

Short communication:

Effect of light and nitrogen concentration on the growth and lipid content of marine diatom, *Chaetoceros calcitrans*

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Received: May 2017

Accepted: July 2017

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Keywords: *Chaetoceros calcitrans*, Light, Nutrient levels, Experimental conditions

Introduction

Microalgae grow in both seawater and freshwater (Carlsson *et al.*, 2007). They have some similarities to terrestrial plants with different growth media and efficient access to water, carbon dioxide and other nutrients, but have higher growth rates and lipid content with high yield per unit area. There are several important applications of microalgae such as animal feed, playing a valuable role in cosmetics, biodiesel production and pharmaceuticals (Olaizola, 2003; Soares *et al.*, 2006; Patil *et al.*, 2008; Huang *et al.*, 2010). In addition, they are more beneficial than corn, soybean and palm oil in biofuel production in the world (Chisti, 2007; Lora and Andrade, 2009; Mata *et al.*, 2010). Most diatoms microalgae such as

Chaetoceros calcitrans are cultivated in bioreactors and as a cosmetic and pharmaceutical than food production at the moment (Chisti, 2007; Janaun and Ellis, 2010). These diatoms with its high lipid content has some major pigments such as chlorophyll *a*, *c*₁ and *c*₂, xanthophylls (fucoxanthin) and carotenes which are used mainly as reserve substances (Lourenco, 2006; De stefano *et al.*, 2009).

C. calcitrans produces more lipid under unfavourable and stress conditions such as nutrient deficiency and low light densities (Hu *et al.*, 2008). Under desirable growth conditions, the *C. calcitrans* synthesize fatty acids for esterification into membrane lipids constituting about 20-50% of their dry weight, while in

unfavourable conditions, the lipid content changes via biosynthetic pathways for the formation and accumulation of neutral lipids, such as the triacylglycerols (TAG). Furthermore, stress causes some change in bioenergetics and biochemical components by increasing the biopolymers and lipids (Alyabyev *et al.*, 2007).

The growth of microalgae and their compositions are influenced by light and nutrients in the growth media which are essential for suitable growth and lipid production in diatoms. Although there are many studies on the effects of light and nutrients on microalgal growth, few studies are related to nutrient and light limitations in *C. calcitrans* (Krichnavaruk *et al.*, 2004). Nutrients and light are the two significant factors in microalgal growth that occur simultaneously. The main hypothesis of this research is to find how light and nutrients are required to culture *C. calcitrans* to obtain the high yield and lipid amounts. This study aimed to evaluate the growth and lipid contents of the diatom, *C. calcitrans* cultivated at different light and nutrient levels.

Materials and methods

This experiment was carried out in the algal laboratory of the National Inland Water Aquaculture Institute (NIWAI), Bandar-eAnzali, Iran. *C. calcitrans* obtained from the Shahed University, Tehran, Iran. F/2 medium used in this experiment was prepared according to the procedure of Guillard (Guillard, 1975). 75 g NaNO₃ and 5 g NaH₂PO₄

H₂O were dissolved in 1 liter of filtered sea water and labelled as solution A (Wati Pal *et al.*, 2013).

C. calcitrans pure sample was placed in a controlled environment to grow or reproduce. The procedure used for this experiment was adapted from Probert and Klaas (1999). 100 mL of the prepared F/2 media was filled into 250 mL Conical culture flasks which were covered using corks and aluminum foil and autoclaved. Then, the flasks were removed and left overnight to cool in the algae room. All the flasks were inoculated with 10% of 2-3 days of exponentially grown *C. calcitrans*.

The above setup was prepared in the laminar flow cabinet to avoid contamination. Once the inlet and exhaust lines were fixed, the flasks were ready for setup in the algae room. Some of the parameters that were kept constant were: temperature 18 °C, pH 7-9, salinity 20-35 ppt. Aeration was controlled by opening or closing the switch valves linked to the inlet line and the cell starter culture the cells were calculated in terms of cells mL⁻¹. the inoculation required 10% of the starter culture. During the preparation of all the twelve flasks, efforts were made to ensure that approximately the same number of cells was transferred.

The light density was measured as the $\mu \text{ mol m}^{-2} \text{ s}^{-1}$ that is a standardized unit of measurement of the light intensity (which can also be called “illuminance” or “illumination”) - as an example for reference purposes.

The flasks were placed at 80, 160, and 240 $\mu \text{ mol m}^{-2} \text{ s}^{-1}$ from the light

source. The growth performance was measured in terms of cell density mL^{-1} . Eighteen (12) trails were carried out that was six treatments with three replicates of each. Totally twelve treatments were based on the different light intensity; 80,160 and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 25, 50, 75 and 100% nutrient levels for 30 days experiments. The F/2 media was kept as a control.

The cell density was estimated by Hemocytometer by counting at a day interval time until the culture reached a stationary stage (Polkinghorne, 2010). Lipid content was analyzed during the stationary phase of culture (20 days). A sample of 1 mL from every flask was taken and averages of 3 counts were done. This experiment was repeated two times to confirm and reconfirm results that were obtained in the first set.

Biomass concentration was determined every day by measuring the optical density at 680 nm with 2000 spectrophotometer.

The statistical analysis used in this experiment was 2-way ANOVA. The comparison of the differences between treatments was made by the LSD Test. A p value less than 0.05 was used as significant difference.

Results and discussion

C. calcitrans microalgae require number of factors such as light and nutrients for optimum growth. Experimental results showed that the growth of *C. calcitrans* was highly influenced by nutrition levels and light density. As indicated in the Table 1, maximum growth was reached in cell

count of *C. calcitrans*, slowed down and then started to decrease. The highest cell count (402×10^6) of the *C. calcitrans* was obtained at 100% nutrient level compared with the lowest at 25% (279×10^6) 20 days after inoculation (Table 1). There was no significant difference between control (400×10^6) and 100% nutrient 20 days after algae cell inoculation ($p > 0.05$).

According to the average growth rate, the cell count increased gradually between 5 to 10 days of inoculation with the lowest on the 25th day. Results showed no significant differences between treatments after 5 days of media enrichment with the experimental nutrients.

The growth of the 100% enrichment was 3.33% less than the control at the end of experiment, although there were no significant differences between the treatments. On the 15th day, the higher concentration was also observed for the 100%, followed by the 75, 50 and 25%. No difference was observed between the 100, 75 and 50% nutrient treatments, while the 25% treatment showed a decline of growth by 16% compared to the 50% treatment. The maximum and minimum variations between the control and 100% concentration were observed on the 25th and 20th days after enrichment, respectively. Also, the minimum difference in cell count during the study was observed on the 20th day.

Results showed a similar growth pattern in the initial rate until the 10th day. The growth increased from the 10th day going up with the enrichment from 200×10^6 to 400×10^6 on the 20th day of

enrichment. On the 20th day, a significant reduction in the cell concentration was obtained compared with the 20th day (25% treatment). The maximum growth phase was from 5-10th day. The 3 times increase was

observed on the 5th to 10th days for the control treatment. The cell count on the 15th and 30th day was similar at 300×10^6 cells (Table 1).

Table 1: The average cell count of *Chaetoceros calcitrans* for the different nutrient treatments.

Nutrient (%) / day	Average cell count $\times 10^6$					
	5	10	15	20	25	30
Control	65	200	300	400	380	300
25	40	125	240	279	240	220
50	45	150	290	299	245	230
75	50	160	300	395	295	260
100	55	170	325	402	310	290

Table 2: The average Chlorophyll *a* and cell density within different nutrient concentrations and light intensity.

Nutrient (%)	80 $\mu\text{mol m}^{-2} \text{s}^{-1}$		160 $\mu\text{mol m}^{-2} \text{s}^{-1}$		240 $\mu\text{mol m}^{-2} \text{s}^{-1}$	
	Chlorophyll <i>a</i>	Cell density $\times 10^6$	Chlorophyll <i>a</i>	Cell density $\times 10^6$	Chlorophyll <i>a</i>	Cell density $\times 10^6$
control	0.99	0.06	1.05	0.062	1.13	0.065
25	1.17	0.07	1.31	0.074	1.43	0.076
50	1.41	0.0703	1.52	0.076	1.59	0.080
75	1.63	0.081	1.78	0.083	1.89	0.085
100	1.86	0.084	1.9	0.087	1.99	0.090

Optical Density ($\text{OD}_{680\text{nm}}$) and cell counts at 4 different light densities showed a significant positive correlation between them (Fig. 1). The optical density (OD_{680}) in the control, 80, 160, and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were 1.12, 1.15, 1.05 and 0.78, respectively.

The growth curves obtained from the cultures grown at salinities of 15 and 25 were fairly similar, with very sharp exponential growth phases, and only differed on the last culture day, when cell density at a salinity of 25 was significantly higher (Fig. 2). In the cultures performed at a salinity of 25, the growth curve demonstrated a lower development of algal population throughout the culture.

C. calcitrans was cultivated under 4 different light intensities. *C. calcitrans* yield was the highest at the lowest light density at 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$. As shown in Fig 1, the algae reached to maximum growth, slows and starts to decrease. This Fig. indicates the phases: exponential phase (growth phase), stationary phase (maximum point reached) then the death phase.

Results showed that the lipid yield decreased significantly with increasing light levels. The total lipid was significantly higher (14.41%) in the control compared with other treatment. The total lipid at 80, 160, and 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were 13.31, 12.09 and 10.17%, respectively.

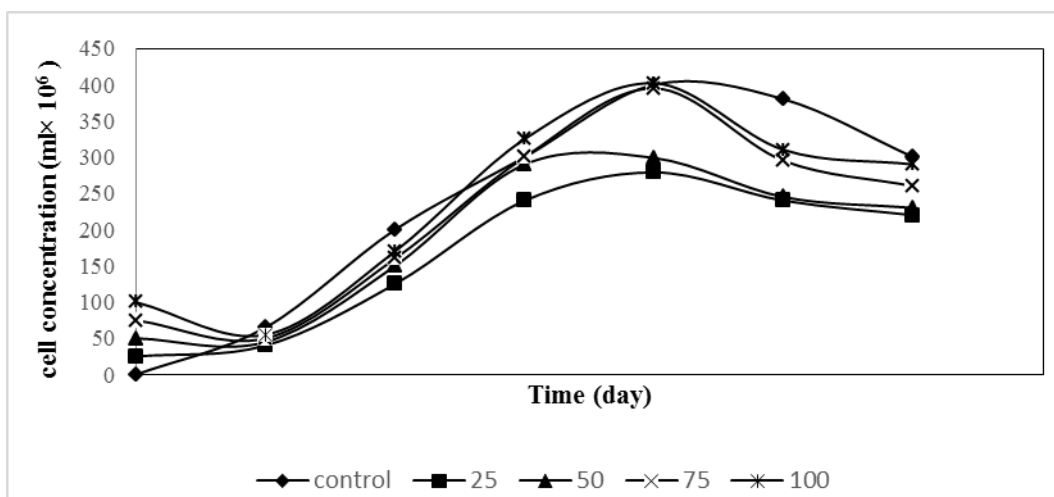


Figure 1: Growth (cell concentration) of *Chaetoceros calcitrans* cultivated under different levels of nutrients.

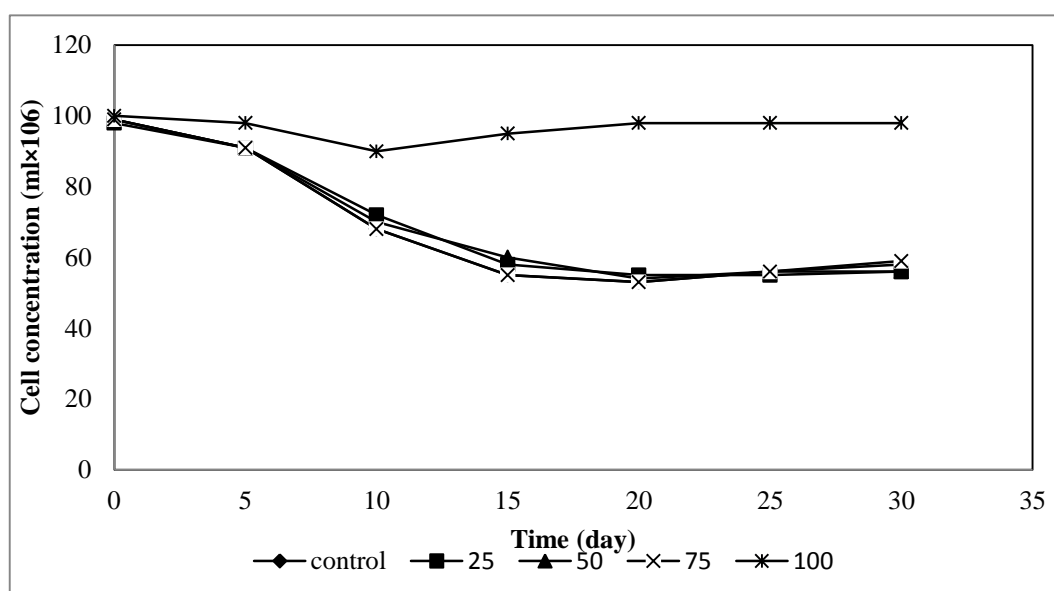


Figure 2: Growth (cell concentration) of *Chaetoceros calcitrans* cultivated under different levels of nutrients and light intensity.

The results of this experiment showed that unlike the nutrient availability, the light density has significant effects on the growth, cell concentration, and chlorophyll *a* content of *C. calcitrans*. The culture media enrichment causes increase in the cell concentration which is in agreement with findings of Cucchiari *et al.* (2008). Similar to the results of Herndon and Cochlan (2007) and De la Cruz *et al.* (2006) who showed that the growth of *Chaetoceros*

algae and cell concentrations decreased with increasing nutrients. *C. calcitrans* under low nutrient levels the light, in the present study, was more effective on the growth and cell concentration. This experiment is in support of Del la Cruz *et al.* (2006) who showed that decrease of nutrient concentration caused *C. calcitrans* to grow exponentially. Unlike the findings of Fogg and Thake (1987), this experiment showed that the death phase of *C.*

calcitrans was not correlated with the nutrient levels. This result is in agreement with Blair *et al.* (2014) who showed that the light accelerated was optimal for the growth of *C. calcitrans* species. This can be explained by previous results that high light intensity ($240 \mu \text{ mol m}^{-2} \text{ s}^{-1}$) causes cell damage and reduces the biomass productivity (Ruyters, 1984).

C. calcitrans lipid content showed high variation with environmental conditions including light and nutrient levels. As mentioned the previous studies, the influence of environmental factors is specific specific. According to present results and that of Pernet *et al.* (2003) the lipid content of *C. calcitrans* increased significantly when the media at different stages of growth showed a decline of environmental factors.

In agreement with Khatoon *et al.*, (2010) the results of nitrogen nutrients effects on *C. calcitrans* growth and cell concentration showed a biomass decrease. They also found that biomass increased by decreasing the nutrients which is in accordance with Ghezlbash *et al.* (2008), who showed that microalgae cultivated at low nutrient levels had the highest biomass.

In conclusion, the variation of nutrients and light factors in the culture medium had significant effects on the growth and the lipid yield of *C. calcitrans*. This experiment showed that the growth and lipid production increased with increasing nutrient and light levels, although more research is needed on this species to produce high growth and lipid yield.

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