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***A N I N T E R N A T I O N A L J O U R N A L***

# Carbon Dioxide Biofixation and Production of *Spirulina* sp. LEB 18 Biomass with Different Concentrations of $NaNO_3$ and NaCl

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## ABSTRACT

*Microalgae are efficient at using solar energy to turn $CO_2$ and nutrients into biomass containing lipids, proteins, carbohydrates and other compounds that may be used to produce bioproducts for human and animal consumption and pharmaceutical use. The aim of this study was to assess the effect of the $NaNO_3$ and NaCl concentration on the growth kinetics, the biomass composition and the ability to biofix $CO_2$ using the microalga* Spirulina *sp. LEB 18. The assays were carried out according to a $2^2$ central composite design (CCD) with different concentrations of $NaNO_3$ (1.25, 1.88 and 2.50 g $L^{-1}$) and NaCl (1.00, 15.0 and 30.0 g $L^{-1}$). The assays were carried out in 2 L vertical tubular photobioreactors at 30°C, 12 h light/dark and an injection of 12.0% v/v of $CO_2$ at 0.3 vvm. The best growing results ($X_{max}$ = 1.60 g $L^{-1}$, $P_{max}$ = 0.109 g $L^{-1}$ $d^{-1}$, $\mu_{max}$ = 0.208 $d^{-1}$) and $CO_2$ biofixation rate (197.4 mg $L^{-1}$ $d^{-1}$) were observed in the assay with 1.25 g $L^{-1}$ $NaNO_3$ and 1.00 g $L^{-1}$ NaCl. Increasing the NaCl concentration produced biomass with a higher carbohydrate content, while increasing the $NaNO_3$ concentration reduced the protein concentration. According to the results, in addition to using* Spirulina *as a source of protein, it can also be used as a source of carbohydrates and to biologically remove $CO_2$ from the atmosphere.*

**Keywords**: $CO_2$; fixation; growth kinetics; microalgae; nutrients.

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## INTRODUCTION

In light of the growing concern about global warming and the sustainable management of resources, microalgae biotechnology is a promising alternative for reducing excess $CO_2$ emissions and for treating wastewater because it can produce biomass, which has several uses (Hende et al. 2012).

Microalgal biomass can be a source of biocompounds, including lipids, proteins, carbohydrates, vitamins and pigments (Spolaore et al. 2006). These compounds can be processed into products with high added value, such as biopolymers, pharmaceuticals, cosmetics, human food and animal feed (Ho et al. 2011). Microalgae have many favorable characteristics: they present high biomass productivity, are tolerant to seasonal variations and have a high photosynthetic efficiency to fix $CO_2$ and accumulate quantities of compounds, such as lipids and carbohydrates (Subhadra and Edwards, 2010).

The growth kinetics and biochemical composition of microalgae can be influenced by several factors: temperature, pH, nutrient concentration of the medium, salinity, illuminance and growth phase. The definition of these parameters, whether qualitative or quantitative, can significantly change the biomass composition, inhibiting or stimulating the synthesis of biomolecules (Hu, 2004). Exposing the cultivations to conditions of stress, such as limited phosphorus and nitrogen (Mutlu et al. 2011), illuminance (Ho et al. 2012) and high salinity (Rao et al. 2007), may trigger the accumulation of biocompounds reserve.

*Spirulina* is a filamentous and alkaliphilic cyanobacterium. Its biomass is exceptional because it contains between 60 and 70% (w $w^{-1}$) proteins, pigments (such as chlorophyll and phycocyanin) and poly-unsaturated fatty acids (such as ɣ-linolenic). *Spirulina's* carbohydrate concentration can vary from 12 to 20% (w $w^{-1}$) and the lipid concentration from 6 to 13% (w $w^{-1}$) (Cohen, 1997). Given these low values of reserve compounds, changes in the concentration of nutrients in the culture medium can increase the content of lipids and carbohydrates.

The carbon source that is required for *Spirulina* cultivation represents 60.0% of the costs with nutrient (Alava et al. 1997). The $CO_2$ concentration of 10-15% is the average value found in the combustion gases of power plants (Lee et al. 2002). The use of carbon source alternative present in the combustion gases can minimize the problems that are caused by the emission of this greenhouse gas and reduce costs with this nutrient (Hughes and Benemann, 1997) for cultivation.

Therefore, the aim of this study was to investigate the effect of the $NaNO_3$ and NaCl concentration on the growth kinetics, biomass composition and ability of *Spirulina* sp. LEB 18 to biofix $CO_2$.

## MATERIALS AND METHODS

### Microorganism and cultivation medium

The microalga used was *Spirulina* sp. LEB 18 (Morais et al. 2008), which was maintained in Zarrouk medium (Zarrouk, 1966). This strain belongs to the Cultures Collection of the Laboratory of Biochemical Engineering, Federal University of Rio Grande (FURG).

### Adaptation of the inoculum

The inoculum was prepared from an initial volume of 3.0 L and biomass concentration of 1.0 g $L^{-1}$. This inoculum was filtered in a sterile environment. The cells that were collected in the filter paper were washed with sterile distilled water to remove the salts from the Zarrouk medium. The washed cells were recovered in 1.0 L of modified Zarrouk medium. The modification of the culture medium involved a reduction of 50% $NaNO_3$ (adaption to the lowest point of the experimental design) in the absence of $NaHCO_3$ (original carbon source of Zarrouk medium). Then, this inoculum was maintained, with carbon dioxide ($CO_2$) as the new carbon source, in the concentration of 5.0% v/v, flow rate of 0.3 vvm (volume of gaseous mixture per volume of medium per min) for 15 min, every 2 h, in the light period. The $CO_2$ specific flow rate was of 1.35 $L_{CO2}$ $L_{medium}^{-1}$ $d^{-1}$ ($R_{CO2fed}$ = 1.35 $d^{-1}$).

**Cultivation conditions**

The assays were carried out according to a $2^2$ central composite design (CCD) (4 assays + 2 central points) (Table 1). The independent variables that were assessed in the culture medium were the concentrations of $NaNO_3$ and NaCl.

**Table 1.** $2^2$ CCD matrix with coded and actual levels of the variables

| Assay | $X_1$ ($NaNO_3$) | $X_2$ (NaCl) | $NaNO_3$ (g $L^{-1}$) | NaCl (g $L^{-1}$) |
|---|---|---|---|---|
| 1 | -1 | -1 | 1.25 | 1.0 |
| 2 | +1 | -1 | 2.50 | 1.0 |
| 3 | -1 | +1 | 1.25 | 30 |
| 4 | +1 | +1 | 2.50 | 30 |
| 5 | 0 | 0 | 1.88 | 15 |
| 6 | 0 | 0 | 1.88 | 15 |

The experiments were carried out in fed-batch mode with $CO_2$ in 2 L vertical tubular photobioreactors with a working volume of 1.5 L at 30 °C, 12 h light/dark photoperiod, and 41.6 $\mu mol_{photons}$ $m^{-2}$ $s^{-1}$ (Morais and Costa, 2007) for 14 d. The stirring of the cultivations was promoted by the continuous injection of compressed air at 0.3 vvm, and during the light phase, the gas mixture was enriched with 12.0% v/v of $CO_2$ for 15 min (Morais and Costa, 2007) every 2 h ($R_{CO2fed}$ = 3.24 $d^{-1}$). In these conditions, but with no $CO_2$ addition and with standard Zarrouk medium, a control assay was carried out. The daily variation in volume due to evaporation was corrected with the addition of sterile distilled water until the working volume of the photobioreactor was restored.

**Analytical determinations**

Samples were collected daily to monitor the biomass concentration. The biomass concentration (BC) was determined spectrophotometry using a standard curve of *Spirulina* sp. LEB 18 (Absorbance = 1.2515*BC + 0.0478, with $R^2$ = 0.9934, and estimated error of 3.38%). This curve was obtained by measuring the optical density of the *Spirulina* inoculum in a spectrophotometer at 670 nm, by relating the optical density and dry weight biomass, as performed by Costa et al. (2002). At the end of the experiments, the biomass was centrifuged at 18,000 g for 20 min at 25 °C, resuspended three times in distilled water and then centrifuged again under the same conditions to remove salts from the medium. Subsequently, the biomass was dried at 40 °C for 24 h and stored at -20 °C until its characterization.

The concentrations of carbon (C) and nitrogen (N) in the biomass were determined in an elemental analyzer (Perkin Elmer 2400, USA) using acetanilide as the standard. The protein concentration was calculated from the concentration of elemental nitrogen using 5.22 as the conversion factor, which is specific for cyanobacteria (Lourenço et al. 2004). The lipid concentration was determined using the Folch method (Folch et al. 1957). The concentration of carbohydrates in the biomass was determined by the adapted 3.5-DNS method (Miller, 1959) with the prior acid hydrolysis of polysaccharides.

**Assessment of the growth parameters**

The maximum biomass concentration ($X_{max}$, g $L^{-1}$) of the *Spirulina* cultivations was obtained for each assay, and the kinetic parameters were calculated. The biomass productivity ($P_x$, g $L^{-1}$ $d^{-1}$) was calculated according to Equation 1, where $X_t$ (g $L^{-1}$) is the biomass concentration at time t (d), and $X_0$ (g $L^{-1}$) is the biomass concentration at time $t_0$ (d). The maximum specific growth rate ($\mu_{max}$, $d^{-1}$) was obtained from the exponential regression of the logarithmic phase of the microalgal growth.

$$P_x = \frac{X_t - X_0}{t - t_o} \quad (1)$$

**$CO_2$ biofixation rate ($R_{CO2}$)**

The $CO_2$ biofixation rate ($R_{CO2}$, mg $L^{-1}$ $d^{-1}$) was calculated according to Toledo-Cervantes et al. (2013), as shown in Equation 2. In this Equation $P_{max}$ (mg $L^{-1}$ $d^{-1}$) was the maximum

productivity for each assay, $x_{cbm}$ ($mg_{carbon}$ $mg_{biomass}^{-1}$) was the mass fraction of carbon as determined using an elemental analysis of biomass, and $M_{CO2}$ and $M_C$ were the molar masses of carbon dioxide and carbon, respectively.

$$R_{CO_2} = P_{max} * x_{cbm} * \frac{M_{CO_2}}{M_C} \quad (2)$$

**Statistical Analysis**

The responses that were obtained in the experiments were evaluated by experimental design methodology use Statistica 6.0 software (StatSoft Inc., USA). The standard error was calculated based on the replications of the central points (pure error) (Teófilo and Ferreira, 2006) with a 95.0% confidence level.

## RESULTS AND DISCUSSION

The profiles of the biomass concentration and biomass productivity of *Spirulina* sp. LEB 18 are shown in Figure 1. The microalga did not present a lag phase of growth due to the pre-adaptation of the inoculum, and the exponential phase was defined between the third and sixth days (Fig 1A), with coefficients of determination ($R^2$) greater than 0.99 for all assays.

The biomass concentration was similar for the seven assays during the 4 and 5 d of cultivation, even in those with the lowest $NaNO_3$ concentrations (1.25 g $L^{-1}$) and highest NaCl concentrations (30 g $L^{-1}$). The highest profile of biomass concentrations (Fig 1A) and biomass productivity (Fig 1B) were observed in the assay using 1.25 g $L^{-1}$ $NaNO_3$ and 1.0 g $L^{-1}$ NaCl and were observed lower profiles of these parameters in the control assay.

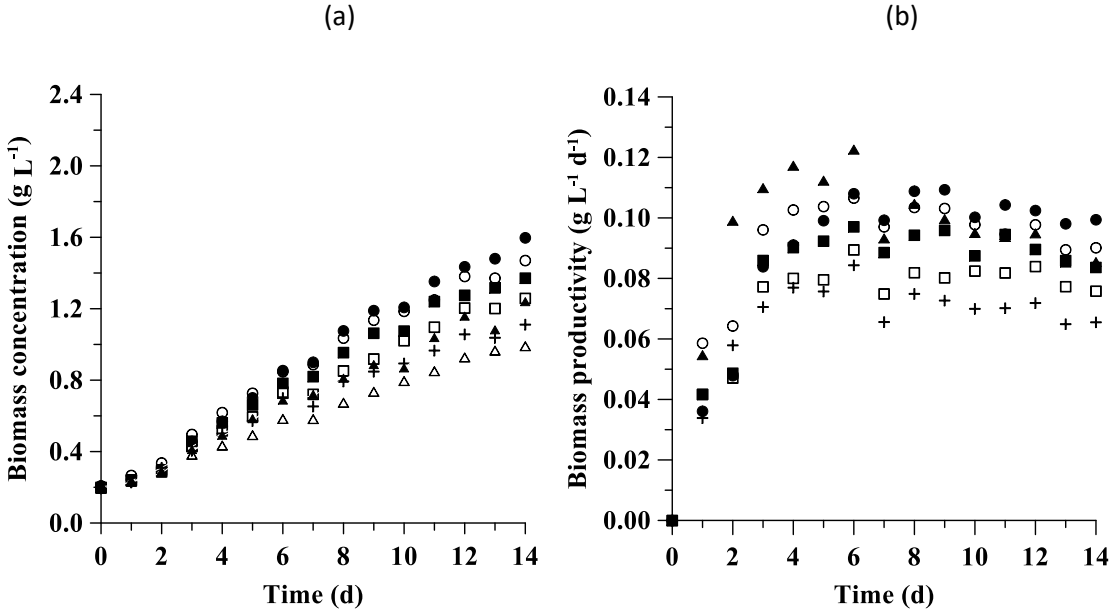


**Figure 1.** Profiles of biomass concentration (a) and biomass productivity (b) as a function of time for assays 1 (●), 2 (○), 3 (■), 4 (□), 5 (+), 6 (▲) and control (Δ) as obtained with *Spirulina* sp. LEB 18 cultivation

Regarding the growth kinetics, the $NaNO_3$ and NaCl concentrations in the culture medium and the interaction between the variables had no significant effect ($p > 0.05$) on the maximum biomass concentration, maximum biomass productivity or maximum specific growth (Table 2). *Spirulina* cultivations normally use Zarrouk medium, which contains 2.5 g $L^{-1}$ $NaNO_3$, 1.0 g $L^{-1}$ NaCl and 16.8 g $L^{-1}$ $NaHCO_3$. This study confirmed *Spirulina* tolerance to the changes in the concentrations of nutrients in the culture medium because the biomass productivity was maintained and was able to use $CO_2$ as the carbon source, presenting better results than the control assay with Zarrouk medium.

The growth that was observed in the cultivations with the highest tested NaCl concentrations tested (15.0 and 30.0 g $L^{-1}$) corroborates the fact that *Spirulina* tolerates higher osmotic pressures compared to the concentration that Zarrouk medium usually provides, which is 1.0 g $L^{-1}$ salt. Many cyanobacteria are considerably tolerant to saline stress because they have various physiological mechanisms, including the accumulation of inorganic or organic osmoregulators (Reed et al. 1986; Mackay et al. 1984) and the active extrusion of intracellular sodium (Gabbay-Azaria et al. 1992; Peschek et al. 1994).

**Table 2.** Real levels of the variables and maximum biomass concentration ($X_{max}$), maximum biomass productivity ($P_{max}$) and maximum specific growth rate ($\mu_{max}$) of *Spirulina* sp. LEB 18 cultivation

| Assay | $NaNO_3$ (g $L^{-1}$) | NaCl (g $L^{-1}$) | $X_{max}$ (g $L^{-1}$) | $P_{max}$ (g $L^{-1}$ $d^{-1}$) | $\mu_{max}$ ($d^{-1}$) |
|---|---|---|---|---|---|
| 1 | 1.25 | 1.0 | 1.60 | 0.109 | 0.208 |
| 2 | 2.50 | 1.0 | 1.47 | 0.107 | 0.177 |
| 3 | 1.25 | 30 | 1.37 | 0.097 | 0.177 |
| 4 | 2.50 | 30 | 1.26 | 0.089 | 0.175 |
| 5 | 1.88 | 15 | 1.11 | 0.084 | 0.177 |
| 6 | 1.88 | 15 | 1.23 | 0.080 | 0.174 |
| Control* | 2.50 | 1.0 | 0.99 | 0.062 | 0.104 |

*Control assay carried out with no $CO_2$ addition

The highest values of biomass concentration, biomass productivity, and specific growth rate were observed in assay 1, with 1.0 g $L^{-1}$ NaCl and 1.25 g $L^{-1}$ $NaNO_3$ (Table 2). This result shows that the 50.0% reduction in the nitrogen source was not a limiting factor for the growth of the microalga. When there is a shortage of a nitrogen source, the microalga's metabolism can use chlorophyll and other pigments as an intracellular nitrogen source (Jiang et al. 2011). Colla et al. (2007) found that a 75.0% reduction in the $NaNO_3$ concentration of a *Spirulina* cultivation did not reduce the biomass productivity.

Our study corroborates this result, as the concentration of $NaNO_3$ was reduced by 50.0% compared to the standard concentration of Zarrouk medium.

Increased of salinity normally harms cell growth due to the higher level of ionic and osmotic stress. This damage is due to changes in the ionic cellular proportions and to the selective permeability of the cell membrane to ions (Brand, 1984; Glass, 1983). This study found that 15.0 and 30.0 g $L^{-1}$ NaCl in the culture medium reduced the maximum biomass concentration by 30.6% and the biomass productivity by 26.6%, taking into account the maximum and minimum values that were reached among the assays for both studied responses.

Some studies have shown that 30 g $L^{-1}$ NaCl in the medium may affect the activity of photosystem II and the energy transfer processes of the proteins in *Spirulina's* thylakoid membrane (Sudhir et al. 2005). Ravelonandro et al. (2011) cultivated *Spirulina platensis* in Zarrouk medium with 13.0 and 30.0 g $L^{-1}$ NaCl and found a reduction in the maximum biomass concentration (from 1.6 to 1.0 g $L^{-1}$) and maximum biomass productivity (from 0.08 to 0.05 g $L^{-1}$ $d^{-1}$). The results that were obtained in this study (assay 3, $X_{max}$= 1.37 g $L^{-1}$ and $P_{max}$= 0.097 g $L^{-1}$ $d^{-1}$) showed that the genus *Spirulina* can produce superior results, even when submitted to the same saline stress (30.0 g $L^{-1}$) and a 50.0% reduction of the nitrogen source.

In the cultivation of *Spirulina* sp. LEB 18 and *Scenedesmus obliquus* LEB 22 in three photobioreactors in series and with the addition of 12% $CO_2$ was obtained the average biomass productivity among three reactors of 0.080 and 0.050 g $L^{-1}$ $d^{-1}$, respectively (Radmann et al. 2011). These results are lower than those in this study, demonstrating that the concentrations of NaCl and $NaNO_3$ in the culture medium can be manipulated to not promote losses of biomass productivity. In the control assay employed were the same $NaNO_3$ and NaCl concentrations of assay 2, however without the addition of $CO_2$. In this context, it is believed that $CO_2$ employment was better assimilated as carbon source and promoted greater growth.

**Table 3.** Concentration of proteins, carbohydrates, lipids and elemental carbon in the biomass and $CO_2$ biofixation rate ($R_{CO2}$) for *Spirulina* sp. LEB 18

| Assay | Proteins (%w $w^{-1}$) | Carbohydrates (% w $w^{-1}$) | Lipids (%w $w^{-1}$) | Carbon (%w $w^{-1}$) | $R_{CO2}$ (mg $L^{-1}$ $d^{-1}$) |
|---|---|---|---|---|---|
| 1 | 62.4 | 11.3 | 10.3 | 49.2 | 197.4 |
| 2 | 60.1 | 15.3 | 16.7 | 48.6 | 189.7 |
| 3 | 60.3 | 16.7 | 9.2 | 49.5 | 176.2 |
| 4 | 55.8 | 14.8 | 10.3 | 46.0 | 150.9 |
| 5 | 55.6 | 20.8 | 15.9 | 48.3 | 149.5 |
| 6 | 56.0 | 20.6 | 12.6 | 48.3 | 141.5 |
| Control* | 58.3 | 15.4 | 12.8 | 48.6 | - |

*Control assay carried out with no $CO_2$ addition

Limiting the nitrogen source in the cultivation of microalgae encourages the metabolism of lipids and carbohydrates. The carbon that is present in the cells will be directed to the production of reserve compounds. Therefore, competition between the synthesis of lipids and carbohydrates will occur, given that the latter are the first reserve compounds to be synthesized, followed by lipids, which are stored when the period of nitrogen shortage is longer (Siaut et al. 2011).

Table 4 presents the estimate of the main effects (L) of the concentration of $NaNO_3$ (g $L^{-1}$), the concentration of NaCl (g $L^{-1}$) and the effect of the interaction between them on the concentration of proteins, carbohydrates and lipids found in the biomass of *Spirulina* and in the $CO_2$ biofixation rate by *Spirulina* cultivation.

**Table 4**. Estimate of the main effects (L) and interaction between the variables for the concentrations of proteins, carbohydrates, lipids in the biomass and $CO_2$ biofixation rate ($R_{CO2}$) for *Spirulina* sp. LEB 18

| Factor | Proteins | | Carbohydrates | | Lipids | | $R_{CO2}$ | |
|---|---|---|---|---|---|---|---|---|
| | Effect | p | Effect | P | Effect | p | Effect | p |
| Mean | 58.4 | 0.001 | 14.5 | 0.002 | 12.5 | 0.050 | 167.7 | 0.009 |
| Curvature | - | - | 12.4 | 0.010 | - | - | - | - |
| $NaNO_3$ (L) | -3.39 | 0.048 | 1.04 | 0.090 | 3.77 | 0.360 | -16.4 | 0.211 |
| NaCl (L) | -3.18 | 0.052 | 2.44 | 0.040 | -3.78 | 0.360 | -29.9 | 0.118 |
| $NaNO_3$ * NaCl (L) | -1.10 | 0.147 | -2.97 | 0.030 | -2.63 | 0.470 | -8.82 | 0.363 |

The concentration of $NaNO_3$ in the culture medium had a significant ($p < 0.05$) and negative (-3.39) effect on the protein concentration in the biomass, indicating that increasing the concentration of this nutrient in the culture medium decreases the concentration of proteins in the microalgal biomass. Thus, the maximum concentration of proteins in biomass (62.4%) was found in assay 1, which had a lower nitrogen concentration (1.25 g $L^{-1}$ of $NaNO_3$). However, the most pronounced reduction of protein (4.5%) occurred when it increased the concentration of nitrogen (1.25 to 2.50 g $L^{-1}$) and maintained the sodium chloride concentration at 30 g $L^{-1}$. In this context, it is believed that the use of NaCl at thirty times the normal level may have had a synergistic effect with increasing the nitrogen source.

Nitrogen is an essential element and is indispensable for the growth of microalgae because the formation of amino acids, proteins, enzymes, coenzymes and chloroplasts, and so forth is associated with the uptake of this compound (Turpin, 1991). Therefore, the results that were obtained in this study can be explained by the fact that the same concentration of $NaNO_3$ was used for the inoculum adaptation. Another explanation for the higher content of proteins being found with the lowest concentration of $NaNO_3$ is that, according to Colla et al. (2007), at 30 °C, the absorption of the culture medium's nitrogen by the microalgae is limited.

In all of the assays that were carried out, regardless of the concentrations of NaCl and $NaNO_3$ that were used in the cultivation, the concentrations of proteins in the *Spirulina* sp. LEB 18 biomass were superior to the results found by Ravelonandro et al. (2011). These authors found that the protein concentration in the biomass of *Spirulina platensis* decreased from 50.0 ± 2.0% to 38.0 ± 1.0% when the NaCl concentration increased from 13.0 to 35.0 g $L^{-1}$.

The protein concentration is an important factor when assessing the nutritional value of microalgae. *Spirulina* cultivation can be an alternative to produce proteins for human or animal

consumption (Spolaore et al. 2006). In Zarrouk culture medium, on bench and pilot scales, the protein concentration in *Spirulina* can reach values between 62.0% (w $w^{-1}$) (Borges et al. 2013) and 86.0% (w $w^{-1}$) (Morais et al. 2009), respectively.

The NaCl concentration and the interaction between the variables ($NaNO_3$ X NaCl) had a significant effect ($p < 0.05$) on the concentration of carbohydrates in the biomass of *Spirulina* sp. LEB 18. The concentration of NaCl had a positive effect (2.44), which shows that increasing the concentration of this compound in the culture medium may increase the amount of carbohydrates of the microalgal biomass. However, the interaction had a negative and, in modulus, higher effect (-2.97) regarding the concentrations of carbohydrates in the biomass. Therefore, this effect demonstrates that an increase in the amount of NaCl and $NaNO_3$ in the culture medium may reduce the concentration of carbohydrates of the biomass.

The experimental design contemplated the optimal point (central points) for carbohydrates concentration of the *Spirulina* biomass compared to the tested concentrations of $NaNO_3$ and NaCl. This was in such a way that the estimate of curvature was statistically significant (Table 4), and the realization of axial points (-1.41 and 1.41) of the experimental design would probably lead to the optimization of the concentration of the microalgal carbohydrate.

A low nitrate, phosphate and bicarbonate concentration combined with an increased concentration of sodium chloride in the culture medium results in a substantial increase in the carbohydrate fraction of the microalgal cells and reduces the total protein content (Tadros, 1991). Vonshak et al. (1988) and Martel et al. (1992) reported that *Spirulina platensis* that was subjected to saline stress increased the metabolism of carbohydrate production in cells. According to Vonshak (1997), in the presence of 0.5 mol $L^{-1}$ (29.3 g $L^{-1}$) NaCl in the cultivation, the maximum accumulation of carbohydrates in the *Spirulina* biomass was 64.4% (w $w^{-1}$).

When the microalga *Scenedesmus obliquus* was deprived of nitrogen, there was a 26.0% reduction in protein concentration, whereas the concentrations of lipids and carbohydrates increased by approximately 11.0% and 14.0%, respectively. In this study, the carbohydrate concentrations increased with the time of deprivation and peaked at 51.8% after 7 d of cultivation (Ho et al. 2012).

The concentration of lipids in the biomass of *Spirulina* sp. LEB 18 did not present reproducibility in the central points (Table 3). The concentration of lipids was not significantly ($p > 0.05$) influenced by the levels of $NaNO_3$ and NaCl in the culture medium or by the interaction between these variables (Table 4). However, the lipid concentrations that were observed in this study were higher than the 5.0% that was reported by Borges et al. (2013) when using *Spirulina* sp. LEB 18 and to the 4.0% found by Batista et al. (2013) when using the biomass of *Spirulina maxima*.

**$CO_2$ Biofixation by *Spirulina***

The $CO_2$ biofixation rate by *Spirulina* was not significantly influenced by the concentrations of $NaNO_3$ and NaCl in the culture medium or by the interaction between the variables ($p > 0.05$) (Table 4).

In addition to nitrogen limitation and higher salinity, adding $CO_2$ to the cultivation can increase biocompounds, such as carbohydrates and lipids, due to the greater incorporation of carbon by the microalga (Hsueh et al. 2009).

Microalgae contain approximately 50.0% (w $w^{-1}$) carbon in their composition (Amaro et al. 2011). The elemental concentration of carbon for the assays was maintained in the range of 46.0 to 49.5%, with the maximum (49.5%) found in assay 3 (Table 3), with 1.25 g $L^{-1}$ $NaNO_3$ and 30 g $L^{-1}$ NaCl.

The maximum $CO_2$ biofixation rate was 197.4 mg $L^{-1}$ $d^{-1}$ (assay 1), 38.7% higher than the lowest $R_{CO2}$ that was obtained in assay 6 (142.0 mg $L^{-1}$ $d^{-1}$). In assays 3 and 4, the reductions of $R_{CO2}$ were also checked against assay 1. This behavior indicates that the concentrations of 15.0 and 30.0 g $L^{-1}$ NaCl may result in decreased biomass productivity and thus in the reduction of $CO_2$ biofixation by the microalga.

The $CO_2$ biofixation rates that were found in this study (Table 3) were higher than the maximum $R_{CO2}$ that was obtained in assays with *Spirulina* sp. LEB 18 (118 mg $L^{-1}$ $d^{-1}$), *Chlorella vulgaris* LEB 106 (124 mg $L^{-1}$ $d^{-1}$) and *Scenedesmus obliquus* LEB 22 (88 mg $L^{-1}$ $d^{-1}$) in Zarrouk medium and 12% v/v of $CO_2$ (1.08 $d^{-1}$) by Radmann et al. (2011). This result shows that *Spirulina* can achieve high $CO_2$ biofixation rates, even under conditions of saline

stress and nitrogen limitation. Thus, the results that were obtained in this study reveal that the cultivation and production of *Spirulina* biomass can be carried out and that the applicability of biomass can be expanded, for example, in the production of carbohydrates.

## CONCLUSION

The highest growing results ($X_{max}$=1.60 g $L^{-1}$, $P_{max}$=0.109 g $L^{-1}$ $d^{-1}$, $\mu_{max}$=0.208 $d^{-1}$) and $CO_2$ biofixation rate (197.4 mg $L^{-1}$ $d^{-1}$) were obtained when *Spirulina* sp. LEB 18 was cultivation with 1.25 g $L^{-1}$ $NaNO_3$ and 1.0 g $L^{-1}$ NaCl. Increasing the NaCl concentration in the culture medium can increase the concentration of carbohydrate in the biomass. The concentration of lipids was not significantly affected by the concentrations of NaCl and $NaNO_3$. Regarding the protein concentration in the biomass, increasing the concentration of $NaNO_3$ in the medium can reduce the concentration of this compound in the biomass. Thus, osmotic stress and the nitrogen deficiency increased the biosynthesis of carbohydrates and did not alter the protein concentration of the *Spirulina*. Besides, in these conditions, *Spirulina* was able biofix of $CO_2$, reducing the emissions of this greenhouse gas.

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