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Light and Nitrogen Deficiency Effects on the Growth and Composition of *Phaeodactylum tricornutum*

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ABSTRACT

The marine microalgae *Phaeodactylum tricornutum,* with a high lipid content constituting 20–60% of its dry weight under controlled growth conditions (*1*), has recently come into focus as a potentially rich source of dietary marine vegetable oil. In particular, this species has a characteristic high content of eicosapentenoic acid (EPA), which has potential benefits in human nutrition, since it cannot be synthesized in vivo in the human body. Some factors that could alter the biochemical composition of *Phaeodactylum tricornutum* in favor of lipid production have been examinated in this study.

Index Entries: *Phaeodactylum tricornutum;* light intensity; nitrogen deficiency; lipid.

INTRODUCTION

Growth conditions, especially light intensity and nitrogen supply, affect not only growth rate and cell yield in culture, but also the lipid content and composition of algae. Many microalgal cells grown under nitrogen-limiting conditions have enhanced lipid content. In green algae, a lipid content ranging from 35 to 58% of their dry weight under nitrate limitation has been reported (*1*). Orcutt and Patterson (*2*) have shown

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that when *Phaeodactylum tricornutum* was grown at light intensities from 2.1 to 21 klx, the lipid content of the cells increased from 20.8 to 22.8%. The concentration of cellular lipids can be altered through manipulation of nutrients also. At low nitrogen levels, the lipid content increased from 20 to 24% (3). The synthesis of a particular lipid will depend on its exact cellular function and the environmental conditions. However, certain conditions that favor production of specific lipid compounds may limit overall biomass production. This study focuses on the changes in total lipid content of *Phaeodactylum tricornutum* with variations in light intensity and nitrogen deficiency. We here report the amount of total lipid, protein, and carbohydrate content of this species grown under various culture conditions. Data on total composition are combined with culture growth rates to estimate the production potential of lipids of nutritional interest.

MATERIALS AND METHODS

Experimental Conditions

Axenic culture of *Phaeodactylum tricornutum* was used for all studies unless otherwise indicated. All experiments were performed in 2-L airlift glass cylinders (8×54 cm) capped with a rubber stopper penetrated by an aeration inflow pipet, outflow line, and a septum allowing for aseptic syringe sampling. Inflow air from an aquarium pump was filter-sterilized by a 0.22-μm Millipore filtration unit. The cultures were positioned in a temperature-controlled room at 22°C with illumination by four cool white fluorescent bulbs. Further mixing was achieved by placing a sterile magnetic stir bar in the cylinder and setting the cylinder on top of a magnetic stirrer set at a low speed. *Phaeodactylum tricornutum* was grown on modified F/2 medium with the composition as follows (stock solutions) (g/100 mL): $NaNO_3$ 15.0, $NaH_2PO_4 \cdot 2H_2O$ 1.0, $CoCl_2 \cdot 6H_2O$ 0.002, $CuSO_4 \cdot 5H_2O$ 0.00196, $MnCl_2 \cdot 4H_2O$ 0.036, $Na_2MoO_4 \cdot 2H_2O$ 0.0013, $Na_2SiO_3 \cdot 9H_2O$ 0.5, $ZnSO_4 \cdot 7H_2O$ 0.0044, $FeCl_3 \cdot 6H_2O$ 0.244, $Na_2 \cdot EDTA \cdot 2H_2O$ 0.189. Furthermore, 0.5 mL of each stock solution was added to 1 L of sea water. The sea water was conditioned by mixing with activated charcoal and double-filtered with Whatman No. 1 filter paper.

Growth Measurement

Growth was monitored by cell count using a hemocytometer and by changes in optical density (OD) at 540 nm in 1 cm pathlength. The initial cell number in each experiment was about 5×10^4 cells mL^{-1}. After inoculation, the cultures were sampled daily for periods up to 10 d. The growth rate (k) in divisions d^{-1} was calculated from cell count data by the equation:

$$K = \text{Log}_2 N/N$$

where N = final cell number, N_0 = initial cell number during the exponential phase of growth.

Light Intensity

Light intensities were varied by adjusting the distance from 4×20W cool white fluorescent bulbs to the surface of the culture vessels. The three condition were 72 $\mu E \cdot M^{-2} \cdot sec^{-1}$, 36 $\mu E \cdot M^{-2} \cdot sec^{-1}$ and 18 $\mu E \cdot M^{-2} \cdot sec^{-1}$. Illumination was provided either continuously or on a 12-h light, 12-h dark cycle.

Nitrogen Supply

The culture was harvested by centrifugation at 3500 rpm for 15 min, and the cells rinsed with sterile F/2 medium lacking nitrogen, and resuspended into F/2 medium with 1/2 nitrogen and nitrogen-free F/2 medium. The cultures were then recultured under identical experimental conditions.

Protein Assay

Total protein was analyzed by the method of Dorsey et al. (4), a modification of Lowrey's method. One to five milliliters of the algal culture were filtered onto a Whatman 2.5-cm GF/C filter and stored at −20°C.

For the assay, the filter was thawed, and 5 mL of freshly prepared Biuret reagent were added. The samples and BSA standard tubes were then incubated at 100°C in a water bath for 60 min. Then 0.5 mL of Folin-phenol reagent (0.5*N*, Sigma) was added immediately. The tube were then cooled and equilibrated to room temperature. The tubes were centrifuged at 3000 rpm for 5 min, and the absorbance of the supernatant read at 660 nm in a Beckman-DU50 spectrophotometer.

Total Carbohydrate Assay

Total carbohydrates were determined by the method of Kochert (5). From 5–20 mL of the algal culture were filtered onto 2.5 cm GF/C glass-fiber filters and rinsed with isotonic ammonium formate. The filters were then stored at −20°C until assayed. Following thawing at room temperature, 5 mL of 1*M* H_2SO_4 were added and heated at 100°C in a water bath for 60 min, cooled to room temperature, and centrifuged at 3000 rpm for 5 min. The supernatant was analyzed for total carbohydrate content. Total carbohydrate content of the algal sample was determined graphically from glucose standard curve at 485 nm.

Total Lipid Extraction and Determination

Total lipid was determined according to the method of Kochert G. (5). Samples ranging from 20–25 mL of algal culture were filtered onto a 2.5-cm GF/C, washed with 10 mL isotonic ammonium formate solution,

and stored at −20°C until assayed. For assay, the filters were thawed and 10 mL of ice-cold 0.2*N* $HCLO_4$ added for extraction of low-molecular-weight components. The pellets contain the macromolecular components. Ten milliliters of chloroform–methanol solution (2:1 v/v) were added to the pellets from the $HCLO_4$ extraction. The pellets were resuspended for 5 min at room temperature, and the samples were centrifuged at 3500 rpm for 30 min and supernatants retained. The extraction was repeated with 5 mL of chloroform–methanol, and the supernatants combined and retained for lipid determination.

To 3 mL of water were added to the 15 mL of combined chloroform–methanol extracts of the cells. The solution was shaken for 5 min to mix well and centrifuged at 3500 rpm for 20 min to separate the phases. The organic phase was collected and the aqueous phase discarded. The chloroform–methanol solution was evaporated under a stream of N_2 to a final volume of 2 mL. 0.1, 0.2, 0.3, 0.4, and 0.5 mL of lipid standard solution (palmitic acid, 1 mg mL^{-1}) were transferred to marked 10 mL screw-capped tubes. All tubes were evaporated to dryness under a stream of N_2; 2 mL of dichromate solution were added to all tubes, capped with Teflon™-lined caps, and set in a boiling water bath for 45 min. After cooling, the tubes were diluted to 10 mL with water and the absorbance of each tube read at 350 nm. Total lipid content of the algal samples was determined graphically from the palmitic standard curve.

RESULTS

The Effect of Light Intensity and Illumination Cycle on Growth

The cell count data for *Phaeodactylum tricornutum* under three different light intensities are presented in Table 1 and as plots of logarithm of cell number vs time in Fig. 1. The three light intensities produced the light-limited growth rates (k) of 2.19 (72 $\mu E \cdot M^{-2} \cdot s^{-1}$, 2.08 (36 $\mu E \cdot M^{-2} \cdot s^{-1}$) and 1.08 (18 $\mu E \cdot M^{-2} \cdot s^{-1}$) during the exponential phase.

The cell number increased as the incident light intensity increased, with markedly reduced growth at the low light intensity. The cell number increased 1230-fold, 965-fold, and 95.4-fold in the 8 d at the light intensities from high to low respectively.

When the illuimination was provided either continuously or on a 12-h light, 12-h dark cycle, the dependence of growth rate on illumination over the entire range of light intensities is indicated in Table 2 and Fig. 2. A lower growth rate was apparent under 12:12 h LD cycle than continuous light. The experimental resolution in this light intensity region was not sufficient to provide statistical confidence limits.

Table 1
Daily Cell Count Data ($\times 10^4$ cells mL^{-1})
and Cell Density (OD at 540 nm) in Light-Limited Growth Experiments
on *Phaeodactylum tricornutum* under Three Different Light Intensities

	72 $\mu E \cdot M^{-2} \cdot s^{-1}$		36 $\mu E \cdot M^{-2} \cdot s^{-1}$		18 $\mu E \cdot M^{-2} \cdot s^{-1}$	
Days	Cell number	OD	Cell number	OD	Cell number	OD
0	5×10^4	0.005	5×10^4	0.005	5×10^4	0.005
1	9×10^4	0.005	5×10^4	0.005	5×10^4	0.005
2	19×10^4	0.020	16×10^4	0.011	6×10^4	0.005
3	-	-	-	-		
4	-	-	-	-		
5	840×10^4	0.491	545×10^4	0.426	53×10^4	0.075
6	2110×10^4	0.843	1275×10^4	0.707	116×10^4	0.146
7	2920×10^4	0.917	2615×10^4	0.887	273×10^4	0.232
8	6150×10^4	1.000	4825×10^4	0.981	477×10^4	0.366
9	5525×10^4	0.954	4800×10^4	0.939	680×10^4	0.466

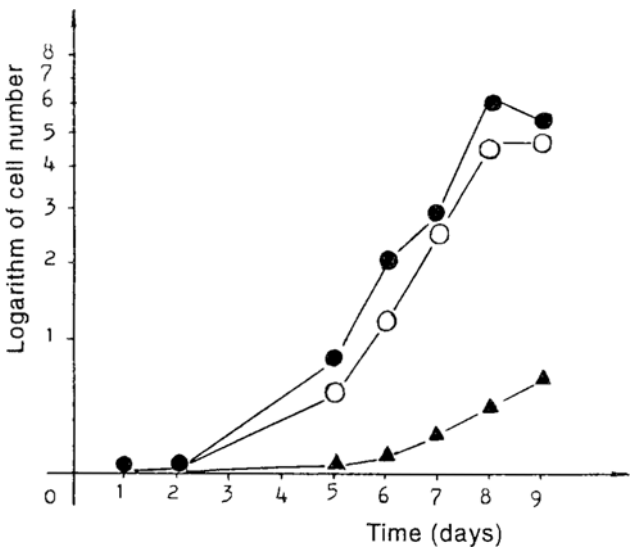

Fig. 1. The growth curves of *Phaeodactylum tricornutum* vs light intensities. ● 72 $\mu E \cdot M^{-2} \cdot s^{-1}$, ○ 36 $\mu E \cdot M^{-2} \cdot s^{-1}$, ▲ 18 $\mu E \cdot M^{-2} \cdot s^{-1}$.

The growth rate of *Phaeodactylum tricornutum* was dependent on the supply of light energy, i.e., the cell number increased 555-fold and 334-fold in the 11 d at continuous light and 12:12 h LD cycle, respectively. The mean growth rate (K) was equal to 1.70 and 1.48, respectively.

Table 2
Daily Cell Count Data ($\times 10^4$ cells mL^{-1})
and Cell Density (OD at 540 nm) under the Illumination
of Continuous Light and 12:12 h LD cycle on *Phaeodactylum tricornutum*

	Continuous light		12:12 h LD cycle	
Days	Cell number	OD	Cell number	OD
0	5×10^4	0.005	5×10^4	0.005
1	9×10^4	0.006	6×10^4	0.006
2	–	–	–	–
3	–	–	–	–
4	225×10^4	0.224	102×10^4	0.080
5	490×10^4	0.391	232×10^4	0.182
6	940×10^4	0.490	404×10^4	0.292
7	1025×10^4	0.597	648×10^4	0.414
8	1220×10^4	0.657	1018×10^4	0.497
9	–	–	–	–
10	–	–	–	–
11	2775×10^4	0.820	1670×10^4	0.555

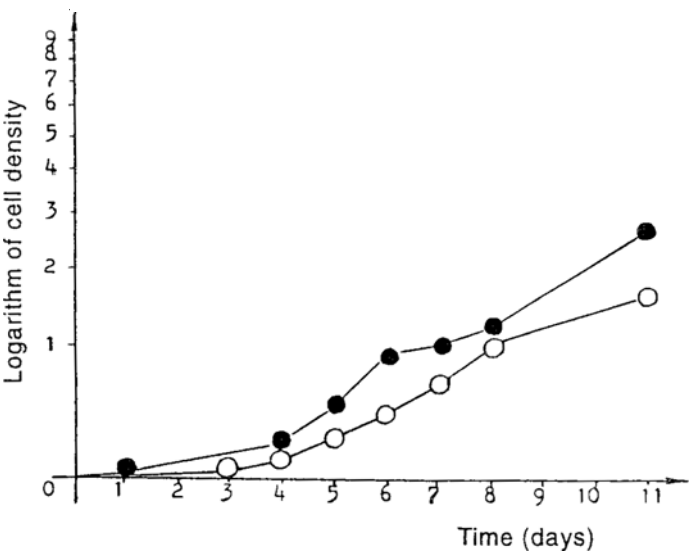

Fig. 2. The growth curves of *Phaeodactylum tricornutum* vs LD cycle. ● Continuous light, ○ 12:12 h LD cycle.

The Effect of Nitrogen Starvation

Under the conditions of nitrogen starvation, the growth of nitrogen-starved cultures was nonexponential, with a mean growth rate, K, over 9 d at -0.59. The cell number increased only marginally over the whole growth period. *Phaeodactylum tricornutum* exhibited a constant, exponential growth in the 0.88 mM N medium, and a reduced calculated growth

Table 3
The Effect of Nitrogen Concentration (0.88 m*M*, 0.44 m*M*, and Nitrogen-Free Medium) on Daily Cell Count Data ($\times 10^4$ cells mL^{-1}) and Cell Density (OD at 540 nm) of *Phaeodactylum tricornutum*

	8.88 m*M* N		0.44 m*M* N		N-free	
Days	Cell number	OD	Cell number	OD	Cell number	OD
0	43×10^4		43×10^4		43×10^4	
1	62×10^4	0.058	51×10^4	0.061	52×10^4	0.064
2	129×10^4	0.112	94×10^4	0.117	67×10^4	0.080
3	650×10^4	0.256	354×10^4	0.254	68×10^4	0.069
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	2060×10^4	0.677	1205×10^4	0.552	79×10^4	0.078
7	2210×10^4	0.728	1640×10^4	0.583	123×10^4	0.072
8	2935×10^4	0.944	1645×10^4	0.743	108×10^4	0.078
9	4860×10^4	0.981	2235×10^4	0.762	97×10^4	0.086
10	4365×10^4	0.854	1945×10^4	0.680	87×10^4	0.079

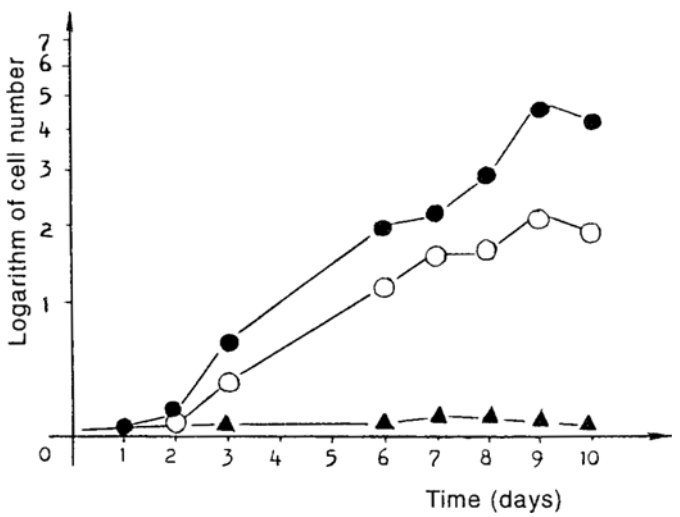

Fig. 3. The growth curves of *Phaeodactylum tricornutum* vs the different nitrogen concentrations in medium. ● 0.88 m*M* N, ○ 0.44 m*M* N, ▲ N free.

rate of about a half of *K* at 0.88 m*M* N when grown in the 0.44 m*M* N medium. The mean growth rate (*K*) over the 9 d was 1.10 and 0.76 in 0.88 m*M* and 0.44 m*M* N medium respectively (Table 3 and Fig. 3).

Cellular Composition

The total lipid as percent dry weight increased as light intensity increased under the different light intensities (Table 4 and Fig. 4).

Table 4
Total Lipid and Biochemical Composition of *Phaeodactylum tricornutum* under the Various Light Intensities after Growth in F/2 Medium for 9 d

Light intensity	Dry weight, mg mL^{-1}	Total lipid, %	Protein, %	Carbohydrate, %
72 $\mu E \cdot M^{-2} \cdot s^{-1}$	0.464	29.52	37.85	17.50
36 $\mu E \cdot M^{-2} \cdot s^{-1}$	0.324	15.90	46.48	24.90
18 $\mu E \cdot M^{-2} \cdot s^{-1}$	0.186	9.20	50.90	27.70

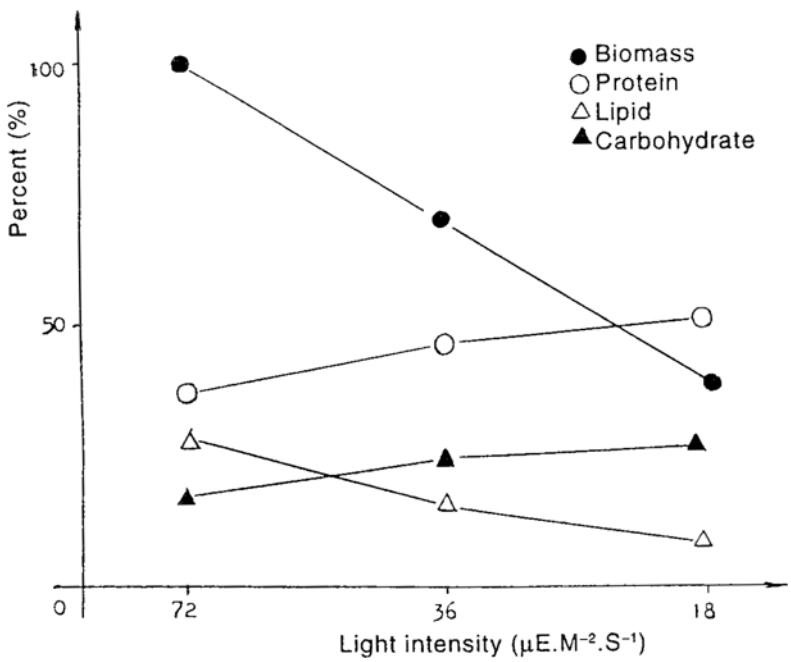

Fig. 4. The biochemical composition (lipid, protein, carbohydrate, and biomass) of *Phaeodactylum tricornutum* under different light intensities in F/2 medium for 9 d.

Lower total lipid level was associated with faster growing cultures in N-sufficient medium (Table 5). The total lipid (in percent dry weight) increased in N-deficient medium with higher light intensity, subsequently, a decline in content of protein and carbohydrate occurred. There was a very sharp decline in biomass at nitrogen-free medium as shown in Table 5 and Fig. 5.

Overall, the biomass of *Phaeodactylum tricornutum* declined sharply as both light intensity decreased from higher to lower and nitrogen level decreased from N-sufficient to N-free conditions with higher light intensity. Although the lipid content was enhanced under the N-limited medium with higher light intensity, it was accompanied by the decline both of protein content and carbohydrate content (Fig. 6).

Table 5
The Total Lipid, Protein, and Carbohydrate Content of *Phaeodactylum tricornutum* at N-Sufficient, N-Limited, and N-Free Medium after 10 d of Growth in the Highest Incident Light Intensity (72 $\mu E \cdot M^{-2} \cdot s^{-1}$)

Nitrogen concentration	Dry weight, mg mL^{-1}	Lipid, %	Protein, %	Carbohydrate, %
0.88 m*M*	0.615	28.46	32.20	18.50
0.44 m*M*	0.380	31.80	24.76	16.70
N-free	0.130	41.30	15.78	14.60

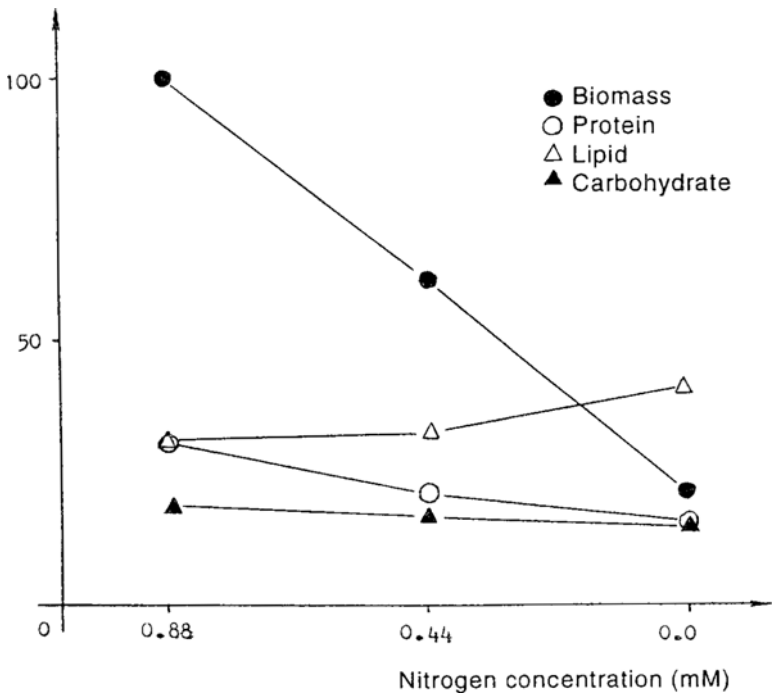

Fig. 5. Changes in composition of lipid, protein, and carbohydrate with biomass of *Phaeodactylum tricornutum* under different nitrogen concentrations after 10 d of growth.

DISCUSSION

This study was undertaken in the laboratory, using axenic conditions to ascribe definitively any changes in the lipids to physiological responses by the algae *Phaeodactylum tricornutum*. *Phaeodactylum tricornutum* was capable of maintaining higher rates of cell division in environments characterized by higher light intensities that allow *Phaeodactylum tricornutum* to have a growth rate (K) of 2.19. There is a particular scarcity of experiments conducted in low light intensities. The logarithmic growth rate was

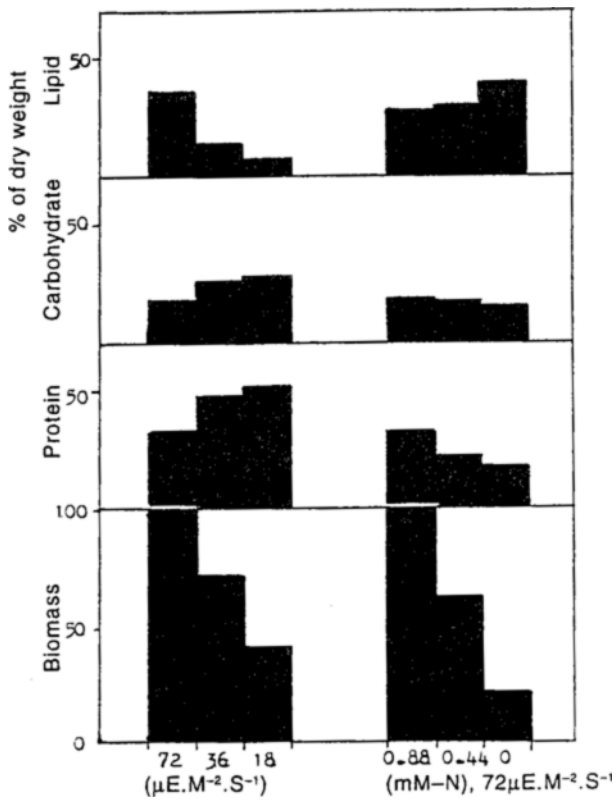

Fig. 6. Interchange of content on cellular lipid, protein, and carbohydrate under the different light intensities and nitrogen levels. Simultaneously, the biomass growth under the above conditions is shown at the bottom.

inhibited at suboptimal concentration of nitrogen until the culture reached nitrogen deficiency. However, higher light intensity and nitrogen sufficiency play an important role in maintaining higher yield with higher growth rate. In the conditions of this experiment, the growth rate of *Phaeodactylum tricornutum* was a function of irradiance and nitrogen-sufficient conditions.

Environmental factors that affect growth rate and cell yield in culture also affect the lipid content and composition of *Phaeodactylum tricornutum*. Nutrient deficiencies, other than N deficiency, may lead to an increase in the cell lipid content. In all the algae, increasing N levels led to an increase in chlorophyll as well as biomass and protein content. At low N levels, green algae had a high lipid content of 35 to 58% of the dry weight. This compares to a total lipid content of 11 to 20% of dry weight at high N levels. Differences in biochemical composition were also apparent in higher irradiance and nitrogen deficiency in *Phaeodactylum tricornutum* in this experiment.

The percentage of the total protein fraction was negatively correlated with irradiance, but this change in percentage is owing to increases in the

cell content of the other fractions, particularly lipid. For the most part, a decrease in protein was compensated for by an increase in irradiance and with increasing nitrogen limitation in the percentage incorporated into lipid. Polysaccharides are produced at a high rate during the light period and consumed in the dark. In this study, the high percentage lipid of *Phaeodactylum tricornutum* is accompanied by a low percentage content of total carbohydrate. In addition, for *Phaeodactylum tricornutum,* lipid content increased with irradiance, perhaps indicating that it is serving at least in part as a long-term storage product. Thus, the awareness that *Phaeodactylum tricornutum* is a source of high content of lipids under controlled growth conditions, especially high light intensity and nitrogen deficiency, may lead to greater advances in the commercial mass culture of this alga.

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