***Artículo original de investigación***

# Effect of culture medium and nutrient concentration on fatty acid content of *Chaetoceros muelleri*

**Juan Manuel Pacheco Vega[1*], Marco Antonio Cadena Roa[1], M. del Pilar Sánchez Saavedra[2], Dariel Tovar Ramírez[3] y Carlos Rangel Dávalos[1]**

[1]Universidad Autónoma de Baja California Sur (UABCS), Apartado Postal 19-B. C.P. 23080. La Paz, Baja California Sur, México.
[2]Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). Departamento de Acuicultura, Kilómetro 107 Carretera Tijuana-Ensenada. Apartado Postal 2732 C.P. 22860. Ensenada, Baja California, México.
[3]Centro de Investigaciones Biológicas del Noroeste (CIBNOR) S.C. Apartado Postal 128, C.P. 23000. La Paz, Baja California Sur, México.

[*]Author of correspondence: pacheco@uabcs.mx

## Resumen

Los ácidos grasos en microalgas bajo cultivo pueden ser modificados por las condiciones de cultivo. La propuesta de este trabajo fue determinar el efecto de las formas de nitrógeno y su concentración en el contenido de ácidos grasos polinsaturados (PUFA) de *Chaetoceros muelleri*. La microalga *C. muelleri* fue mantenida en cultivos estáticos a 19°C y una intensidad de luz de 150 mmol $m^{-2}s^{-1}$. El medio fue utilizado como control. Las microalgas se cultivaron en tres diferentes concentraciones de medio de cultivo (f, f/2 y f/4). El medio de cultivo experimental fue preparado con fertilizantes agrícolas líquidos (AF) en tres diferentes concentraciones (AF, AF/2 y AF/4). La microalga se cosechó en la fase exponencial. La extracción de ácidos grasos fue por transterificación directa y su cuantificación por cromatografía de gases. El mayor porcentaje de ácidos grasos altamente insaturados (HUFA) se obtuvo al utilizar el medio f/2 (8.65%), mientras que el menor porcentaje se obtuvo al utilizar el medio a base de fertilizantes agrícolas (5.78%). Se encontró una relación directa entre la cantidad de HUFA y la concentración de nutrientes en ambos tipos de medio de cultivo. Se concluye que para obtener el mayor contenido de HUFA en *C. muelleri*, ésta debe ser cultivada en el medio f/2 o su equivalente AF/2. Adicionalmente, dicha práctica disminuye el costo de nutrientes.

**Palabras clave:** *microalgas, Chaetoceros muelleri, ácidos grasos, fertilizantes agrícolas, medio de cultivo.*

## Abstract

Fatty acid content in microalgae can be modified by culture conditions. The purpose of this study was to determine the effect of nitrogen sources and their concentration in the polyunsaturated fatty acid (PUFAs) contents of cultured *Chaetoceros muelleri*. *Chaetoceros muelleri* was batch cultured at 19°C and a continuous light intensity of 150 mmol $m^{-2}s^{-1}$. Three different concentrations of culture medium (f, f/2 and f/4) were used. The experimental medium was prepared with liquid agricultural fertilizers (AF) in three concentrations (AF, AF/2 and AF/4).

*Chaetoceros muelleri* was harvested at lag phase. Extraction of fatty acids was performed by direct transesterification and quantified by gas chromatography. The highest HUFA percentage was obtained with f/2 medium (8.65%), while the lowest was obtained with AF/2 medium (5.78%). A direct relationship between HUFA concentration and nutrient concentration in both culture media was found. It is concluded that *C. muelleri* must be cultured with f/2 medium or its equivalent AF/2 in an effort to obtain the highest HUFAs content. Additionally, the use of AF/2 medium results in a more cost effective option.

**Key words**: *microalgae, Chaetoceros muelleri, fatty acids, agricultural fertilizers, culture medium.*

## 1. Introduction

Microalgae cultures are used to feed fish larvae, crustacean larvae and mollusks in all stages (Meireles *et al*. 2003). Survival and growth of these organisms is determined by the nutritional quality of these cultures, which is also related to the content of highly polyunsaturated fatty acids (HUFAs) (Spektorova *et al*. 1986; Meireles *et al*. 2003). Polyunsaturated fatty acids (PUFAs) have specific physiological functions such as being structural components of phospholipid biomembranes (Trautwein 2001, Miliou *et al*. 2006). Several groups of marine organisms (e.g. fishes and shrimps) require an exogenous incorporation of PUFA due to their inability to synthesize them. The biochemical composition of microalgae can be modified as a function of culture conditions such as temperature, light intensity and spectral composition, source and concentration of nitrogen (Reitan *et al*. 1994). Among the different microalgae species used in aquaculture, *C. muelleri* is one of the most commonly used due to its biochemical composition and ease of production.

Agricultural fertilizers are widely used in the preparation of media for microalgae in outdoor cultures for commercial aquaculture in Northwest México (López-Elías *et al.* 2005). However, fertilizers frequently contain more than one nitrogen source. This may affect their relative availability for microalgae uptake and growth, causing variations in biomass yield and biochemical composition (Lourenco *et al*. 2002; Richmond 2003). The aim of this study was to compare the production of HUFA by *C. muelleri* grown with different concentrations of a standard medium (f medium) and with a non-conventional medium obtained from agricultural fertilizers containing different nitrogen sources (e.g. urea, ammonium and nitrate).

## 2. Materials and methods

### 2.1 *Culture maintenance*

The *C. muelleri* strain used in this study was obtained from the microalgae culture collection of the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). The main strain was isolated from the Oceanic Institute of Hawaii, USA in 1981. The culture was kept non-axenic and in f/2 medium (Guillard 1975) at 19 ºC with continuous white light at 150 µmoles $m^{-2}s^{-1}$. The experiments in this study were conducted at Pichilingue Aquaculture Laboratory (latitude: 24°16'12.80"N, longitude: 110°19'19.20"W) at the Universidad Autonoma de Baja California Sur.

Seawater was filtered through 10, 5 and 1 µm cotton filters and UV irradiated. For culture maintenance, seawater was sterilized as described by Guillard and Ryther (1962). For plastic bag cultures, seawater was

disinfected as mentioned by Hemerick (1973). Agricultural fertilizers were prepared with 72% phosphoric acid as the source of phosphorous plus a liquid fertilizer, with 32% total nitrogen provided as urea (16.4%) and ammonium nitrate (15.6%). These proportions matched the phosphorous and nitrogen concentrations of the f/2 medium. The f/2 medium (Guillard 1975) was used as control. For both culture media, three nutrient concentrations were used: f, f/2, f/4 and AF, AF/2, AF/4 (Table 1)

**Table 1.** Nutrient composition and concentration of the different culture media used in the present study.

| **Component /** | **AF** | **AF/2** | **AF/4** | **f** | **f/2** | **f/4** |
|---|---|---|---|---|---|---|
| ***Macronutrients*** | *mL $L^{-1}$* | *ml $L^{-1}$* | *mL $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* |
| Nitrogen: Nitrate (7.8%), Ammonium (7.8%), Urea (16.4 %). | 118.4 | 59.2 | 29.6 | 150* | 75* | 37.5* |
| Phosphoric acid | 4.8 | 2.4 | 1.2 | - | - | - |
| Sodium phosphate | - | - | - | 10 | 5 | 2.5 |
| Silica | 1000.0 | 500.0 | 250.0 | 60 | 30 | 15 |
| ***Micronutrients*** | *g $L^{-1}$* | *g $L^{-1}$* | *g $L^{-1}$* | *g $L^{-1}$* | *g $L^{-1}$* | *g $L^{-1}$* |
| EDTA-Fe | 8.6 | 4.3 | 2.1 | 8.6 | 4.3 | 2.1 |
| Copper sulfate | 19.6 | 9.8 | 4.9 | 19.6 | 9.8 | 4.9 |
| Zinc sulfate | 44.0 | 22.0 | 11.0 | 44.0 | 22.0 | 11.0 |
| Cobalt chloride | 20.0 | 10.0 | 5.0 | 20.0 | 10.0 | 5.0 |
| Manganese chloride | 360.0 | 180.0 | 90.0 | 360.0 | 180.0 | 90.0 |
| Sodium molybdate | 126.0 | 63.0 | 31.5 | 126.0 | 63.0 | 31.5 |
| ***Vitamins*** | *mg $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* | *mg $L^{-1}$* |
| Biotin | 2.0 | 1.0 | 0.5 | 2.0 | 1.0 | 0.5 |
| Cyanocobalamin ($B_{12}$) | 2.0 | 1.0 | 0.5 | 2.0 | 1.0 | 0.5 |
| Thiamine HCl ($B_1$) | 40.0 | 20.0 | 10.0 | 40.0 | 20.0 | 10.0 |

*Only nitrate

### 2.2 *Acclimation of the microalgae in the culture media*

Triplicate non-axenic cultures were kept for eight days in 250 ml Erlenmeyer flasks with 150 ml of the three control nutrient concentrations (f, f/2, f/4) and non-conventional (AF, AF/2, AF/4) culture media. For each culture condition plastic translucent bags of 28 cm x 66 cm (9 L), were conducted in triplicate.

Cell concentrations were measured daily with a Beckman DU 640 spectrophotometer at 550 nm. The pH was measured daily with an Orion 301 pH meter and maintained at 7-8. The specific growth rate was measured as described by Fogg and Thake (1987). The cultures were maintained at 19 °C with continuous light at 150 µmoles $m^{-2}s^{-1}$. Triplicate samples were used to evaluate biomass production and cell composition. Total dry weight was determined gravimetrically according to Sorokin (1973). Protein content was determined according to Lowry *et al*. (1951) modified by Malara and Charra (1972). Carbohydrates were determined according to White (1987) and Dubois *et al*. (1956). Lipids were determined colorimetrically according to

Pande *et al*. (1963), after extraction with the method of Bligh and Dyer (1959) modified by Chiaverini (1972).

### 2.3 *Extraction and characterization of fatty acids*

Samples of each experimental condition (400 mL) were collected on the fourth day of culture post acclimation. Each sample was concentrated to 30 mL by centrifugation at 3000 rpm for 18 min using an IEC Centra GP8R. Samples were then lyophilized with a LABCONCO freeze dry system/freezone 4.5. The extraction of fatty acids was done by direct transesterification (Carrapiso and Garcia 2000). Injection of the samples was then performed in a gas chromatograph, Varian CP-3800, fitted with a Mass Detector 1200L. The samples were injected in 1 µl volumes into a capillary column Omegawax 250 of Funded Silica with dimensions of 30m X 0.25 mm X 0.25 um (SUPELCO). The carrier gas was helium (99%) at a flow-rate of 1.2 ml $min^{-1}$. Identification of fatty acids was made by comparing the obtained retention times with those of commercial standards of methyl-esters of PUFA (Kit, SIGMA). In addition, further confirmation was performed by comparing the masses of the obtained compounds with those in a mass spectra (NBS75K and NIST98) library.

### 2.4 *Statistic analysis*

Statistical analysis was performed using STATISTIC 7.0 software for Windows (Statsoft, USA). Percentage data obtained from the proximal composition and fatty acid profiles were arcsine-square-root transformed prior to analysis. Differences in cell concentration, specific growth rate, dry weight and proximal composition of *C. muelleri* cultured under the experimental conditions were determined by one-way analysis of variance (ANOVA). When variances were homogeneous and residuals were normally distributed, a Tukey *a posteriori* test was used to identify significant differences among treatments ($P< 0.05$). Differences in the percentage of each fatty acid as a function of the culture media and concentration of nutrients were determined with a two-way analysis of variance.

## 3. Results

Cell concentration and growth rate was significantly different among treatments ($P<0.05$). The lowest density was obtained in the treatment AF/4, while maximum density was obtained with the AF treatment. Significant differences were found in the growth rate among treatments ($P<0.05$) (Table 2). The microalgae had the slowest growth rate in AF/4 medium, while maximum growth rates were observed with the f, f/2 and AF/2 media (Table 2).

In terms of the proximal composition, the highest protein content was observed with the AF, AF/2, AF/4, f and f/2 treatments, which f/4 had lower protein content ($P<0.05$). Regarding lipid content, no significant differences were observed for the culture media treatments ($P>0.05$). The carbohydrate content was not significantly different among treatments ($P>0.05$) (Table 2).

The major saturated fatty acid (SFA) was obtained AF/4 medium, while AF, AF/2, f, f/2 and f/4 had lower content (Table 3). There were significant differences in the fatty acid profiles of monounsaturated (MUFA) polyunsaturated (PUFA) and highly unsaturated fatty acids (HUFA) of C. muelleri due to the culture medium and concentration ($P< 0.05$).

**Table 2.** Mean values and standard deviations of cell concentrations (106 cells $ml^{-1}$), specific growth rate (divisions day-1), dry weight (µg per 106 cells) and proximate composition (as percentage of dry weight) of *Chaetoceros muelleri* cultures in f and AF media prepared with agricultural fertilizer (AF) at different nutrient concentrations. Standard deviation is indicated in brackets; values with the same letter within the same row indicate that they are not significantly different (P>0.05; a>b>c>d>e>f).

| **Parameter** | **Culture medium** | | | | | |
|---|---|---|---|---|---|---|
| | **f** | **f/2** | **f/4** | **AF** | **AF/2** | **AF/4** |
| Cell concentration | 1846.8 | 2482.1 | 488.1 | 3818.7 | 2907.5 | 803.7 |
| | (152.9)$^{d}$ | (206.9)$^{f}$ | (45.1)$^{d}$ | (478.3)$^{a}$ | (141.2)$^{b}$ | (19.4)$^{e}$ |
| Specific | 0.832 | 0.914 | 0.479 | 0.860 | 0.548 | 0.378 |
| growth rate | (0.02)$^{a}$ | (0.11)$^{a}$ | (0.11)$^{b}$ | (0.13)$^{a}$ | (0.10)$^{b}$ | (0.07)$^{c}$ |
| Proteins | 28.9 | 29.9 | 22.7 | 25.1 | 27.2 | 29.6 |
| | (3.4)$^{a}$ | (0.6)$^{a}$ | (0.2)$^{b}$ | (0.7)$^{a}$ | (3.2)$^{a}$ | (3.6)$^{a}$ |
| Lipids | 6.5 | 7.0 | 6.1 | 6.5 | 7.3 | 7.2 |
| | (0.1)$^{a}$ | (0.2)$^{a}$ | (0.6)$^{a}$ | (0.9)$^{a}$ | (0.2)$^{a}$ | (0.3)$^{a}$ |
| Carbohydrates | 16.4 | 17.8 | 18.0 | 18.2 | 19.6 | 21.4 |
| | (2.0)$^{a}$ | (2.6)$^{a}$ | (1.2)$^{a}$ | (0.1)$^{a}$ | (2.7)$^{a}$ | (2.2)$^{a}$ |
| Dry weight | 57.5 | 58.0 | 71.4 | 54.6 | 52.6 | 77.4 |
| | (5.2)$^{b}$ | (2.0)$^{b}$ | (6.8)$^{a}$ | (5.0)$^{b}$ | (2.2)$^{b}$ | (6.8)$^{a}$ |

The lowest SAFA concentration was found in f media (50.33%) and the highest in AF/2 media (63.95%). The lowest MUFA concentration was found in the AF/4 treatment (28.45%) and the highest value was for F/4 treatment (36.51%). The lowest PUFA was for AF/4 media (4.62%) while that the highest value was for f/2 treatment (17.18%).

Significant differences were obtained in the concentration of HUFAs ($P$<0.05) such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) due to culture medium and concentration.

The lowest HUFA percentages were observed in treatment AF/4 (1.25%) and the highest percentage was for treatment f/2 (8.65%). The EPA (20:5 n-3) levels in both culture media and all concentrations were significantly different ($P$<0.05).

Arachidonic acid (20:4 n-6) was not detected in the AF treatment. Docosohexaenoic acid (22:6 n-3) was only detected in the AF/2 treatment (Table 3).

## 4. Discussion

The biochemical composition of *C. muelleri* can be altered by the culture medium and the nutrient concentration in the medium. As expected, those media with the lowest concentrations of nutrients (f/4 and AF/4) showed the lowest cell densities, possibly due to the effect of low nutrient concentration on the metabolic processes of the microalgae (Darley 1987). Different nitrogen sources could have affected microalgae growth, as it is well known that in plant tissues nutrient assimilation and transport is modulated by nitrogen sources. For instance, reduced nitrogen forms (ammonium and urea) are preferred over more oxidized forms, such as nitrate or nitrite (Iriarte *et al*. 2007).

**Table 3**. Mean values of fatty acids of *Chaetoceros mulleri* cultures in f and AF media with different nutrient concentrations. Data is presented as percentage of fatty acids; values with the same letter within the same row indicate that they are not significantly different ($P$>0.05; a>b>c>d>e>f).

| Fatty acid | Culture medium | | | | | |
|---|---|---|---|---|---|---|
| | **f** | **f/2** | **f/4** | **AF** | **AF/2** | **AF/4** |
| **Saturated** | | | | | | |
| 14:00 | 18.35 $^{b}$ | 24.19 $^{ab}$ | 13.69 $^{c}$ | 25.68 $^{a}$ | 15.32 $^{bc}$ | 13.78 $^{bc}$ |
| 15:00 | 1.10 $^{a}$ | 1.05 $^{a}$ | 1.19 $^{a}$ | 1.40 $^{a}$ | 1.17 $^{a}$ | 1.37 $^{a}$ |
| 16:00 | 28.70$^{cde}$ | 21.55 $^{d}$ | 36.28 $^{b}$ | 25.07 $^{de}$ | 32.38 $^{ce}$ | 47.15 $^{a}$ |
| 17:00 | Trace | 0.05 $^{c}$ | 0.10 $^{a}$ | 0.08 $^{b}$ | 0.04 $^{c}$ | 0.11 $^{a}$ |
| 18:00 | 1.25 $^{b}$ | 3.38 $^{a}$ | - | 0.28 $^{d}$ | 0.72 $^{c}$ | - |
| 19:00 | 0.48 $^{b}$ | 0.57 $^{b}$ | 0.15 $^{a}$ | 0.17 $^{d}$ | 2.11 $^{a}$ | 0.33 $^{c}$ |
| 20:00 | 0.45 $^{c}$ | 0.80 b | 0.80 $^{b}$ | 0.63 $^{b}$ | 1.48 $^{a}$ | 1.21 $^{a}$ |
| 21:00 | - | - | - | - | 0.28 $^{a}$ | - |
| **Sum SAFA** | **50.33** | **51.59** | **52.21** | **53.31** | **53.5** | **63.95** |
| **Monounsaturated** | | | | | | |
| 16:1 (n-7) | 29.04 $^{bc}$ | 32.15 $^{ab}$ | 36.11 $^{a}$ | 27.98 $^{bc}$ | 33.39 $^{ab}$ | 27.85 $^{bc}$ |
| 16-1 (n-5) | 0.41 $^{ab}$ | 0.37 $^{b}$ | 0.18 $^{b}$ | 0.65 $^{ab}$ | 0.29 $^{b}$ | 0.14 $^{b}$ |
| 17:1 | 0.47 $^{a}$ | 0.19 $^{b}$ | 0.08 $^{c}$ | 0.18 $^{b}$ | 0.06 $^{c}$ | |
| 22:1 (n-7) | - | - | - | 0.81 $^{a}$ | 0.17 $^{c}$ | 0.46 $^{b}$ |
| 22:1 (n-9) | 4.69 | - | - | - | - | - |
| 20:1 (n-6) | - | 1.09 $^{b}$ | 0.13 $^{c}$ | - | 2.11 $^{a}$ | - |
| **Sum MUFA** | **34.61** | **33.80** | **36.51** | **29.62** | **36.02** | **28.45** |
| **Polyunsaturated** | | | | | | |
| 16:2 (n-4) | 3.96 $^{a}$ | 3.30 $^{b}$ | 1.04 $^{c}$ | 2.21 $^{b}$ | 1.58 $^{c}$ | 0.73 $^{c}$ |
| 16:3 (n-3) | 5.50 $^{ab}$ | 4.35 $^{b}$ | 0.81 $^{c}$ | 6.77 $^{a}$ | 3.95 $^{b}$ | 0.89 $^{c}$ |
| 18:2 (n-6) | 1.07 $^{ab}$ | 0.17 $^{d}$ | 1.11 $^{b}$ | 0.52 $^{c}$ | 0.44 $^{c}$ | 1.14 $^{a}$ |
| 18:3 (n-3) | 0.23 $^{c}$ | 0.71 $^{a}$ | 0.34 $^{bc}$ | 0.34 $^{b}$ | 0.95 $^{a}$ | 0.43 $^{b}$ |
| 18:4 (n-3) | trace | trace | 0.03 $^{b}$ | - | trace | 0.18 $^{a}$ |
| 20:4 (n-6) | - | 1.24 $^{b}$ | 0.50 $^{c}$ | - | 0.26 $^{c}$ | 0.13 $^{d}$ |
| 20:5 (n-3) | 4.06 $^{e}$ | 7.41 $^{a}$ | 0.99 $^{d}$ | 4.80 $^{b}$ | 4.58 $^{c}$ | 1.12 $^{f}$ |
| 22:6 (n-3) | - | - | - | - | 0.94 | - |
| **Sum HUFA** (ARA, EPA and DHA) | **4.06** | **8.65** | **1.49** | **4.80** | **5.78** | **1.25** |
| **Sum PUFA** | **14.82** | **17.18** | **4.82** | **14.64** | **12.70** | **4.62** |

Trace= detection of fatty acids present in trace amounts, - not detected.

South and Whittick (1987) described that nitrogen sources that are reduced to ammonium ($NH^{+4}$) are preferably used for amino acid biosynthesis. The low protein concentration in the f/2 medium could have had an effect on the low cell densities obtained. The nitrogen deficiency in the microalgae, due to nitrogen limitation during the log phase, possibly affected protein synthesis, thus reducing the amount of soluble protein available for metabolic processes. This was also observed by Matos Moura *et al*. (2007).

An increase in the lipid content of the cultures with lower nutrient content was observed, since the accumulation of lipids in microalgae can be induced by nitrogen limitation in the medium (Sánchez-Saavedra and Voltolina 2005, Medina-Reyna and Cordero-Esquivel, 1998). In other studies, results obtained with *Nannochloris atomus* and *Tetraselmis* sp. showed that the lipid content decreases when a limitation of phosphorus exists (Reitan *et al*. 1994). We found no differences in the percentages of carbohydrates. Collectively, these results suggest that by the third day of culture (harvest day), the microalgae had not been able to accumulate carbohydrates. The low dry weight in the microalgae cultured with f/2 and AF/2 is attributed to the decline of nutrients in the media, which eventually leads to a decrease in the cellular content.

Desaturases catalyze the formation of double bonds for the conformation of unsaturated fatty acids (Martin *et al*. 2007). Previous studies with *Euglena gracilis* found that the desaturase acitivty is stimulated when cultures are in the presence of $NH^{+4}$. Also, quantitative differences between distinct forms of nitrogen can be determined from the intake of nutrients by cells. In cyanobacteria, it is known that there is a periplasmic protein that makes solute binding easier. These are integral cytoplasmic proteins with ATPase activity associated that regulate the intake of $NO^{-3}$ and $NO^{-2}$ into the cell (Wu and Stewart, 1998; Martinez-Espinosa 2003). However, in eukaryotic cells such as *C. muelleri*, $NH^{4+}$ permeates through biological membranes (Martinez-Espinoza 2003). Therefore, our results show that the nutrient concentrations of f/2 and AF/2 facilitate cell intake, thus producing in this way the highest levels of HUFA.

Results in this study also show that the fatty acid profile of *C. muelleri* can be modified by the concentration and source of nitrogen. Liang and Mai (2005) found that in *C. gracilis* the total amount of MUFA increased while that of PUFA decreased with culture age. These trends were also observed in the present study. The biosynthesis of fatty acids in chloloroplasts may be regulated by acetyl coenzime-A and malanolin-ACPs, which are enzymes that initiate the formation of ACP protein transporters (Mcginnis and Sommerfeld 2000). In addition, the proportion of PUFA in *C. muelleri* may vary when different nitrogen sources are used (Post-Beittenmiller *et al.* 1992 and Liang *et al*. 2006). For instance, in a study by the later authors, urea was the only chemical form that yielded a high quantity of 20:5 n-3 fatty acids, followed by nitrates and ammonia. In that same study, no significant differences were found in the content of other HUFAs, such as DHA. The present results do not show a clear relationship among the contents of HUFAs. Low (f/4 and AF/4) and high (AF) nutrient concentration diminished the HUFA concentration in *C. muelleri*. On the other hand, induced stress by reducing nutrient concentration in the culture media increased the total lipid content of the microalgae. However, this not always applies to the HUFA proportion.

The results presented in this work are in agreement with those of Pernet *et al*. (2003), who mentioned that total lipids in *C. muelleri* could increase because of nutrient deficiency. The relative proportion of nutrients can modify the fatty acid profile of the microalgae, increasing SAFA and MUFA proportion and in smaller amount PUFA content. The percentage of phosphorus was found to be the limiting nutrient related to the synthesis of phospholipids. Nevertheless, fatty acid biosynthesis and proportion may vary according to the microalgae species (Guan-Qun 2007).

## 5. Conclusions

Based on the results of the present study it is concluded that, under the described conditions, the specific growth of a four-day culture of *C. muelleri* is affected when the culture medium contains a low concentration of nutrients (f/4 and AF/4). Depending on the desired major cellular component (proteins, lipids or carbohydrates) it is recommended to use the culture medium accordingly. To get the highest value of HUFAs, the use of f/2 or the proposed AF medium (agricultural fertilizers) is recommended.

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