Enhancing Growth and Lipid Productivity of the Marine Diatom Chaetoceros sp

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Abstract: This research was conducted to study the effect of cultivating the diatom *Chaetoceros sp* under different nitrogen concentrations and varying light and dark periods on growth and lipid productivity. The diatom *Chaetoceros sp* was cultivated using the F2 nutrient medium, prepared with three different nitrogen concentrations (10%, 50%, and 100%) as a percentage of the recommended concentration for preparing the F2 medium, and under different light and dark cycles (18:6 and 12:12 hours of light: dark). Growth rate, chlorophyll *a* content, dry weight, and total lipid content were measured and evaluated as response factors to the interaction of the studied growth conditions. The results showed that cultivating *Chaetoceros sp* in the nutrient medium with 10% nitrogen inhibited growth rate and significantly reduced chlorophyll *a* content and dry weight. Although there was a noticeable decrease in total lipid content under the same conditions, the effect was less pronounced. The results also indicated a significant decrease in growth rate and dry weight of cultures grown in the nutrient medium with 50% reduced nitrogen concentration. However, the total lipid content recorded a significant increase (21.12%), particularly under the 12:12 (light: dark) regime, while chlorophyll *a* content did not show significant variation compared to the medium supplemented with a higher nitrogen concentration (100%).

Keywords: *Chaetoceros* sp., enhancing, growth, lipid, nitrogen, light

Introduction:

Diatoms are a distinct group of photosynthetic microalgae known for their unique siliceous skeleton, or frustule. They are regarded as cosmopolitan phytoplankton because they inhabit nearly every aquatic environment, including both freshwater and marine ecosystems, with approximately 200,000 recognized species. Diatoms are dynamic microorganisms with rich biodiversity due to their ability to adapt to diverse environments, including extreme and oscillating conditions (e.g., solar, osmotic, oxidative and nutrient stress). Diatoms are an outstanding source of diverse substances with potential applications in various industries, including bioenergy, pharmaceutical, nutraceutical, chemical, feed and food [1,2]. *Chaetoceros* is widely used in aquaculture due to its high nutritional value (lipids, sterols, proteins, vitamins), *Chaetoceros* sp. is also easily fed and digested by aquatic organisms, which can improve the survival rate of fish, shrimp and crab larvae, and is an important bait for aquaculture [3]. However, the low growth rate and poor light adaptability of *Chaetoceros* sp. limit their applications as aquatic food. It is well known that nitrogen is a critical and essential element for seaweeds, playing a key role in the growth and biosynthesis of various structural and physiological compounds, including proteins, nucleic acids, and pigments [4, 5].

Previous studies have demonstrated that nitrogen deficiency typically leads to reduced growth rates and lower levels of nitrogenous compounds in algae, including chlorophyll *a*, phycobiliproteins, proteins, and amino acids [6, 7]. Conversely, under nitrogen stress, soluble polysaccharides and lipids in algae tend to increase [5, 6]. During nitrogen starvation, reductions in pigment content are often accompanied by decreased quantities and activity of Photosystem II, [8, 9]. Achieving the right balance between maximum growth and maximum lipid content and productivity is the primary goal of many experimental works to ensure cost-effective biodiesel production from microalgae. The aim of this study is to optimize the culturing conditions and enhance the growth and as a consequence, the biomass production of *Chaetoceros*, along with improving its biochemical composition.

Material and Methods

Chaetoceros sp. culturing and preparation

In this research, the f/2 medium was used as the basic culturing medium [3]. The cultivation took place on a shaker table with a temperature of 25 ± 0.5 °C, at light intensity 2500 ± 100 lx. The seawater was filtered by silicon nitride nanomembrane filters and media were sterilized at 121 °C for 20 min and then cooled. Batch cultures, conical flasks each 500 ml, were implemented and inoculated with the target diatom species. Three nitrogen concentrations were tested (free nitrogen media, 50% nitrogen reduced media and full nitrogen enriched media), each treatment was implemented triplicates under two light: dark regimes (18: 6 and 12: 12 light: dark cycle).

Growth rate

Growth rate was estimated by cell counting using Improved Neubauer haemocytometer (Germany) as described by Wood *et al*. (2005), Growth rate was calculated according to the formula:

$$r = \ln (F1-F0)/t1-t0$$

Where F1 is the number of cells at t1 (time1), F0 is the number of cells at t0 (time 0).

Chlorophyll *a* content

Chlorophyll *a* was determined following the method of Henriques [10], where aliquots were collected from each growing culture at the mid- exponential growth phase, centrifuged, the suspension was discarded and the pellet was re-suspended in distilled water to remove any salts that might retain on the biomass. Centrifugation was repeated three times, then ten milliliter of acetone 90% was added to each tube containing the pellet with strong vortex mixing for 15 seconds, and stored in a refrigerator in the dark overnight. After incubation, the aliquots were then centrifuged at 3500 rpm for 15 minutes. The supernatant was transferred to another tube and the volume was made up to 15ml using acetone 90%. The absorbance of the resulting pigment extracts against the blank (acetone 90%) were measured by spectrophotometer. Chlorophyll *a* content (µg chlorophyll *a*/ml culture) in the extracts were determined using the following equation:

$$\text{Chl. } a \text{ } (\mu g \text{ } ml^{-1}) = (11.85A664 - 1.54A647 - 0.08A630)v/lV$$

Where, Axxx is the absorbance at xxx nm, after subtracting the sample absorbance at 750 nm

Estimation of the dry weight

Aliquots were collected from each growing culture at the mid-exponential growth phase, and centrifuged at 3500 rpm for 10 minutes, the suspension discarded and the pellet re-suspended in distilled water to remove any salts that could have been retained with the biomass, centrifuged again, and the same procedure repeated three times. The pellet was dried in an oven at 105˚C for 24 hours, taken out the oven and kept in a desiccator, and weighed using analytical balance.

Dry weigh (dw) expressed as mg/l-1, and calculated according to the following formula:

$$\text{Dry weight (dw mg/l-1)} = (wt-wi)/v$$

Measurement of total lipid content

Total lipid content was extracted according to Bligh and Dyer using mixture of organic solvents and estimated gravimetrically as described by Sündermann [11]. Aliquot was collected and centrifuged, then the pellet was freeze-dried (lyophilized) and weighed. For extraction, the freeze-dried material re-suspended in 4 ml dH_2O, then 10 ml of methanol and 5 ml of chloroform were added, resulting in a 10:5:4 ratio of methanol: chloroform: water, respectively. After overnight extraction at room temperature, 5 ml of water and 5 ml of chloroform were added, tubes centrifuged at 3500 rpm for 10 minutes, the chloroform lower layer which contains the extracted lipid was removed and placed into a pre-weighed vial, then the chloroform evaporated by heating the vials in a water bath at 55±1˚C, followed by 1 hour in an oven at 105±1˚C, vials weighed again, where the difference in the weight represents the weight of the total extracted lipid. Total lipid content represented as percentage of total dry weight.

Statistical analysis

Data was statistically analyzed using SPSS (two-way ANOVA), tukey's post hoc test was used following an ANOVA with a significant result.

Results and discussion

Table 1. summarizes the results obtained; figures 1-4 presenting the interactions of the two factors tested for each of the parameters measured. Results showing that growth rate and chlorophyll *a* content were restricted under nitrogen starvation conditions across both light regimes tested (figure 1, 2); this suggests that nitrogen is a limiting factor for growth under such these conditions. The highest growth rate, coupled with the greatest dry weight (0.82 d/day^{-1} and 198 mg/l^{-1} respectively) were observed under the 18: 6 light: dark regime with fully nitrogen-supplemented media; notably, the growth rate was consistently significantly ($p \leq 05$) higher under the 18:6 light regime and with full nitrogen supplementation. On the other hand, the two nitrogen formulations (50% and 100%), and both of light: dark cycles tested, had no significant effect on chlorophyll *a* content (figure 2); this might be attributed to the time where samples were collected for chlorophyll *a* estimation, as mentioned in the previous section, all samples were collected at mid-exponential phase and after six hours of light exposure, where nitrogen and other nutrients were available in sufficient amounts before cultures reach stationary phase, as the cultures received adequate light exposure for synthesizing chlorophyll *a* molecules. In contrast, total lipid content was significantly ($p \leq 05$) higher under 12: 12 light: dark regime compare to 18: 6 light: dark regime, this was noticeably higher under 12: 12 light: dark cycle (figure 4), this was only observed when nitrogen was available. It has been reported that reducing the content of nitrogen and phosphorus in the culture medium is widely used to change the content and productivity of lipids in microalgae [12]. Previous studies showed that maintaining diatoms batch cultures under an optimized Guillard f/2 growth medium with four additions of concentrated macronutrient solution, resulted in higher protein content and lower content of carbohydrates and lipids [2]. Increasing

in total lipid content of microalgae under various light: dark regimes was also reported [13].

Table 1. Effect of two light: dark regimes and three nitrogen enriched growth media on the Growth rate, Chlorophyll *a*, Dry weight and Total lipid content

light: dark cycle	Nitrogen conc (%)	Growth rate (d/day^{-1})	Chlorophyll a (µg/ml^{-1})	Dry weight (mg/l^{-1})	Total lipid content (%)
18: 6	N10%	0.14 ± 0.01	0.09 ± 0.01	72.2 ± 4.2	11.49 ± 1.16
	N50%	0.53 ± 0.01	1.74 ± 0.05	149.8 ± 2.4	16.97 ± 2.01
	N100%	0.82 ± 0.02	1.81 ± 0.11	198.3 ± 2.1	14.69 ± 1.86
12:12	N10%	0.13 ± 0.01	0.11 ± 0.08	74.1 ± 3.3	10.09 ± 1.28
	N50%	0.48 ± 0.02	1.80 ± 0.06	149.5 ± 3.1	21.12 ± 2.09
	N100%	0.66 ± 0.01	1.78 ± 0.02	181.2 ± 1.7	18.19 ± 0.96

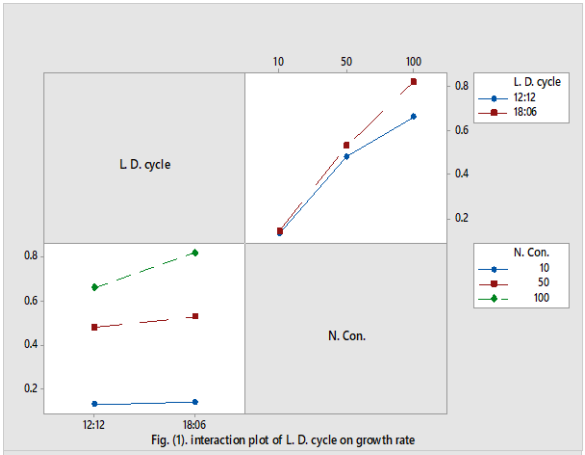

Fig. (1). interaction plot of L. D. cycle on growth rate

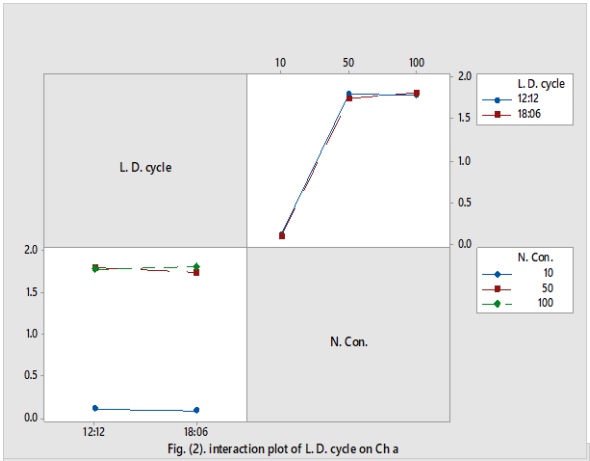

Fig. (2). interaction plot of L. D. cycle on Ch a

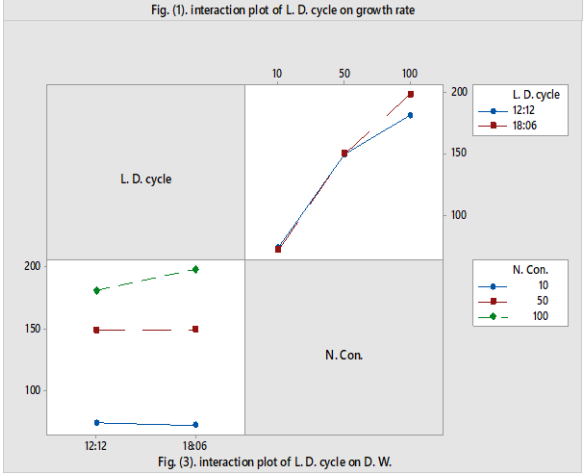

Fig. (3). interaction plot of L. D. cycle on D. W.

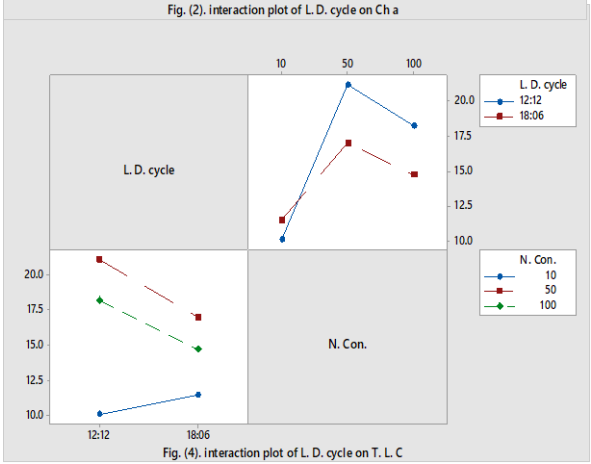

Fig. (4). interaction plot of L. D. cycle on T. L. C

*L.D. cycle (light dark cycle 18:06, 12:12); N.Con. 10%, 50%, 100% (nitrogen concentration as (%) of the original f2 media); Ch a (chlorophyll *a* content); T.L.C. (total lipid content)

Conclusions

This study provides insights into the physiological changes of *Chaetoceros* sp. under nitrogen stress conditions and varying light-dark periods. It offers foundational knowledge for develop in cultivation strategies for this species, demonstrating that manipulating growth conditions plays an important role in enhancing the growth and productivity of microalgae

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