Revista Ciência Agronômica, v. 51, n. 1, e20165285, 2020
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Influence of nutrients on biomass and oil yield from microalgae *Chlorella vulgaris* for biodiesel production[1]

Influência dos nutrientes no rendimento de biomassa e óleo da microalga *Chlorella vulgaris* para a produção de biodiesel

Glacio Souza Araujo[2]*, Dilliani Naiane Mascena Lopes[3], Clarice da Silva Santiago[4], José William Alves da Silva[2] and Fabiano André Narciso Fernandes[5]

ABSTRACT - Microalgae are commonly used in aquiculture as feed for postlarval mollusks, fish and crustaceans because they are easy to grow, small in size, grow rapidly and have high levels of fatty acids. These microorganisms also accumulate high amounts of oil, which can be extracted and converted into biodiesel using chemical processes. In this work, freshwater microalgae *Chlorella vulgaris* was grown in the Live Food Production Laboratory (LABPAV/IFCE Aracati Campus), with urea (stock solution 1), triple superphosphate (stock solution 2) and vitamins (stock solution 3), in growth medium, in triplicate, using three different quantities of stock solutions 1 and 2, but with a constant amount of vitamins. The quantities of 0.5, 1 and 2 mL (T0.5, T1 and T2, respectively) were used for both stock solutions. We then monitored the growth of the microalgae, flocculated through chemical flocculation by adding a NaOH 2N solution, air-oven dried at 60 ºC for 24 hours, weighed the dried biomass on a semi-analytical balance, and extracted the oil using solvents. We thus observed that algal growth intensified and dry biomass increased as the amount of nutrients increased in the growth media; inversely, the best oil level was observed in the treatment using the lowest amount of nutrients in the growth media where the microalgae developed (20.13±0.19%). Finally, in Treatment T2, even with the lowest percentage of oil (18.95%), the amount of biomass produced compensates in the oil productivity, and using a lower amount of nutrients in the media of culture.

Key words: Biodiesel. Microalgae. Oil.

RESUMO - As microalgas são bastante utilizadas na aquicultura como alimento para pós-larvas de moluscos, peixes e crustáceos em decorrência da facilidade de cultivo, ao pequeno tamanho, acentuada velocidade de crescimento e alto teor de ácidos graxos. Esses microrganismos também acumulam grandes quantidades de óleo, que podem ser extraídos e convertidos por processos químicos em biodiesel. Nesse trabalho, foi cultivada a microalga de água doce *Chlorella vulgaris* com uréia (solução estoque 1), superfosfato triplo (solução estoque 2) e vitaminas (solução estoque 3), nos meios de cultivo, em triplicata, usando três diferentes quantidades das soluções estoque 1 e 2, mas com uma quantidade constante de vitaminas, com 0,5; 1 e 2 mL (T0,5; T1 e T2, respectivamente). Com isso, acompanhamos o crescimento das microalgas, floculamos através de floculação química, com adição de uma solução de NaOH 2N, secamos em estufa com renovação de ar a 60 ºC por 24 horas, pesamos a biomassa seca em balança semi analítica e extraímos o óleo utilizando solventes. Assim, observamos que quanto maior a quantidade de nutrientes nos meios de cultivo ocorreu o mais elevado crescimento das algas e obtenção de biomassa seca, mas inversamente a isso, o melhor teor de óleo foi observado quando as microalgas foram cultivadas utilizando a menor quantidade de nutrientes nos meios de cultura (20,13±0,19%). Por fim, no Tratamento T2, mesmo tendo o percentual menor de óleo (18,95%), a quantidade de biomassa produzida compensa na produtividade de óleo, e utilizando uma menor quantidade de nutrientes nos maios de cultivo.

Palavras-chave: Biodiesel. Microalgas. Óleo.

DOI: 10.5935/1806-6690.20200008
*Autor for correspondence
Received for publication in 03/09/2016; approved in 09/08/2019
[1]Trabalho extraído da Tese do primeiro autor apresentada ao Programa de Pós-Graduação em Engenharia de Pesca, Universidade Federal do Ceará/UFC
[2]Instituto Federal de Educação, Ciência e Tecnologia do Ceará/IFCE, *Campus* Aracati, rodovia CE 040, km 137,1, Aeroporto, Aracati-CE, Brasil, 62.800-000, glacio@ifce.edu.br (ORCID ID 0000-0002-9968-0610), jose.william@ifce.edu.br (ORCID ID 0000-0002-0345-4193)
[3]Doutoranda da Rede Nordeste de Biotecnologia - RENORBIO/UFC, Fortaleza-CE, Brasil, naianemascena88@gmail.com (ORCID ID 0000-0003-0958-2847)
[4]Graduanda em Engenharia de Aquicultura, Instituto Federal de Educação, Ciência e Tecnologia do Ceará/IFCE, *Campus* Aracati, Aracati-CE, Brasil, claricesantiaggo@gmail.com (ORCID ID 0000-0001-5673-399X)
[5]Departamento de Engenharia Química, Universidade Federal do Ceará/UFC, Campus do Pici, Fortaleza-CE, Brasil, fabiano@ufc.br (ORCID ID 0000-0003-3495-5975)

INTRODUCTION

Microalgae are commonly used in aquiculture as feed for postlarval mollusks, fish and crustaceans because they are easy to grow, small in size, grow rapidly and have high levels of fatty acids (DERNER *et al*., 2006).

The choice of growth medium is extremely important for mass microalgae production. Inadequate use can affect the growth rate and biochemical composition of cells (SÁNCHEZ; MARTINEZ; ESPINOLA, 2000). For each species of microalgae, cell yield and biochemical composition depend strongly on growth type and nutrient profile of the medium (GUEDES; AMARO; MALCATA, 2011).

Microalgae also accumulate large amounts of oil, especially triglycerides, which can be extracted and converted into biodiesel using chemical processes (URI; TATYANA; MEIRA, 2010).

Currently, the focus of biodiesel production is moving towards the use of non-edible sources, such as the reuse of cooking oil, use of low-quality animal fats, and microalgae cultivation (DEVAPPA *et al*., 2010). Some species of microalgae are promising sources for large-scale biodiesel production (CHISTI, 2008), with higher yields than other oilseeds (GAO *et al*., 2010), making them a viable solution from an economic and environmental standpoint, and an excellent alternative to fossil fuels (ZENG *et al*., 2011). Moreover, its production does not compete with food products, as microalgae can be grown in non-farmable regions (SUBHADRA; EDWARDS, 2010).

The composition of the growth medium is important in microalgae cultivation in order to obtain a high final cell concentration. Furthermore, the components of the medium must meet the basic requirements for production and accumulation of cell metabolites, providing proper energy for biosynthesis and cell maintenance (AZMA *et al*., 2011). Among the main physical and chemical factors that affect the growth of microalgae are light, temperature, salinity, and nutrient availability and quality (RICHMOND, 2004).

Growth rate and oil production are also directly related to the concentration of nutrients in the growth medium of microalgae. Nitrogen plays an important role in controlling the yield of these organisms, and biomass and lipid production can be maximized at a certain nitrogen concentration (DE LA HOZ *et al*., 2011). Dragone *et al*. (2011) mention that limiting nutrients in the growth medium causes starch levels to rise, making bioethanol production viable.

Wijffels (2007) comments that biofuel production from microalgae will depend on the growth rate of the species being grown and its oil content. Microalgae with high lipid production are the most desirable for obtaining biodiesel. Microalgae produce different types of lipids, hydrocarbons and other complex oils, depending on the species.

Araujo *et al*. (2011) grew ten species of microalgae (seven chlorophytes and three diatoms) under two different salinity levels (25 and 35) and observed that microalgae *Nannochloropsis oculata*, *Isochrysis* sp., *Dunaliella* sp., *Tetraselmis tetrathele* and *Chlorella vulgaris* grown at 35 salinity showed oil yield above 20.00% – the latter featuring a 52.49% yield. As such, these species can be used as sources of biodiesel, as was the case for microalgae *Chaetoceros mulleri*, *N. oculata*, *Tetraselmis chui*, *Tetraselmis tetrathele* and *C. gracilis* at 25 salinity – the latter showed the best yield response when salinity was decreased, going from 15.50% to 60.28%.

In this context, the objective of this work was to evaluate the influence of nutrients on the yield of biomass and oil of microalgae *Chlorella vulgaris* for biodiesel production, as well as the description of the species.

MATERIAL AND METHODS

Freshwater microalgae *Chlorella vulgaris* (Sisgen AADB63D) was grown from a strain belonging to the Center for Biotechnology Applied to Aquiculture (CEBIAQUA/UFC), kept at 22 ± 2 °C, in test tubes, with artificial lighting and a light cycle of 16 h light and 8 h darkness.

The experiment was carried out at the Live Food Production Laboratory (LABPAV/IFCE Aracati Campus). The growth medium consisted of 120 g of urea (stock solution 1), 30 g of triple superphosphate (stock solution 2), dissolved into a liter of distilled water, and vitamins (stock solution 3), in triplicate, using three different quantities of stock solutions 1 and 2, but with a constant amount of vitamins. The quantities of 0.5, 1 and 2 mL (Treatment 0.5, Treatment 1.0 and Treatment 2.0 - T0.5, T1 and T2, respectively) were used for both stock solutions (Table 1). The growth media were prepared with distilled water, previously autoclaved at 121 °C for a period of 15 minutes.

The cultivation of the microalgae started from a volume of 20 mL in a 250 mL Erlenmeyer flask, into which the same volume of medium was added every other day. Cultivation was gradually increased up to three liters of useful volume, receiving constant aeration through diaphragm pumps with airflow of 2 L min^{-1}. From this standard cultivation, the inoculums were utilized for their respective experiments varying the amounts of nutrients in

Table 1 - Description of the different treatments used in this experiment, for a volume of one liter of distilled water. The growth medium consisted of 120 g of urea (stock solution 1), 30 g of triple superphosphate (stock solution 2), dissolved into a liter of distilled water, and vitamins (two ampoules of Citoneurim dissolved into a 50 mL of distilled water - stock solution 3)

Treatment	Stock solution 1	Stock solution 2	Stock solution 3
T0.5	0.5 mL	0.5 mL	0.5 mL
T1	1.0 mL	1.0 mL	0.5 mL
T2	2.0 mL	2.0 mL	0.5 mL

the growth media. It should be noted that about 100 mL of this standard cultivation was used to create the permanent cultures.

For the permanent cultivations we used 2500 mL of previously autoclaved distilled water and varied the amounts of both stock solutions 1 and 2 of these nutrients in the growth media. After preparing the permanent growth media, we added 100 mL of the standard culture, thus totaling 2600 mL of total useful volume.

During the cultivations, irradiance was 60 µE cm^{-2} s^{-1}, constantly provided by two 40 W fluorescent light bulbs, and room temperature in the experiment location stood around 23 ± 1 ºC. The cultivations were carried out with constant volume.

At the start and every other day thereafter, the number of cells was counted in a Neubauer chamber using a binocular microscope. This parameter was useful to monitor cell multiplication. It should be noted that cultivation times of microalgae were variable, because it depended on the viability of the cells.

The chemical flocculation technique was applied to separate the microalgae from the growth medium, by adding a NaOH 2N solution. The supernatant was syphoned and the algal biomass was air dried in an oven at 60 ºC for 24 hours.

Crude protein was measured at the Animal Nutrition Laboratory belonging to the Animal Science Department of the Federal University of Ceará, and followed the method described by the Association of Official Analytical Chemists - AOAC (1995).

The analysis was carried out using the Kjeldahl method, by digestion with sulfuric acid (H_2SO_4), alkaline distillation with sodium hydroxide (NaOH) and titration with sulfuric acid 0.1 N, with 6.25 being the conversion factor of total nitrogen for crude protein (LYNCH; BARBANO, 1999). At the end of titration, the formula below was used to determine the percentage of crude protein:

$$\%CP = \frac{ml\ of\ H_2SO_4 \times Acid\ normality \times Conversion\ factor}{Weight\ of\ the\ sample\ (g)}$$

In which:

%PB = Crude protein of the sample

H_2SO_4 = milliliters of acid spent in titration

Normality of the acid = 0.1 N

Conversion factor = 6.25

Lipid levels were determined in triplicate, following the method by Bligh and Dyer (1959). To that end, five grams of dry microalgae biomass, 25 mL of methanol, 12.5 mL of chloroform and 5 mL of water were added into each 250 mL Erlenmeyer flask.

Each Erlenmeyer flask was sealed and exposed to sonication during 10 minutes in an ultrasonic bath with a frequency of 40 KHz and 80 W of power. Next, another 12.5 mL of chloroform and 12.5 mL of water were added, and sonication was carried out again for five minutes. Lastly, the solid part was vacuum filtered and later oven-dried during 24 h at 60 °C.

The characterization of lipids found in the oil extracted from microalgae *C. vulgaris* was carried out with gas chromatography coupled to a mass spectrometer (GC-MS), using the Ce 2-66 method by the American Oil Chemists' Society (1997). This method uses KOH 0.5 mol L^{-1} in anhydrous methanol (saponification reagent), a solution containing 20 g of NH_4Cl + 600 mL of anhydrous methanol + 30 mL of concentrated H_2SO_4 (esterification reagent), a saturated NaCl aqueous solution (saline solution) and petroleum ether.

To analyze methyl esters, a CGC Agilent-6850, GC SYSTEM-series gas chromatographer was used, attached to a flame ionization detector (FID) and to an integrator, using a DB-23 Agilent capillary column (50% cyanopropyl/polydimethylsiloxane), 60 m in length, 0.25 mm internal diameter, and 0.25 µm liquid film thickness. Column flow was set to 1.0 mL min^{-1}, injected volume was 1.0 µL, and helium (99.95%) was used as carrier gas.

The procedure started by weighing 50 mg of the oil extracted from the dry biomass of *C. vulgaris* in a sealed test tube. Next, 4.0 mL of the saponification reagent was added, the tube was stirred vigorously and heated in boiling

water for five minutes. After the tube cooled, 5.0 mL of esterification reagent was added and the tube was again stirred vigorously in boiling water for five minutes. The tube was cooled again and 4.0 mL of saline solution and 5 mL of petroleum ether were added, stirring vigorously. The tube was left at rest until the phases completely separated, after which a section of the extract containing the methyl esters was taken, which injected into the chromatograph. The same procedure was carried out for mustard oil (reference oil), the ester levels of which were already known.

By comparing the retention time (t_R) of the esters from each oil to the retention time of the esters of mustard oil (analyzed under the same operational conditions), it was possible to identify the percent composition of each component in the microalgae oil. In order to obtain the average molar mass of the methyl esters resulting from the transesterification of plant oils, the equation below was used, as per (VARGAS, 1996).

$$AMM\left(ethyl\ esters\right) = \frac{\sum\left[\left(Ai\right)\chi\left(MMi\right)\right]}{\sum\left(Ai\right)}$$

In which:

Ai = percentage rate of ester i;

MMi = molar mass of ester i (g mol^{-1}).

The molar mass (MM) of the plant oil can be calculated through the equation:

MM (plant oil) = [(3ˣaAMM of methyl esters) - 4]

The data obtained in the present study were submitted to analysis of variance (ANOVA) and, in the case of significant difference, the means were submitted to the Tukey test for the level of confidence of 5% using the program BioEstat 5,0.

RESULTS AND DISCUSSION

The results obtained when assessing the influence of nutrients on the yield and characterization of biomass and oil from microalgae *Chlorella vulgaris* for biodiesel production are shown in Table 2 and Figure 1.

As can be observed, in the treatment in which 0.5 mL of solutions 1 and 2 were utilized for each liter of grown medium prepared (T0.5), the microalgae did not develop, decreasing their cell concentration daily and stopping growth on the sixth day (12±2 cells mL^{-1} x 10^6). Conversely, when using 1 and 2 mL of the solutions (T1 and T2, respectively), the microalgae showed two days of adaptation to grown conditions (induction phase), followed by rapid growth, reaching their peak on the eighth day. After that time, the microalgae entered the decline phase, decreasing growth. At that time the natural flocculation of the crops began (tenth day of cultivation).

In the treatment in which 2 mL of solutions 1 and 2 (T2) were used, the number of cells was much greater than T1, with a maximum of 889±35 cells mL^{-1} x 10^6, compared to only 477±19 cells mL^{-1} x 10^6 (T1) in the eighth day. In the last day of cultivation, the number of cells at T2 was 523±21 cells mL^{-1} x 10^6, compared to 323±11 cells mL^{-1} x 10^6 (T1).

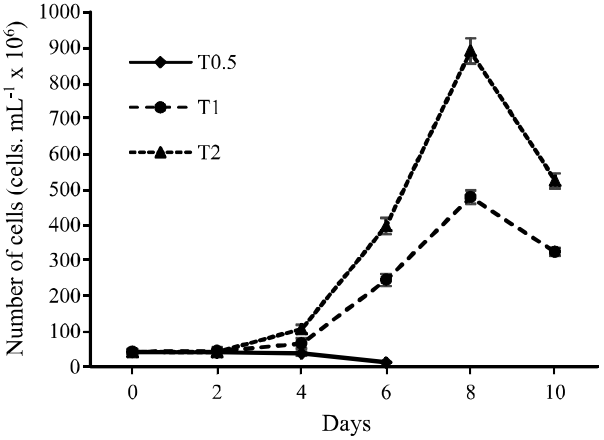
In treatments T1 and T2 a total of 52 mL of NaOH 2N solution was used for flocculation of the 2600 mL in total volumes in both experiments, at the end of the 10 days of microalgae cultivation.

The dry biomass yields for these treatments were 0.025±0.002% and 0.031±0.004%, that is, 0.65±0.05 g and 0.81±0,1 g for T1 and T2, respectively. Lastly, with regard to oil yields, the highest level was observed in the treatment using the smaller quantity of nutrients (T1) (20.13±0.19%). At T2, the rate was 18.95±0.23%, lower than that found at T1.

Table 2 - Cellular densities of microalgae *C. vulgaris* when varying the quantity of nutrients in the yield and characterization of biomass and oil, for biodiesel production. T0.5=0.5 mL of solutions 1 and 2 for each liter of grown medium prepared; T1.0=1.0 mL of solutions 1 and 2 for each liter of grown medium prepared and T2.0=2.0 mL of solutions 1 and 2 for each liter of grown medium prepared

Days	Number of cells (cells mL^{-1} x 10^6)		
	T0.5	T1	T2
0	41 ± 5	42 ± 8	42 ± 7
2	40 ± 6	44 ± 10	41 ± 3
4	37 ± 6	65 ± 14	106 ± 12
6	12 ± 2	244 ± 17	396 ± 23
8	-	477 ± 19	889 ± 35
10	-	323 ± 11	523 ± 21

Figure 1 - Growth in the number of cells of the microalgae *C. vulgaris* when varying the quantity of nutrients in the yield and characterization of biomass and oil, for biodiesel production. T0.5=0.5 mL of solutions 1 and 2 for each liter of grown medium prepared; T1.0=1.0 mL of solutions 1 and 2 for each liter of grown medium prepared and T2.0=2.0 mL of solutions 1 and 2 for each liter of grown medium prepared



The lowest amount of dry biomass (0.025 ± 0.002%), obtained at T1, was higher than that found by Zheng *et al*. (2011) while growing the same species in saltwater.

Frumento *et al*. (2013) grew microalgae *C. vulgaris* in two different tubular photobioreactors – one helical and the other horizontal – both 2.0 liters in 5 klux using 0.2 g L^{-1} of bicarbonate in the growth medium with CO_2, in both treatments. The helical photobioreactor showed the best result for biomass yield (84.8 mg L^{-1} d^{-1}). However, the tubular photobioreactor showed the best lipid yield (10.3 mg L^{-1} d^{-1}) due to the higher rate of oil in the biomass (22.8%).

Shen *et al*. (2015) cultivated microalgae *Chlorella vulgaris* adapted to the variation of 0 to 50 g L^{-1} of salinity to remove nutrients in saline wastewaters, growth and lipid accumulation, and found that the efficiency of removal of total nitrogen and total phosphorus were 92.2-96.5% and 99%, respectively, at the end of 20 days. Lipid accumulation was observed during nitrate depletion (<5 mg L^{-1}) and 40% of this compound was obtained, in a saltwater recirculation system in a photobioreactor.

In wastewaters, He *et al*. (2013) also grew microalgae *C. vulgaris* in high levels of ammonia for biodiesel production and observed that the specific growth rate was 0.92 d^{-1} in 17 mg L^{-1} of ammonia, decreasing to 0.33 d^{-1} in 39-143 mg L^{-1} of ammonia. In 39 mg L^{-1} of ammonia, the highest yield was obtained (23.3 mg L^{-1} d^{-1}), falling drastically under the highest rate of ammonia.

Doan, Sivaloganathan and Philip (2011) conducted a study to evaluate the potential of certain marine microalgae as raw materials for biodiesel production. The results revealed that microalgae *Nannochloropsis* sp. showed both the highest concentration of dry biomass (0.4 ± 0.003 g L^{-1}) and highest lipid yield (44.9%), making it quite promising as a source for biodiesel production, with much higher values than those in the aforementioned study.

With these results, although the microalgae *Chlorella vulgaris* cultivated with 1mL of solutions 1 and 2 (T1) has presented lower yields of dry biomass at the end of the cultures (0.65 g), but higher yields of oil (20.13%) when compared to the culture with 2 mL of solutions 1 and 2 (T1) (0.81 g and 18.95%, respectively), we suggest the T2 treatment as the best amount of nutrients as a promising source of biodiesel, yielding a final oil yield of 0.15 g, compared to only 0.13 g of oil. This means that in Treatment T2, even with the lowest percentage of oil (18.95%), the amount of biomass produced compensates in the productivity of this compound, and using a lower amount of nutrients in the growth medium.

Nutritional stress due to nitrogen shortage inhibits microalgae cell growth due to its inability to synthesize proteins leading the organism to produce energetics reserve biomolecules, such as lipids and carbohydrates, increasing cell volume (SILVA; LOURENÇO; CHALOUB, 2009).

The strategies that provide the increase of lipid production by microalgae under programmed stress conditions, caused by the limitation of nutrients or variations of cultivation conditions (temperature, pH and salinity), may canalize the metabolic flow of lipid biosynthesis during photosynthesis when the energy source (light) and the carbon source (CO_2) are abundantly available and when the cellular mechanisms for photobiosynthesis are active (COURCHESNE *et al*., 2009). The lipid productivity takes into account both the lipid concentration of lipids inside the cells, as well as the total biomass obtained, being a useful indicator of the potential production costs liquid biofuels. Currently, to cause stress by nitrogen restriction in the culture medium to promote the rising of lipid production in microalgae is one of the most studied strategy (WIDJAJA; CHIEN; JU, 2009).

The crude protein levels in microalgae *C. vulgaris* grown in different amounts of nutrients (1.0 and 2.0 mL of solutions 1 and 2 for each liter of grown medium prepared), which showed positive performance were 25.85±0.24% and 27.46±0.59%, very similar, for the treatments with 1.0 and 2.0 mL of solutions 1 and 2, respectively. Thus, the treatment in which 2.0 mL of solutions 1 and 2 was used showed the highest rate of proteins, when compared to the

treatment where the highest concentration of nutrients was used in the grown media.

In wastewater, He *et al*. (2013) cultivated the microalgae *C. vulgaris* in high amounts of ammonia and obtained 12% protein in 17 mg L^{-1}, and in 207 mg L^{-1} ammonia the value was 42% protein. In addition, the carbohydrate in the dry biomass was in the range of 14 to 45%, with a maximum value occurring in 143 mg L^{-1} of ammonia.

The results obtained by the respective authors were lower than that found in the respective work, at 17 mg L^{-1} of ammonia. However, in 207 mg L^{-1} of ammonia, the amount of protein was higher than in the respective study. This is likely due to the different variation of ammonia in the research in question, as well as in the formulation of the growth media of the microalgae.

Total proteins in microalgae *C. vulgaris* vary according to growth conditions and also with the type of nitrogen (SAFI *et al*., 2013).

The identification of the methyl esters found in the oil of microalgae *C. vulgaris* grown in different amounts of nutrients, in both treatments that showed positive performance, (T1 and T2), revealed the presence of palmitic, oleic and linoleic acids, of which almost 40% is palmitic acid, a fatty acid with no unsaturation (Table 3).

Table 3 - Composition of fatty acids found in oil obtained from *C. vulgaris* grown outdoors

Identified fatty acids	Amount (%)	
	T1	T2
16:0 (palmitic)	38.6	36.4
18:1 (oleic)	22.7	21.8
18:2 (linoleic)	16.3	18.7
18:3 (linolenic)	3.0	3.5
Not identified (C20 to C25)	19.4	19.6

The absence of or insignificant unsaturation in the carbon chain results in higher quality of oil for biodiesel, as carbon chains with few unsaturation undergo less oxidation that more unsaturated chains, but they freeze in higher temperatures.

In both treatments (T1 and T2), almost 20% of fatty acids were not identified, among which might be the series of long-chain unsaturated fatty acids (C20 to C25), which are quite important from a nutritional standpoint but could not be identified using the method employed herein.

The method of extraction of the oil used was Bligh and Dyer (1959), because it is widely used in the scientific environment due to the ease and for using methanol, chloroform and water.

Mandal and Mallick (2009) grew freshwater microalgae *Scenedesmus obliquus* under different nutrient concentrations in order to analyze lipid content, and also detected that palmitate and oleate were the main lipids of that microalgae, which makes it a suitable source for biodiesel production.

Zheng *et al*. (2011) cultivated the same species of *Chlorella* used in this work and observed that the oil composition was 71.76% and 28.24% of unsaturated and saturated fatty acids, respectively. Of total unsaturated fatty acids, 45% and 23% were oleic (18:1) and palmitoleic (16:1) acids, respectively. With regard to saturated fatty acids, 23% was palmitic acid (16:0). These differences in comparison to the present study are likely die to growth conditions, particularly with regard to different quantities of nutrients in the growth media.

He *et al*. (2013) grew microalgae *C. vulgaris* in wastewaters with high levels of ammonia for biodiesel production and observed that long-chain unsaturated fatty acids (C16 to C18) accounted for 80% of total fatty acids obtained. The increase in the amount of ammonia from 17 mg L^{-1} to 207 mg L^{-1} favored the emergence of short-chain fatty acids and unsaturated fatty acids.

In this work, we found the same class of long-chain unsaturated fatty acids (C16 to C18) as those found by the authors of the aforementioned study.

Thus, the physical-chemical conditions of the crops directly influence the quality and quantity of fatty acids produced by the microalgae, which makes it quite important to characterize the lipids in the same species any time when growth conditions are altered.

The induction of programmed nutritional stress caused by nitrogen restriction, besides increasing the lipid content by the organism, causes a gradual change in the composition of the fatty acids free of triglycerides (WIDJAJA; CHIEN; JU, 2009). This happens because the scarcity of nitrogen facilitates the activation of the enzyme *diacylglycerol acyltransferase*, responsible for the conversion of acyl-coA to triglycerides (TAKAGI *et al*., 2000). According to Meng *et al*. (2009) triglycerides are more useful for the production of biodiesel.

CONCLUSIONS

1. The results of this work demonstrate that using 1 and 2 mL of the solutions, the microalgae showed two days

of adaptation to grown conditions (induction phase), followed by rapid growth, reaching their peak on the eighth day;

2. The dry biomass yields for these treatments were 0.025 ± 0.002% and 0.031 ± 0.004%, that is, 0.65 ± 0.05 g and 0.81 ± 0.1 g for T1 and T2, respectively. Lastly, with regard to oil yields, the highest level was observed in the treatment using the smaller quantity of nutrients (T1) (20.13 ± 0.19%). At T2, the rate was 18.95 ± 0.23%, lower than that found at T1;
3. In spite of these oil yield data, we suggest the T2 treatment as the best quantity of nutrients as a promising source of biodiesel, because it presents a final oil yield of 0.15 g, compared to only 0.13 g of oil in the T1 treatment. This means that in Treatment T2, even with the lowest percentage of oil (18.95%), the amount of biomass produced compensates in the productivity of this compound, and using a lower amount of nutrients in the growth medium;
4. The identification of the methyl esters found in the oil of microalgae *C. vulgaris* grown in different amounts of nutrients, in both treatments that showed positive performance, revealed the presence of palmitic, oleic and linoleic acids, of which almost 40% is palmitic acid, a fatty acid with no unsaturation. It is worth noting that biodiesel is the fuel composed of alkyl esters of long chain carboxylic acids, produced from the transesterification and/or esterification of greases, from fats of vegetable or animal origin, and that in Brazil, 75% of production is made with soybean oil, 20% with animal fat and the rest with several other sources, such as palm oil, cottonseed oil and canola.

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