2 | 24.8 | 13.3 | 5.14 | 5.93 |
| 50 | -N* | 12.7 | 16.5 | 22.2 | 14.8 | 5.38 | 6.31 |
| | Mean (-N) | 18.2±4.8 | 16.8±0.4 | 22.1±2.3 | 13.1±1.4 | 5.24±0.02 | 5.88±0.31 |

*Extreme N deficiency.

In N-sufficient cells the protein yield (% protein times cell yield) ranged from 3.1 to 5.7 g $m^{-2}$ $day^{-1}$ and the lipid yield was 1.6 to 2.9 g $m^{-2}$ $day^{-1}$. Nitrogen deficiency reduced the protein yield to 1.4 g $m^{-2}$ $day^{-1}$ and the lipid yield was not changed very much (1.6 g $m^{-2}$ $day^{-1}$).

Nitrogen deficiency did not change the mean caloric content of the cells. In N-sufficient cells the caloric content was 5.12 cal $mg^{-1}$ dry weight; in deficient cells it was 5.24 cal $mg^{-1}$ dry weight. On an ash-free base N-sufficient cells contained 5.55 cal $mg^{-1}$ and deficient cells contained 5.88 cal $mg^{-1}$.

### 3.2.5 Tetraselmis Main Culture Experiment

This alga was chosen because it gave high cell densities in preliminary culture and because it is often used for food for marine animals in various aquaculture projects (65,66).

#### 3.2.5.1 Narrative

This alga was cultured for 66 days during January, February and March 1982. Figures 3-17 - 3-19 show changes in dry weight, optical density, and cell numbers for this whole period. The light intensity was 93,560 cal $day^{-1}$ over the culture surface. The light was not filtered through $CuSO_4$. The culture temperature was $20^{o}$-$22^{o}$C. Initially the culture was operated in the batch mode for two periods (days 1-8 and days 10-15). Then semicontinuous culture was carried out with removals of 1320-1600 mL $day^{-1}$ from days 17-19. This was too

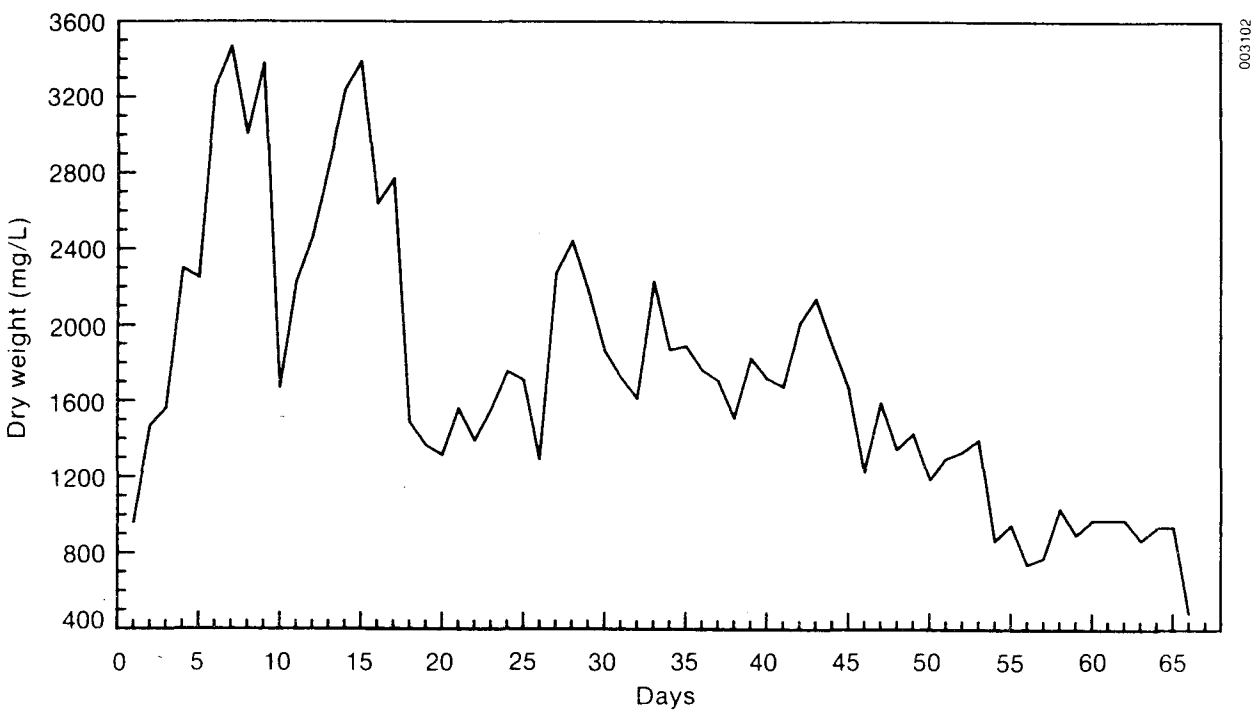


**Figure 3-17. Dry Weight Changes - Tetraselmis**

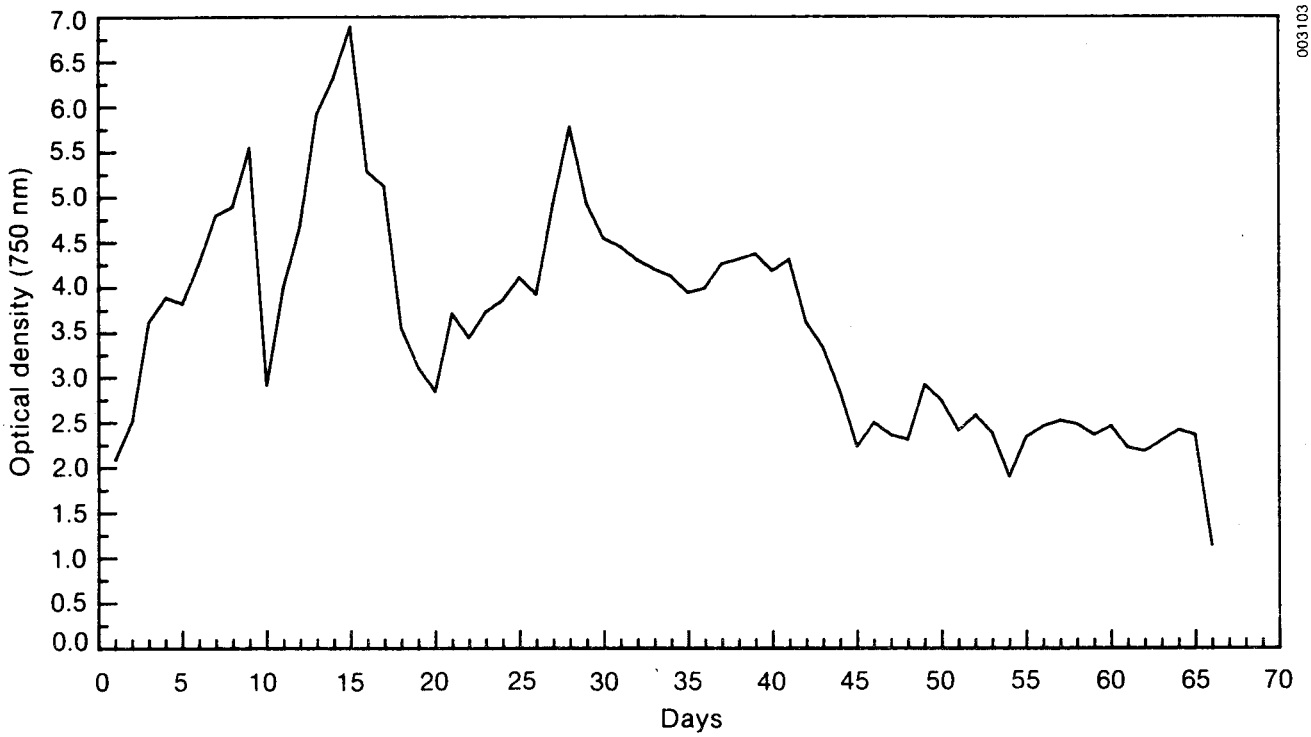


**Figure 3-18. Optical Density Changes - Tetraselmis**

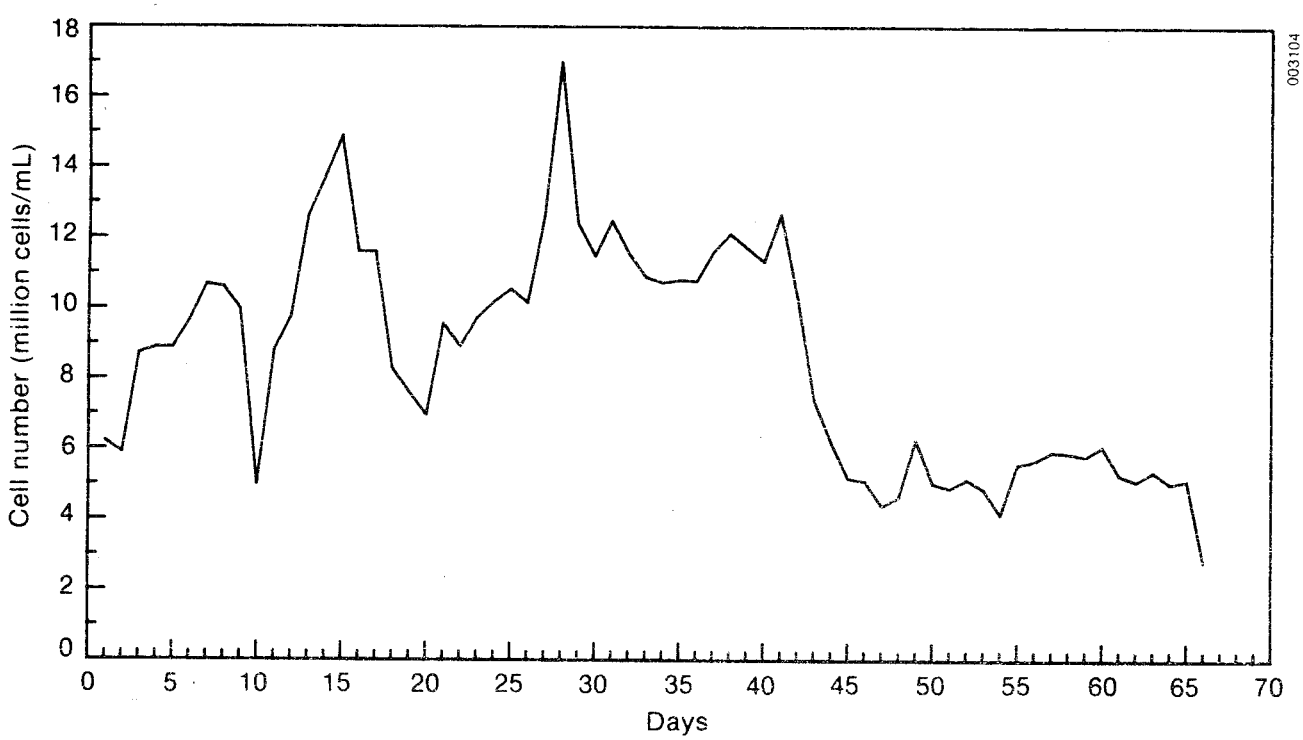


**Figure 3-19. Cell Number Changes - Tetraselmis**

high a rate of removal and cell parameters decreased. From day 21 to day 25 and from day 28 to day 34 the culture was operated in semicontinuous culture at a lower rate of removal (610 to 900 mL $day^{-1}$). Crop parameters were reasonably stable during these periods. On day 35 diluting with N-deificient medium commenced and continued until day 46; 600 mL of culture was removed each day and replaced with N-deficient medium. From days 47 to 53 the culture was operated in the batch mode without any dilution to achieve extreme N deficiency. Then 1300 mL was removed and replaced with N-sufficient medium to achieve recovery. Further growth did not occur, however, and the only evidence of recovery was an increased cellular protein content at the end of the experiment. Even though growth did not occur, the cells were motile and appeared healthy when observed under the microscope.

#### 3.2.5.2 Yields and Efficiencies

Yield and efficiencies during the various culture manipulations are shown in Table 3-12. The highest yields and efficiencies were found during the two initial batch growth periods. Semicontinuous culture, while resulting in a reasonably steady state with regard to crop parameters, resulted in a lower yield and efficiency than batch culture. Nitrogen deficiency reduced the yield and efficiency even more.

#### 3.2.5.3 Proximate Cellular Composition

The proximate composition of N-sufficient and N-deficient Tetraselmis cells is shown in Table 3-13. Deficiency more than halved the protein content, and the

**Table 3-12. Yields and Efficiencies during the Tetraselmis Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 1-8 | Batch growth<br>93,560 cal $day^{-1}$<br>N sufficiency | 19.1 | 7.3 |
| 10-15 | Batch growth<br>93,560 cal $day^{-1}$<br>N sufficiency | 17.0 | 6.5 |
| 21-34 | Semicontinuous culture<br>at 0.20 vol. $day^{-1}$<br>93,560 cal $day^{-1}$<br>N sufficiency | 14.4 | 5.5 |
| 46 | Semicontinuous culture<br>at 0.30 vol. $day^{-1}$<br>93,560 cal $day^{-1}$<br>Incipient N deficiency | 12.7 | 3.6 |

**Table 3-13. Proximate Cellular Composition of Tetraselmis under N-Sufficient and N-Deficient Conditions**

| Culture Day | Culture Conditions | % Dry Weight % Protein | % Carbohydrate | % Lipid | % Ash | Cal $mg^{-1}$ dry wt | Ash-free cal $mg^{-1}$ |
|---|---|---|---|---|---|---|---|
| 36 | +N | 74.6 | 8.7 | 22.3 | 8.0 | 5.16 | 5.61 |
| 37 | +N | 63.4 | 9.8 | 24.0 | 8.0 | 5.02 | 5.45 |
| 38 | +N | 73.9 | 7.8 | 26.8 | 8.6 | 4.94 | 5.40 |
| 39 | +N | 63.3 | 9.8 | 24.7 | 9.7 | 5.10 | 5.65 |
| 40 | +N | 54.7 | -- | 22.1 | 7.3 | 4.71 | 5.08 |
| 41 | +N | 53.7 | -- | 23.0 | 9.4 | 4.32 | 4.77 |
| 66 | +N | 67.6 | 17.4 | 21.0 | 17.7 | 3.90 | 4.74 |
| | Mean (+N) | 64.4±7.7 | 10.7±3.4 | 23.4±1.8 | 9.8±3.3 | 4.73±0.43 | 5.24±0.35 |
| 42 | -N | 33.1 | 43.6 | 16.9 | 8.4 | 4.25 | 4.64 |
| 43 | -N | 30.6 | 40.3 | 15.3 | 9.4 | 4.18 | 4.61 |
| 44 | -N | 25.0 | 53.1 | 12.7 | 9.0 | 4.20 | 4.62 |
| 46 | -N | 28.1 | -- | -- | 8.5 | 3.80 | 4.15 |
| 49 | -N | 25.0 | 57.2 | 15.4 | 13.6 | 3.61 | 4.18 |
| 53 | -N* | 28.1 | 41.4 | 12.9 | 16.1 | 3.88 | 4.62 |
| | Mean (-N) | 28.3±2.9 | 47.1±6.8 | 14.6±1.6 | 10.8±2.9 | 3.99±0.24 | 4.47±0.22 |

*Extreme N deficiency.

carbohydrate level increased nearly five-fold, as metabolism was shifted from protein production. Deficiency reduced the lipid content somewhat. A pie diagram of mean percentages of the varioius components is not presented because the percentages add up to slightly >100%.

In N-sufficient cells (semicontinuous culture) the yields of protein, carbohydrate, and lipid were about 9.3, 1.5, and 3.4 g $m^{-2}/day^{-1}$, respectively. These figures changed to 3.6, 6.0, and 1.9 g $m^{-2}/day^{-1}$, respectively, for protein, carbohydrate, and lipid during incipient N deficiency. Thus the carbohydrate yield was greatly increased by deficiency.

In N-sufficient cells the mean cellular caloric value was 4.73 cal $mg^{-1}$ dry weight. This value decreased to 3.99 cal $mg^{-1}$ dry weight in incipiently N-deficient cells. This might be expected since protein has a higher energy content than carbohydrate.

### 3.2.6 Isochrysis Main Culture Experiment

This alga also gave high cell densities in preliminary culture and is often used for marine animal food in aquaculture projects (66).

#### 3.2.6.1 Narrative

Initially this alga was started in the main culture apparatus in March and April of 1982 (Figures 3-20 - 3-22). However, after 22 days the culture

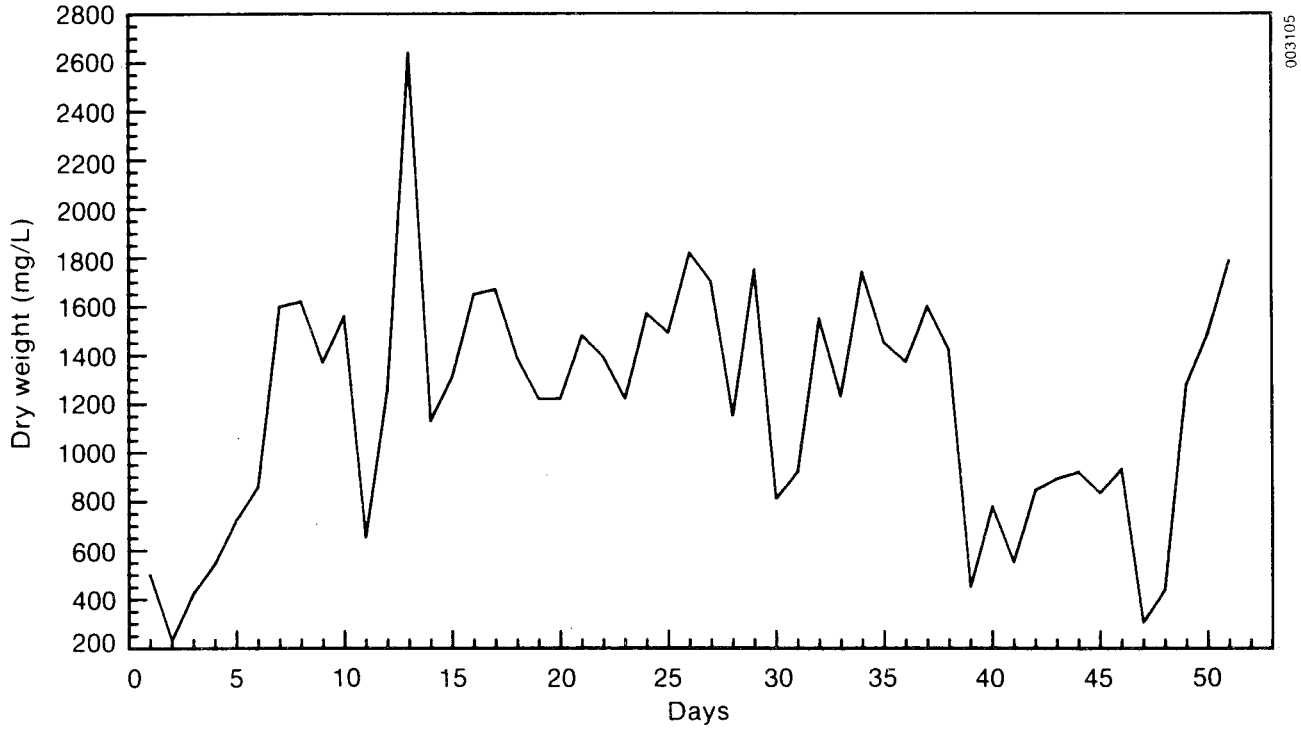


**Figure 3-20. Dry Weight Changes - Isochrysis**

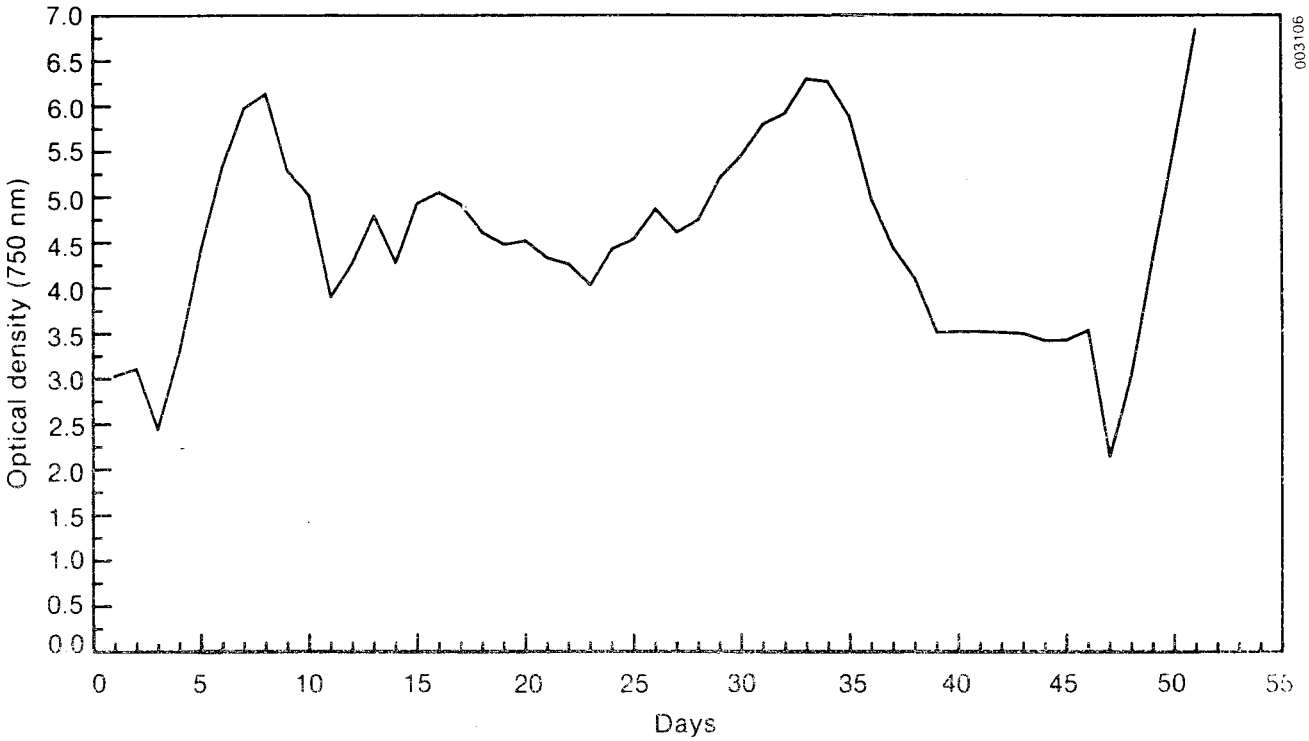


**Figure 3-21. Optical Density Changes - Isochrysis**

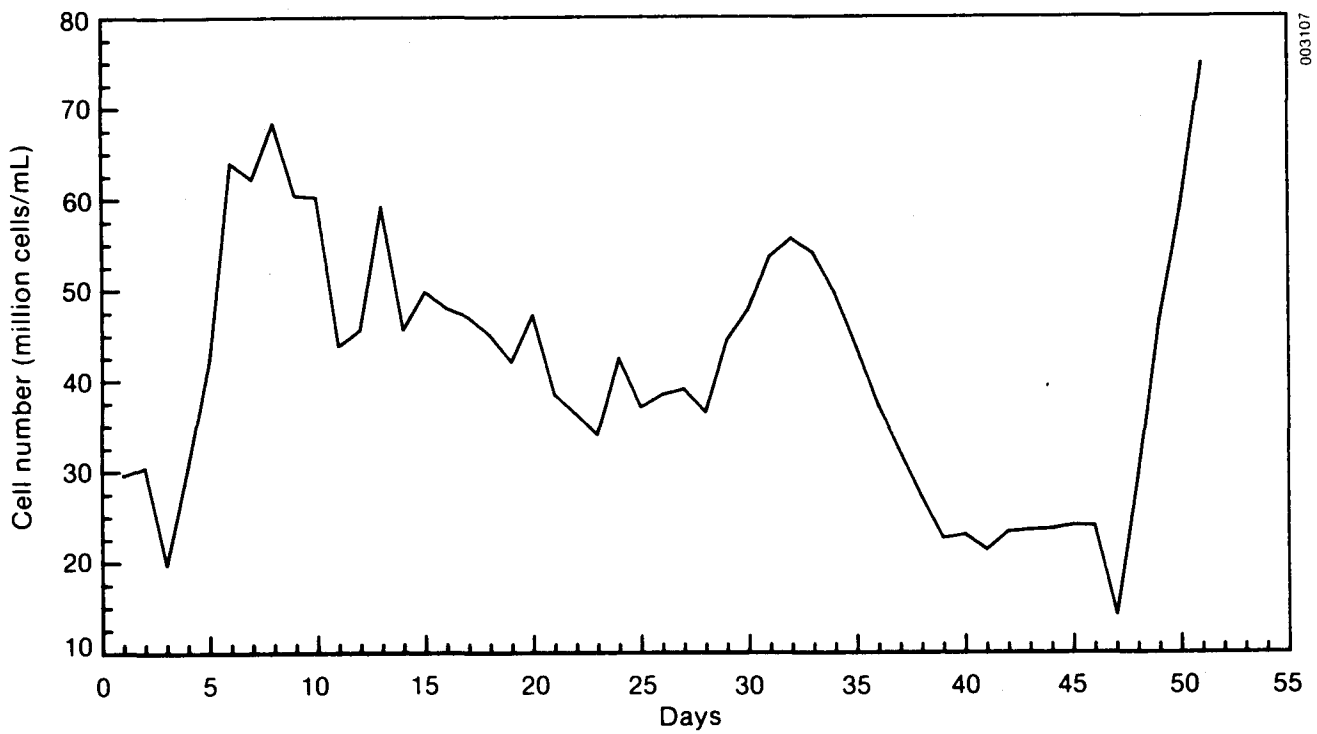


**Figure 3-22. Cell Number Changes - Isochyrsis**

became contaminated by a ciliate which was obviously eating the cells. Therefore the experiment was terminated.

A second experiment was run for 51 days in May, June, and July 1982. No contamination was observed during this experiment. The light intensity was 95,120 cal day$^{-1}$ and the light was not filtered through $CuSO_4$. The temperature ranged from 24°C to 26°C. Figures 3-20 - 3-22 show changes in dry weight, optical density, and cell numbers for the whole experiment. Days 1-2 were a period of adjustment of the culture and no growth occurred. Thereafter (days 3-7) the culture grew well in the batch mode. From days 8-11 the culture was operated semicontinuously with 25% removed each day followed by dilution back to volume with fresh N-sufficient medium. This harvest volume was too great and cell growth could not keep up with dilution. Another period of batch culture (without dilution) was carried out from days 11-13. Then the culture was diluted again and allowed to equilibrate for two days. The culture was then (days 17-24) operated semicontinuously with 15% of the volume removed each day followed by dilution with fresh (+N) medium. The population parameters remained reasonably steady under this culture regime. On day 24, an N-deficiency cycle was commenced. Fifteen percent of the culture volume was removed each day and the culture was brought up to volume with N-deficient medium. It took until day 33 for the culture to become deficient and growth parameters to begin dropping; from days 24-33 populations even increased. The populations reached a minimum because of deficiency on day 38. Thereafter, the culture was not harvested until day 46 (extreme N deficiency). At that time one-third of the culture was removed and replaced with fresh N-sufficient medium to allow recovery to occur. Strong increases in growth parameters occurred from day 47 to day 51 when the experiment was terminated.

Centrifuge harvests during the development of deficiency consisted of a layer of orange material over a layer of green material. Motile cells were present in each layer. The orange layer was gelatinous and sticky and became more yellow in truly deficient material. It was difficult to separate the two layers and ash levels were higher in the upper orange or yellow layer. However, where possible, ash and caloric analyses were made on the green material.

#### 3.2.6.2 Yields and Efficiencies

Yields and efficiencies during the various culture manipulations are shown in Table 3-14. The highest yield and efficiency was found during the second batch growth period (days 11-12). Semicontinuous culture at 15% removal per day was successful in that the culture could be maintained, but the yields and efficiencies were lower than during batch culture. Nitrogen-deficiency reduced the yield and efficiency even more.

#### 3.2.6.3 Proximate Cellular Composition

The cellular composition of Isochrysis during N-sufficient and N-deficient conditions is shown in Table 3-15. Deficiency reduced the protein content and greatly increased the carbohydrate level. Lipid content increased slightly,

**Table 3-14. Yields and Efficiencies during the Isochrysis Main Culture Experiment**

| Days | Culture Conditions | Yield ($g\ m^{-2}\ day^{-1}$) | Efficiency (%) |
|---|---|---|---|
| 3-7 | Batch growth N sufficiency | 11.5 | 2.8 |
| 11-13 | Batch growth N sufficiency | 47.9 | 11.6 |
| 14-24 | Semicontinuous culture at 0.15 vol. $day^{-1}$; N sufficiency | 8.6 | 2.1 |
| 25-33 | Semicontinuous culture at 0.15 vol. $day^{-1}$ Beginning N deficiency | 7.6 | 1.9 |
| 33-38 | Semicontinuous culture at 0.15 vol. $day^{-1}$ Incipient N deficiency | 5.5 | 1.5 |
| 47-51 | Batch growth; recovery from N deficiency | 19.4 | 4.7 |

**Table 3-15. Proximate Cellular Composition of Isochrysis under N-Sufficient and N-Deficient Conditions**

| Culture Day | Culture Conditions | % Dry Weight: % Protein | % Carbohydrate | % Lipid | % Ash | Cal $mg^{-1}$ dry wt | Ash-free cal $mg^{-1}$ |
|---|---|---|---|---|---|---|---|
| 22 | +N | 54.6 | 23.4 | 29.0 | 37.2 | 3.13 | 4.98 |
| 24 | +N | -- | 26.2 | 32.9 | 29.5 | 3.90 | 5.53 |
| 25 | +N | 53.7 | 26.8 | 31.8 | 37.1 | 3.06 | 4.86 |
| 26 | +N | 44.2 | 18.2 | 20.8 | 36.2 | 3.50 | 5.48 |
| 27 | +N | 44.4 | 18.6 | 22.7 | 41.9 | 2.60 | 4.48 |
| 28 | +N | 60.7 | 23.2 | 32.9 | 39.7 | 2.74 | 4.54 |
| 29 | +N | 44.0 | 13.4 | 22.1 | 31.2 | 3.38 | 4.91 |
| 32 | +N | 44.0 | 18.7 | 30.5 | 29.3 | 3.63 | 5.13 |
| 33 | +N | 55.8 | 35.7 | 36.2 | 27.0 | 3.44 | 4.71 |
| 34 | +N | 42.3 | 31.9 | 26.4 | 24.1 | 3.51 | 4.62 |
| 51 | +N | 48.3 | 18.3 | 27.9 | 20.6 | 4.49 | 5.65 |
| | Mean (+N) | 44.9±15.6 | 23.1±6.3 | 28.5±4.8 | 32.2±6.5 | 3.40±0.50 | 4.99±-0.39 |
| 35 | -N | 32.8 | 41.6 | 28.0 | 26.2 | 3.58 | 4.85 |
| 36 | -N | 27.8 | 45.2 | 27.6 | 23.1 | 3.78 | 4.92 |
| 37 | -N | 26.4 | 71.1 | 42.5 | 21.3 | 3.78 | 4.92 |
| 38 | -N | 23.2 | 62.7 | 35.5 | 22.3 | 3.56 | 4.58 |
| 46 | -N* | 26.1 | 63.7 | 33.4 | 24.2 | 3.62 | 4.78 |
| | Mean (-N) | 27.3±3.1 | 56.9±11.4 | 33.4±5.5 | 23.4±1.7 | 3.66±0.10 | 0.13±0.13 |

*Extreme N deficiency.

while ash levels decreased. In general the ash content of both sufficient and deficient cells was quite high compared to that of other algae. Since percentages of protein, carbohydrate, lipid, and ash add up to >100%, we suspect that these ash levels are unrealistic.

During semicontinuous growth at 0.15 volume removed $day^{-1}$ the yield of protein, carbohydrate, and lipid in N-sufficient cells was 3.9, 1.8, and 2.4 g $m^{-2}$ $day^{-1}$, respectively. In N-deficient cells these values were 1.5, 3.1, and 1.8 g $m^{-2}$ $day^{-1}$, respectively. Thus overall carbohydrate yield increased during deficiency.

In N-sufficient cells the mean caloric value did not differ greatly from that for N-deficient cells even on an ash-free basis.

### 3.2.7 Botryococcus Main Culture Experiment

This alga has been reported to produce high amounts of hydrocarbons (67). Even though it grew slowly in preliminary culture, it seemed desirable to test

it at least briefly in our main culture apparatus with a high light intensity. It was grown for 11 days in the batch mode during May 1982.

### 3.2.7.1 Yield and Efficiency

Growth curves (optical density and dry weight changes) are shown in Figures 3-23 and 3-24. Cell numbers were not assessed since this alga occurs in colonies having variable cell numbers per colony. Even at a high light intensity (93,560 cal $day^{-1}$), the alga grew very slowly; the yield was only 3.4 g $m^{-2}$ $day^{-1}$ and the efficiency was only 1.1%. For the latter, cell calories were calculated from the carbon content in mg × 11.0 (68).

### 3.2.7.2 Proximate Culture Composition

The proximate composition of healthy cells from preliminary cultures is shown in Figure 3-25. Protein content was quite low compared to that of other algae, but the lipid content was high. We did not perform ash analyses; the ash level would be included in the "other" fraction.

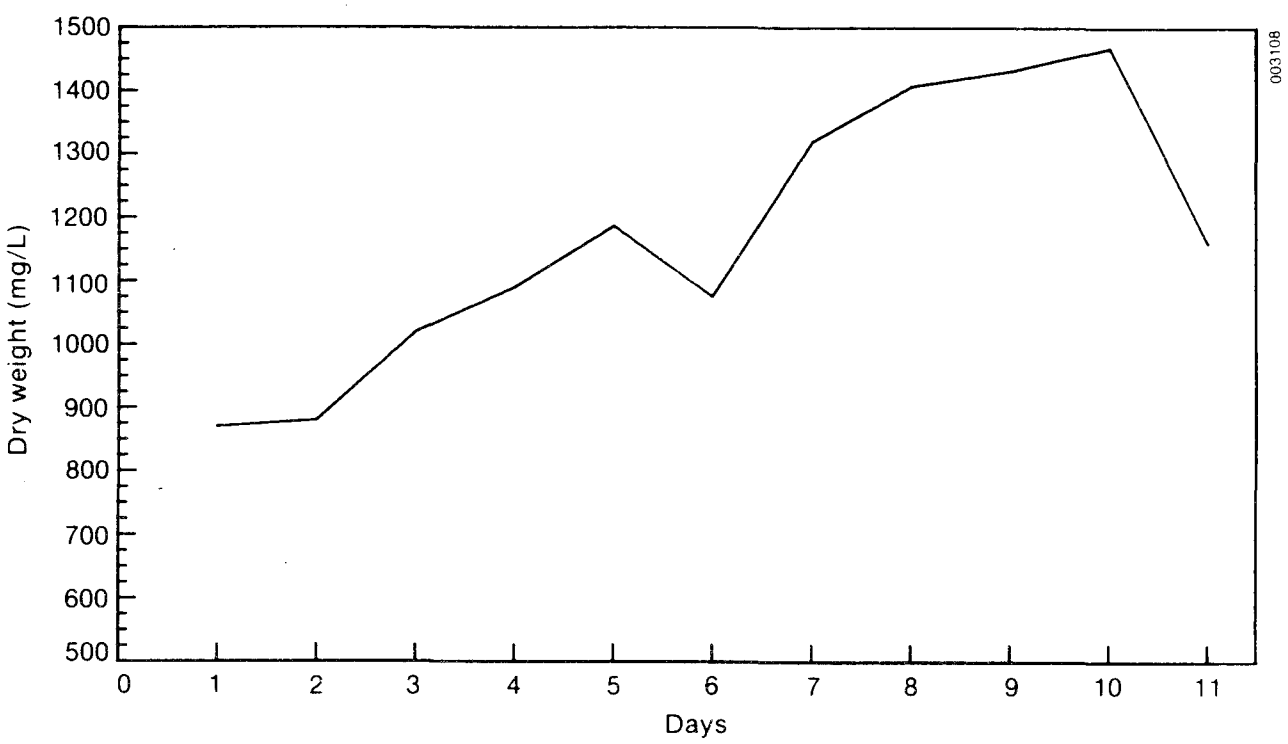


**Figure 3-23. Dry Weight Changes - Botryococcus**

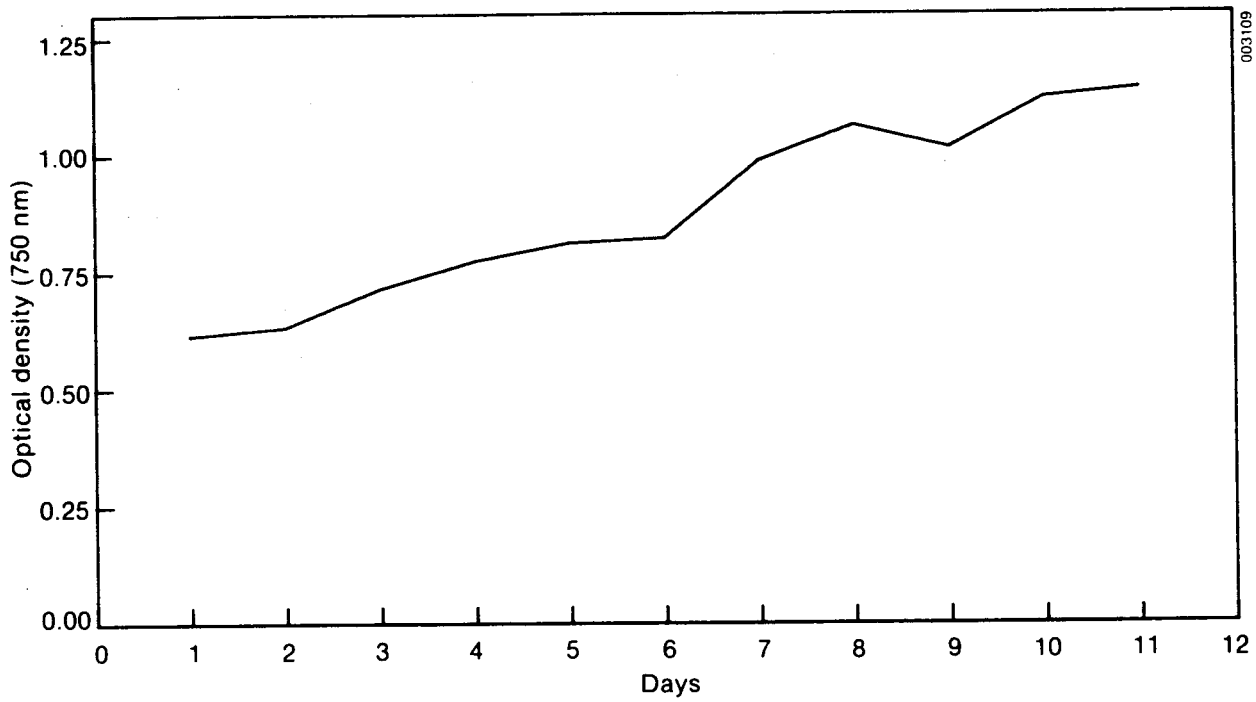


**Figure 3-24. Optical Density Changes - Botryococcus**

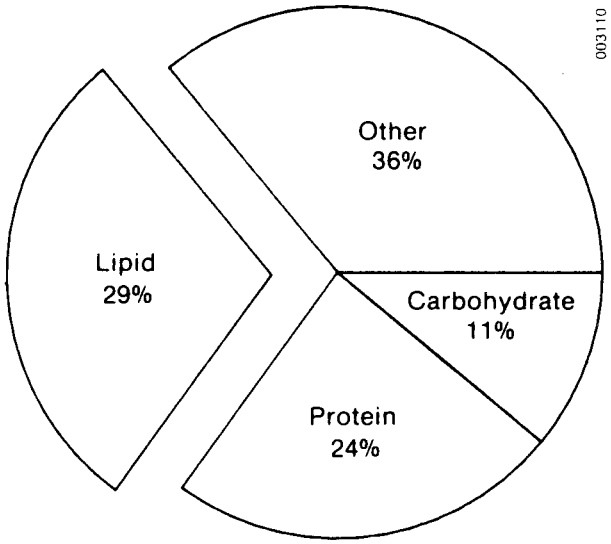


**Figure 3-25. Proximate Composition of Botryococcus Cells**

# SECTION 4.0

# DISCUSSION

## 4.1 YIELDS AND EFFICIENCIES

Yields and efficiencies of various algae can be compared among the species in terms of the preliminary cultures (Section 3.1) and with regard to the results of the main culture experiments (Section 3.2). Species investigated in the latter experiments are more comparable because dry weight measurements were made and good efficiency measurements could be made.

In preliminary cultures, Phaeodactylum outgrew all other species (Tables 3-1 and 3-2), but all species investigated could be "pushed" to achieve very dense cultures.

Yields and efficiencies for six species in the main culture experiments are summarized in Table 4-1. Yield values ranged from 1.1 to 21.7 g $m^{-2}$ $day^{-1}$ and efficiencies of light utilization ranged from 1.1% to 12.2%. The highest values were found for Phaeodactylum batch culture growth. With this alga yields and efficiencies in continuous culture were less than those in batch culture. This was not always the case for other algae, e.g. Dunaliella and Monallanthus. Other workers have found yields ranging up to 41 g $m^{-2}$ $day^{-1}$ (Phaeodactylum) (18) and in outdoor cultures average yields are about 20 g $m^{-2}$ $day^{-1}$ (6). For most of the algae that we studied, the yields were somewhat less than 20 g $m^{-2}$ $day^{-1}$. Measured efficiencies have ranged up to 20% (8), but most values (9,10,17,18) are less than this, as are our values.

With proper management of algal cultures--use of 100% $CO_2$, proper culture thickness and cell density, adequate turbulence, use of closed systems, etc.--it may be possible to increase yields somewhat. Mauldin et al. (16) reports an average yield of 71 g $m^{-2}$ $day^{-1}$ for Chlorella. For management of our cultures, we determined, from increases in dry weight in batch culture, the amount of material that could be removed continuously or semicontinuously per day, and we have run the cultures in these latter modes. Steady states were achieved successfully, but we believe that much more work needs to be done to determine how to manage mass cultures for maximum sustained yields.

Laws et al. (62) have recently completed a study of Phaeodactylum yields and efficiencies in an outdoor raceway culture in Hawaii. In the winter their yield figures were 3.0 to 4.6 g $m^{-2}$ $day^{-1}$ with efficiencies ranging from 1.3% to 1.8%. Their spring values were 10.5 to 11.6 g $m^{-2}$ $day^{-1}$ and efficiencies increased to 3.4% to 4.2%. Foils placed in the raceway to provide increased turbulence (flasing light) increased the yields from 5.1 to 10.7 g $m^{-2}$ $day^{-1}$ (calculated from their carbon yields). Corresponding efficiency increases were from 1.5% to 3.3%. Our maximum yields and efficiencies were at least double these values and this suggests that, with proper manipulation of the raceway culture, the yields and efficiencies of the Hawaiian workers could be greatly increased. They are presently working on these problems and their studies provide an example of the possibilities of maximizing yields and efficiencies through proper outdoor culture management.

**Table 4-1. Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensities and Nutrient Sufficiency - Main Culture Experiments**

| Alga | Yield (g dry wt $m^{-2}$ $day^{-1}$) | Efficiency (%) | Lipid Yield (g $m^{-2}$ $day^{-1}$) | Protein Yield (g $m^{-2}$ $day^{-1}$) |
|---|---|---|---|---|
| Phaeodactylum (batch culture) | 2.17 | 12.2 | 4.34 | 12.2 |
| Phaeodactylum (continuous culture) | 16.2 | 6.2 | 3.14 | 9.2 |
| Dunaliella (batch culture) | 9.1 | 3.2 | 2.10 | 5.8 |
| Dunaliella (continuous culture) | 12.0 | 4.3 | 2.77 | 7.7 |
| Monallanthus (batch culture) | 8.2 | 4.3 | 1.70 | 3.4 |
| Monallanthus (semicontinuous culture) | 12.0 | 5.8 | 2.48 | 5.0 |
| Tetraselmis (batch culture) | 19.1 | 7.3 | 4.47 | 12.3 |
| Tetraselmis (semicontinuous culture) | 14.4 | 5.5 | 3.37 | 9.3 |
| Isochrysis (batch culture) | 11.5* | 2.8 | 3.28 | 5.2 |
| Isohrysis (semicontinuous culture) | 8.6 | 2.1 | 2.45 | 3.9 |
| Botryococcus (batch culture) | 3.4 | 1.1 | 0.99 | 0.8 |

*One anomalous value for this species was 47.9 g $m^{-2}$ $day^{-1}$.

## 4.2 PROXIMATE COMPOSITIONS

The proximate compositions of N-sufficient cells grown at moderate light intensities are summarized in Table 4-2. Most algae were high in protein and contained smaller amounts of carbohydrate and lipid. The caloric content on an ash-free basis was quite uniform.

**Table 4-2. Proximate Compositions of N-Sufficient Microalgae.**

| Alga | % of Dry Weight | | | | Caloric Content | |
|---|---|---|---|---|---|---|
| | % Protein | % Carbohydrate | % Lipid | % Ash | Cal $mg^{-1}$ dry wt | Ash-free cal $mg^{-1}$ dry wt |
| Phaeodactylum | 55.0 | 10.5 | 19.8 | 11.6 | 4.81 | 5.44 |
| Dunaliella | 64.2 | 12.6 | 23.1 | 11.7 | 4.72 | 5.26 |
| Monallanthus | 41.3 | 13.9 | 20.7 | 8.3 | 5.12 | 5.55 |
| Tetraselmis | 64.4 | 10.7 | 23.4 | 9.8 | 4.73 | 5.24 |
| Isochrysis | 44.9 | 23.1 | 28.5 | 32.2 | 3.40 | 4.99 |
| Botryococcus | 24.0 | 11.0 | 29.0 | -- | -- | |
| -Comparative Values from Parsons et al. (26)- | | | | | | |
| Phaeodactylum | 33 | 24 | 6.6 | 7.6 | -- | -- |
| Dunaliella | 57 | 31.6 | 6.4 | 7.6 | -- | -- |
| Tetraselmis | 52 | 15.0 | 2.9 | 23.8 | -- | -- |

In Table 4-2 our values are compared with those of Parsons et al. (26). For Phaeodactylum we found more protein and less carbohydrate. For Dunaliella and Tetraselmis our protein levels were similar to the others reported. Our carbohydrate levels were lower in Dunaliella and similar in Tetraselmis to those reported previously. The previous lipid levels in all three organisms were very much less than ours. This is probably due to the fact that the previous methods for lipids measured only those fatty acids with >12 carbon atoms (61) while our method measures all the nonpolar material extracted by methanol-chloroform and insoluble in water.

## 4.3 EFFECTS OF NITROGEN DEFICIENCY

The effects of nitorgen deficiency on yield of cellular dry weight, protein, carbohydrate, and lipids and on efficiency are summarized in Table 4-3 for five marine microalgae. These are data that were obtained for algae growing continuously or semi-continuously, not in the batch culture mode.

Yields of dry weight, protein and lipid were reduced by N deficiency as were efficiencies of light utilization. For Dunaliella and Tetraselmis, carbohydrate yields were increased 3- to 4-fold by N deficiency. In these species deficiency might thus be a means of increasing the amount of cellular material that could be anaerobically digested to methane as an energy-producing step.

**Table 4-3. Effects of Nitrogen Deficiency on Yields and Photosynthetic Efficiencies of Several Microalgae at Moderate Light Intensity**

| Alga | N Status | Yield ($g\ m^{-2}\ day^{-1}$) Overall dry wt. | Protein | Carbohydrate | Lipid | Efficiency (%) |
|---|---|---|---|---|---|---|
| Phaeodactylum | +N | 16.2 | 9.3 | 1.78 | 3.14 | 6.2 |
| | -N | 7.7 | 2.3 | 1.23 | 2.69 | 4.1 |
| Dunaliella | +N | 12.0 | 5.8 | 1.51 | 2.10 | 4.3 |
| | -N | 10.2 | 2.7 | 5.15 | 1.69 | 2.8 |
| Monallanthus | +N | 12.0 | 5.0 | 1.67 | 2.48 | 5.8 |
| | -N | 7.5 | 1.4 | 1.26 | 1.66 | 3.7 |
| Tetraselmis | +N | 14.4 | 9.3 | 1.54 | 3.37 | 5.5 |
| | -N | 12.7 | 3.6 | 5.98 | 1.85 | 3.6 |
| Isochrysis | +N | 8.6 | 3.9 | 1.99 | 2.45 | 2.1 |
| | -N | 5.5 | 1.5 | 3.13 | 1.84 | 1.5 |

## 4.4 LIGHT INTENSITY EFFETS IN PHAEODACTYLUM

The effects of light intensity on this alga were investigated in two separate experiments. Generally moderate light intensities (about 40% of sunlight) gave the best yields and efficiencies (Tables 3-3 and 3-7) and removal of the $CuSO_4$ filter increased yields (Table 3-7), probably because of increased amounts of red light (Figure 2-2). Growth was reduced by lower and higher intensities. At reduced intensities the mean intensity seen by each cell in this well-mixed culture was probably less than that required to saturate photosynthesis. At high intensities, the cells were probably photoinhibited.

Mann and Myers (69) determined that photosynthesis ($O_2$ evolution; 1-cm culture thickness; 40 mg dry weight $L^{-1}$) in Phaeodactylum was saturated at an intensity of 0.057 cal $cm^{-2}$ $min^{-1}$, or for our culture vessel area and over a whole 12-hour day, 28,900 cal $culture^{-1}$ $day^{-1}$. Goldman (13) gives a general curve (his Figure 11, top curve) for yield versus light intensity at a similar saturation intensity ($I_s$ = 0.06 cal $cm^{-2}$ $min^{-1}$), but that curve does not include the effects of photoinhibition. At our low intensity in Phaeodactylum experiment 1 (62,715 cal $day^{-1}$) the yield from his curve would be approximately 22 g $m^{-2}$ $day^{-1}$. In batch culture we obtained a yield of 21.7 g $m^{-2}$ $day^{-1}$ at this intensity (Table 3-3). It is probable that in our well-mixed, 5-cm-thick culture with a dry weight approximately 25 times that used by Mann and Myers, the mean light seen by the cells was very close to the saturation intensity they found for a thinner, less dense culture. The agreement in these comparisons is remarkable since Mann and Myers measured short-term photosynthesis and we measured increases in dry weight over a two-day period. Using Goldman's curve to calculate yield at our medium and high

intensities, the values would be approximately 30 and 35 g $m^{-2}$ $day^{-1}$. We attribute our lower yields (Table 3-3) to photoinhibition. Further empirical studies are needed on the effect of intensity on yield and efficiency in thicker culture vessels and at various cell densities. High cell densities were used at various light levels in Phaeodactylum experiment 2, but the yields were less than those in experiment 1. Further optimization experiments on cell density, culture thickness, and intensity would have obvious applications to outdoor mass culturing.

The efficiency of light utilization by Phaeodactylum in our culture system varied greatly with culture conditions. However, our maximum efficiency, 12.2%, was very close to the value of 13% reported by Raymond (18) for this species in outdoor culture. Goldman (13) suggests that the maximum attainable efficiency would be about 20%, and this has been achieved by Myers (7) and by Kok (8). Here again, variations of culture thickness, cell density, light intensity, nutrient supply, and temperature might increase the actual measured efficiency. Further experiments to "push" the growth of any given algae in dense cultures are needed.

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# APPENDIX A

## PAPERS PRESENTED

Three papers have been presented at SERI subcontractors meetings and manuscripts of each have been delivered to SERI. These are as follows:

- Thomas, W. H. 1980. Pushing Phaeodactylum for maximum growth and energy yield. Honolulu, Hawaii, December 1980.
- Thomas, W. H., Seibert, D. L. R., Alden, M., and Neori, A. Effects of light intensity and nitrogen deficiency on yield, proximate composition, and photosynthetic efficiency of Phaeodactylum. Washington, D.C., July 1981.
- Thomas, W. H. 1982. Laboratory selection of microalgae for maximum lipid and protein production. Washington, D.C., June 1982.

# APPENDIX B

## DESERT ALGAE BIBLIOGRAPHY

An annotated bibliography of papers on algae and their habitats in the arid United States Southwest was completed in preparation for research on yields and efficiencies of desert algae. It is stored on microcomputer disk and is searchable by keywords. The bibliography has been published as a SERI technical report.

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5. Publication Date
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7. Author(s) William H. Thomas, Don L. R. Seibert, Martha Alden, Peter Eldridge, Amir Neori


8. Performing Organization Rept. No.


9. Performing Organization Name and Address
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University of California at San Diego


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(G)

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Solar Energy Research Institute
1617 Cole Boulevard
Golden, Colorado 80401

13. Type of Report & Period Covered
Technical Report

14.

15. Supplementary Notes
Technical Monitor: Andy Hill


16. Abstract (Limit: 200 words) The yields, photosynthetic efficiencies, and proximate composition of several microalgae were compared in dense cultures grown at light intensities up to 70% sunlight. Yields ranged from 3.4 to 21.7 g dry weight/$m^2$ day. The highest yield was obtained with Phaeodactylum; the lowest in Botryococcus cultures. The same species had the highest and lowest efficiencies of utilization of photosynthetically active radiation. In nitrogen-sufficient cells of all but one species, most of the dry weight consisted of protein. Lipid content of all species was 20%-29%, and carbohydrate content 11%-23%. Lipid content increased somewhat in N-deficient Phaeodactylum and Isochrysis cells, but decreased in deficient Monallanthus cells. Because the overall dry weight yield was reduced by deficiency, lipid yields did not increase. However, since the carbohydrate content increased to about 65% in N-deficient Dunaliella and Tetraselmis cells, the carbohydrate yield increased. In Phaeodactylum the optimum light intensity was about 40% of full sunlight. Most experiments with this alga included a $CUSO_4$ filter to decrease infrared irradiance. When this filter was removed, the yield increased because more red light in the photosynthetically active spectral range was included. These results should prove useful to workers attempting to maximize yields and efficiencies, but additional studies are needed.


17. Document Analysis

a. Descriptors Algae ; Chemical Composition ; Bench-Scale Experiments ; Culture Media ; Phytoplankton

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