

Evaluation of Culture Media Limited in Nitrogen and Silicates on the Production Response and Lipid Content of the Diatom *Chaetoceros muelleri*

¹López-Elías, José Antonio, ²Carvalho Ruiz, María Gisela, ¹Estrada Raygoza, Leticia Areli, ¹Martínez Córdova, Luis Rafael, ²Martínez-Porchas Marcel, ³Miranda Baeza Anselmo and ²Ramírez Suárez, Juan Carlos

¹University of Sonora, Colosio s/n between Sahuaripa and Reform, Col. Center, C.P. 83000, ²Center for Food Research and Development, A.C., km 0.6 Road to Victory, C.P. 83304, Hermosillo, ³Sonora State University, C.P. 85800, Navojoa, Sonora, México

Abstract: Phytoplankton is a good option to obtain oil for biodiesel production and some marine diatoms contained up to 20% of lipids. Based on this, we evaluated mediums limited in nitrogen and silicate to enhance the production of lipids by *Chaetoceros muelleri* at laboratory condition. The cultures were made by quadruplicate in carboys (20 L). Three treatments (T1 with a reduction of 75% of nitrates; T2 with a reduction of 75% of silicate and T3 with a reduction of 75% of nitrate and silicate from medium f/2) and a Control (C) consisted of medium f/2, were evaluated. In general, cell density and dry biomass were higher in the control; however, the lipid content was greater in the treatments (24 to 34%), when compared to the control (15%). Similarly the production of lipids by cell was higher in the treatments (20 to 42 pcg/cell) as compared to the control (16 pcg/cell), while the production by volume was similar among treatments and control. The fatty acid profile was different between treatments and control; a high proportion of saturated and less of poly unsaturated fatty acids were found in the media limited in nutrients. The results of the study suggest that *C. muelleri*, may be a good-potential specie to produce bio-combustibles due to its high content of lipid when cultured in media reduced in nitrogen and silicates at low production costs.

Keywords: Biodiesel, *chaetoceros muelleri*, limited mediums, lipids, nitrate, silicate

INTRODUCTION

The most studied microalgae belong to the classes Chlorophyta and Bacillariophyta (Hu *et al.*, 2008). The production of microalgae is at present a well-controlled process managed at commercial level; however, some variables of the process such as culture media, pH, temperature, light and other, are susceptible to be modified to provoke a desirable response on the cultured organisms (Martínez-Córdova and Martínez-Porchas, 2013). It has been documented that changes on the culture medium, particularly reductions on some nutrients such as nitrogen and silicates, limit the growth rate of some microalgae, but at the same time increase their content of some cellular components such as lipids (Albarracín, 2007). Nitrogen limitation is considered the most efficient strategy to increase the content of neutral lipids in the microalgae, particularly triglycerides constituted by highly saturated fatty acids (Pal *et al.*, 2011; Huang *et al.*, 2013).

The diatom *Chaetoceros muelleri* is one of the microalgae most commonly used for the nutrition of zooplankton and the larval stages of many farmed species including fishes and shrimp (Mendiola-León,

2008). This is due to its great replication rate and biochemical composition which make it very adequate as larval feed (Palacios *et al.*, 2002; Jaime-Ceballos *et al.*, 2006; Rodríguez *et al.*, 2012). This diatom has a high content of lipids (between 30-46%) which can be increased under certain management strategies, making it a potential source for bio-combustibles production (Garibay-Hernández *et al.*, 2009).

The aim of the present study was to evaluate the effect of culture media limited in nitrogen and/or silicates, on the lipid production by *Chaetoceros muelleri* under laboratory conditions.

MATERIALS AND METHODS

The study was conducted in a laboratory of microalgae equipped with constant and enough illumination by fluorescent neon lamps (40 watts) and air conditioning to maintain temperatures between 21 to 25°C. A single-factor experimental design with 4 replicates were performed for the study. The experimental units consisted of plastic bottles of 20 L, operated at 10 L of culture medium, with constant aeration.

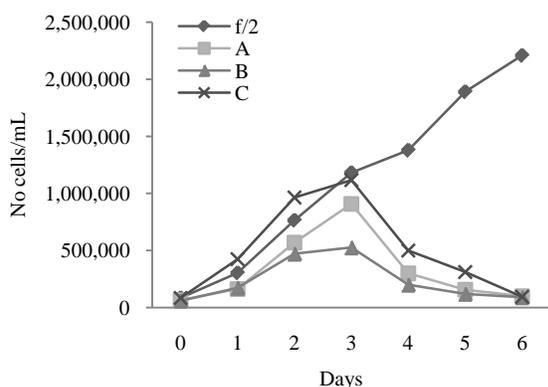


Fig. 1: Growth curve of *Chaetoceros muelleri* grown on the fourth treatments

Three treatments and a control were evaluated. In the Control (C), the standard medium f/2 Guillard and Ryther (1962) was used. Treatment 1(T1) consisted of the standard medium with a reduction of 75% of nitrates; in treatment 2 (T2) a reduction of 75% of silicates was applied and in treatment 3 (T3) nitrates and silicates were reduced in 75%.

A daily monitoring of environmental parameters was done in every one of the experimental units. Temperature was recorded by means a bulb thermometer (-10°C a 50°C; precision±0.5°C); light intensity with a exposure meter Exttech Instruments model L695238; and pH by means a pH meter Hanna Instruments model HI98108.

The cellular concentration was evaluated by direct counting in a microscopy, using anhematocitometer Neubauer (0.1 mm in depth). At the end of the culture period (stationary phase), samples of the culture were taken to measure dry weight biomass, organic matter, lipids and ash. The rest of the culture was flocculated to analyze the lipid profile by means of gas chromatography. The evaluation of biomass was made by filtration of 200 ml of the sample in GFC What man paper (47 mm) pre-calibrated (W1), dried for 8 h in a stove at 75°C and Weighed (W2) in a digital balance Mettler model AJ100. After that the filters were

incinerated in a muffle at 500°C for 8 h and weighed again (W3). The difference between W2-W1 corresponded to total biomass; the difference between W2-W3 corresponded to organic matter and the difference between W3-W1 corresponded to ash (García *et al.*, 2012).

The lipids were extracted by the method of Bligh and Dyer (1959) and then quantified by the micro method of Pande *et al.* (1963). The solvents for extraction were eliminated in a vacuum rotavapor at 45°C and then the fatty acid composition was determined by first an extraction by the method of Folch *et al.* (1957) after a metil-esterification done accordingly to official methods AOAC 969.33, by mean a Capillary Gas chromatography, a silica capillary column (AOAC, 2000).

RESULTS

In general terms the final cell density was greater in the Control (C), with a mean of 2.0×10^6 cells/mL, as compared to the Treatment 1 (T1) and 3 (T3), both with a mean around 1.0×10^6 cells/mL, while in the treatment 2 (T2) the lowest mean, 0.5×10^6 cells/mL, was observed (Fig. 1). However at the third day, the density between control and T1 was similar.

The dry biomass and organic matter were slightly higher in the microalgae cultured in the control but no significant differences were found when compared to the cultured in the media limited in nitrogen and silicates (Table 1). A similar trend showed the total production of lipids which was around 40% higher in the control respect to the observed in the treatments (Table 2).

Regarding to the lipid/biomass proportion, it was greater in the media limited in nitrogen and silicates as compared to the control, reaching values up to 34% in these last.

Similarly, the quantity of lipids by cell was higher in the treatments when compared to the control. The higher value was found in treatment T2 (Table 2).

Table 1: Means±S.D. of biomass, dry weight, organic matter and ash of *Chaetoceros muelleri* grown in the fourth treatments. Different letters in a column means significant differences (p<0.05)

Treatment	Dry weight (g/L)	Organic matter (g/L)	Ashes (g/L)
f/2 (Control)	0.245±0.020 ^b	0.133±0.010 ^b	0.112±0.016 ^b
T1	0.078±0.078 ^a	0.040±0.005 ^a	0.041±0.014 ^a
T2	0.077±0.020 ^a	0.036±0.006 ^a	0.038±0.011 ^a
T3	0.096±0.025 ^a	0.046±0.010 ^a	0.050±0.016 ^a

Table 2: Means±S.D. of lipids production (% , grams/L, picograms/cell) by *Chaetoceros muelleri* in the fourth treatments. Different letters in a column means significant differences (p<0.05)

Treatment	Lipids		
	(%)	(g/L)	(pcg/cell)
f/2 (Control)	15.54±3.06 ^a	0.037±0.009 ^b	16.80±6.40 ^a
T1	28.86±8.53 ^b	0.021±0.005 ^a	22.61±9.48 ^a
T2	34.28±4.79 ^b	0.024±0.007 ^a	42.46±12.22 ^b
T3	24.63±4.13 ^{ab}	0.021±0.006 ^a	20.99±7.79 ^a

Table 3: Fatty acids (weight percentage of total fatty acids) (Means±S.D.) of *Chaetoceros muelleri* in the fourth treatments. Different letters in a column means significant differences (p<0.05)

Culture medium	Saturated fatty acids	Mono unsaturated fatty acids	Poly unsaturated fatty acids
f/2 (Control)	42.63±1.43 ^b	38.46±0.86 ^b	18.48±0.78 ^b
T1	59.72±3.01 ^b	32.24±2.19 ^a	8.02±0.81 ^a
T2	51.17±0.40 ^b	38.83±0.99 ^b	9.87±0.36 ^a
T3	59.82±3.24 ^b	33.40±1.24 ^a	6.77±1.57 ^a

In regard to Fatty acid composition (Table 3), the higher percentage of them corresponded to saturated fatty acid, which ranged from 42.6 to 59.8% of the total lipid content, with a significantly lower proportion in the control when compared to treatments 1 and 3.

The mono-unsaturated fatty acids content varied from 32.2 to 38.8%, being significantly higher in the control and T2 (38.5 y 38.8%, respectively), when compared to T1 and T3 (32.8% for both). With respect to the poly-unsaturated fatty acids, they ranged from 6.8 to 18.5%, with mean percentages in the media limited in nitrogen and silicates around 63% lower than the found in the control.

DISCUSSION

The cell density was similar in all the media up to day 3; afterward, that level was maintained only in the control, where densities reached almost the double as compared to all the media limited in nutrients, which density declined in the day 4. Yu *et al.* (2009) grew out two species of diatoms in a medium limited in nitrogen and silicates, obtaining cell densities around 1.0×10^6 cells/mL, equal to the found in the present study for the media limited in the same nutrients.

The biomass obtained in the media limited in nitrogen and silicates was around 34% lower than the recorded for the control. This decrement is not very great, considering that the limitation in nutrients was 75%. Lee *et al.* (2011) found a similar biomass with the diatom *Chaetoceros calcitrans* in the f/2 medium; while Varfolomeev and Wasserman (2011), also reported very similar biomass for *C. muelleri*. Contrarily, Gouveia and Oliveira (2009) evaluated six microalgae and found that biomass reached from 2.0 to 3.6 g/L, values much greater than those we found; however their culture system was different and they work with other microalgae species.

The mean lipids content in the treatments (28%) was around 50% greater than the obtained in the control (15.5%). Li *et al.* (2008) also reported higher lipid content in diatoms when the nitrogen was limited in the culture medium. Similarly Ryu and Rorrer (2010) found an increase in the lipid content of another diatom limiting the silicates in the medium.

Although the final micro algal biomass was lower when using the media limited in nutrients, the decrement of around 41% is partially compensated by the higher proportion of lipids in the microalgae cultured under those conditions. If additionally, the lower cost of these media is taken into consideration, it

could be economically feasible their use in massive processes for the production of bio-combustibles.

With respect to lipid content, Shalaby (2011) evaluated 17 microalgae and found a lipid content ranging from 4 to 40%; while Andrade-Nascimento *et al.* (2012) documented the lipids percentage in 12 microalgae species which ranged from 13.52 to 49.0% and 10 of them had contents from 13.52 to 28.43% very similar to the values found in the present investigation. Lee *et al.* (2011) reported a content of lipids around 15% for *C. calcitrans*, similar to the found in the present study in the standard medium (control), but much lower than the observed in the treatments with limitation of nitrogen and silicates. Borowitzka and Moheimani (2013) reported for different microalgae a lipid percentage around 30%, near to the level of 34.3% recorded in the present study for treatment 2.

In the present study, it was found that in general the lipid fraction of the microalgae was composed by around 50% of saturated fatty acids, 37% of mono-unsaturated and a much lower percentage of poly-unsaturated. The saturated fraction was higher than the reported by Hu *et al.* (2008) who found from 32.1 to 37.0%; but lower than the 61.3% reported for the diatom *Paheodactylum tricrnutum*, by Griffiths *et al.* (2012). For *Scendesmus* sp. and *Noechloris* sp. Lopes da Silva *et al.* (2009) found a much lower percentage of saturated fatty acids, ranging from 23.7 to 20.8%. This finding is very important since the mono-unsaturated fatty acids may be important sources for the production of bio-combustibles because make the product more stable and with less possibility of rancidity than the poly-unsaturated. Salis *et al.* (2010) suggest that the saturated fatty acid could be also used for that purpose except in very cold regions. Andrade-Nascimento *et al.* (2012) documented that the proportion of mono-unsaturated fatty acids in 12 species of microalgae ranged from 14.63 to 47.36%, except for *Botryococcus braunii*, which had up to 79.61%. The microalgae we studied, *C. muelleri*, had 35.5% in to the range of the mentioned results.

The results of the present investigation suggest that *C. muelleri*, may be a good candidate for using in the production of bio-combustibles because of its production response and lipid content inclusively in media reduced in nitrogen and silicates, which reduce the cost of production. However we consider that more investigation is necessary to improve the process, including more information about the exact reduction in the nutrients and the environmental conditions affecting the growth and composition of the species.

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