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Effect of nitrogen and carbon sources and salt stress on the bioactive components concentration in *Scenedesmus obliquus*

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Abstract

In *Scenedesmus obliquus* culture, the effects of different nitrogen and carbon sources and saline stress on total carotenoids, total proteins and total phenols contents were investigated. Microalgae in Allen & Arnon culture medium were cultivated, until reaching the stationary phase. Then, sodium nitrate or urea as nitrogen source and sodium acetate or glucose as carbon source, were incorporated. Additionally, sodium chloride was added for cause saline stress. Fourteen treatments were carried out, during 40 days. Differences in bioactive components concentration were observed for the different nitrogen and carbon sources from day 26. When 0.24 g/L of urea were used, 3 times more of total carotenoids were obtained than when sodium nitrate was used; while, with 3.68 g/L of glucose, 1.5 times more of total carotenoids were reached than with sodium acetate was used. Total proteins concentration was 1.9 times higher when 0.24 g/L of urea and 5.02 g/L of sodium acetate were utilized as nitrogen and carbon sources, respectively. With 31.00 g/L of urea 4 times more of total phenols were obtained than with sodium nitrate; while, 12 times more of total phenols were achieved with 3.68 g/L of glucose than with sodium acetate. Saline stress caused a reduction in the bioactive components of interest. The highest concentrations obtained were: 513.20±13.21mg β-carotene/g_{dwc}, 7.25±0.34 mg BSA/g_{dwc}, and 16.78±0.54 mg GAE/g_{dwc}. Therefore, this study confirmed that *Scenedesmus obliquus* microalgae developed strategies to adapt to the new conditions caused by the nitrogen and carbon sources incorporation, causing changes in the concentration of bioactive components.

Keywords: *Scenedesmus obliquus*; Carotenoids; Protein; Phenols

1 Introduction

Microalgae have applications in various segments of the industry, e.g. aquaculture, food, pharmaceutical, cosmetics and biofuel production [1].

Nowadays, one of the main interests in the production of microalgae is the extraction of bio-compounds, such as protein, pigments, lipids and other high-value molecules. Subjecting microalgae to environmental stresses are one of the technological options to increase the production of biomass and molecules of interest [2].

In response to abiotic stresses, through photosynthesis and aerobic metabolism, microalgae produce reactive oxygen species which can be toxic and cause cell damages. Microalgae have developed defense strategies. One of them is the synthesis of a heterogeneous group of molecules which have the ability to delay, prevent, or remove oxidative damage

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to the cell. It includes enzymes and non-enzymatic molecules such as carotenoids, phenolic acids, or vitamins C and E that are present in high concentration in some microalgae species [3].

Parameters as temperature, pH, salinity, nutrients and others affect the biochemical content of microalgae. Thus, it would be interesting to study the effects of these parameters to optimize antioxidant production [2, 3]. However, the kind and intensity of stress must be well studied because can adversely influence microalgal growth, diminishing the production of such molecules [2].

The most important macronutrient is carbon. This constitutes 50 % of the microalgal biomass [4]. Carbon is an essential element of proteins, lipids, and carbohydrates, since it plays a critical role in microalgae growth. The lack of this element would reduce the biomass productivity and limit the syntheses of some value-added components in microalgae cells. Carbon resources commonly used for microalgae cultivation include inorganic carbon sources as carbon dioxide, carbonate, bicarbonate, and organic carbon sources such as glucose, acetic acid and glycerol. Not all the organic carbon sources can be assimilated by microalgae cells, and some of the organic carbon sources even have hazardous effects on algae growth [5].

Another nutrient to consider is nitrogen, as it is one of the critical nutrients in the production of biomass and different algal metabolites. Microalgal biomass has a high concentration of nitrogen, which is an essential biochemical compound present in DNA, RNA, proteins, and pigments.

Several algal biotechnological processes need to exploit this feature to improve the performance of specific metabolites of interest, such as carotenoids, proteins and phenolic compounds [2, 6, 7].

Nitrogen forms that microalgae can absorb and utilize include inorganic nitrogen sources (ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen) and organic nitrogen sources (urea, casein, and amino acids) [5, 7].

Types and concentrations of nitrogen sources have a significant effect on the growth and biochemical composition of microalgae. Different algal species have different requirements of type and concentrations of nitrogen sources; therefore it is important to compare various nitrogen sources to select the most appropriate one [7]. Microalgal cells can tolerate concentration up to 100 mM of NO_3^-, but higher concentrations negatively affect microbial growth, probably because of an increase of nitrate reductase activity and simultaneously an increase in NO_2^- and NH_4^+ concentration, in a range that could be toxic for the cells. Organic nitrogen as urea can be metabolized by microalgae. Generally, urea is hydrolyzed to ammonia and carbonic acid, both utilized by microalgae [8].

In addition, algae species show different responses to stress factors. Some microalgae cells develop adaptable strategies as a response to new conditions. As a consequence, they have different mechanisms such as morphological changes and diverse physiological and bio-chemical processes [9].

Some microalgae have the capability to change its growth rate as well as its biochemical composition, being salinity one of the most influential parameters. Algae exposure to lower or higher sodium chloride concentration than their natural levels, can alter growth rate and vary biochemical composition [10]; favoring or not the presence of interest components in microalgal culture.

In recent years, the design of culture media that adapts to the necessities of the strain has increased, as an alternative to traditional media. Therefore, they are designed using specific concentrations of sodium nitrate, potassium phosphate, sodium acetate and other critical nutrients for the production of biomass and deposition of metabolites of industrial interest [6].

In this sense, the aim of this work was to evaluate, in *Scenedesmus obliquus* culture, the effects of different carbon and nitrogen sources and saline stress on concentrations of total carotenoids, total proteins and total phenols.

2 Material and methods

2.1 Microalga and culture medium

Scenedesmus obliquus microalgae (IOAC081F) were utilized in this work, which was isolated from Salto Grande Reservoir (Argentina) [11].

The cells were maintained at 25 ± 2 °C, relative humidity of 65 % inside of photobioreactors under artificial light with a 16:8 h light/dark photoperiod inside a culture chamber (MGC – 400H, China). The inoculum was kept in Allen & Arnon culture medium. This medium includes the following components, in mg/L: 850 $NaNO_3$, 0.239 $NaVO_3$, 1.26 $Na_2MoO_4.2H_2O$, 2.86 H_3BO_3, 1.81 $MnCl_2.4H_2O$, 0.222 $ZnSO_4.7H_2O$, 0.079 $CuSO_4.5H_2O$, 0.0403 $CoCl_2.6H_2O$, 124 $MgSO_4.7H_2O$, 15 $CaCl_2.2H_2O$, 117 NaCl, 0.029 EDTA, 0.025 $FeSO_4.7H_2O$ and 174 K_2HPO_4.

Ratio culture medium: inoculum was 5:1. Each photobioreactor was kept under stirring by air injection at 0.2 v/v/min in order to maintain homogeneous conditions until reaching the stationary phase. Then, studies of carbon and nitrogen sources and saline stress were carried out; using as a reference published studies by our research group.

These studies concluded that the combination of experimental factors which maximized the concentration of total carotenoids and total proteins was: 0.69 g $NaNO_3$/L, 5.02 g CH_3COONa/L and an irradiance of 54.71 µmol/(m^2 s); while for total phenols the best conditions were 87.73 g $NaNO_3$/L, 5.02 g CH_3COONa/L and 45.04 µmol/(m^2 s) of irradiance [12].

The concentration of 0.69 g $NaNO_3$/L is equivalent to 0.11 g N/L and 87.73 g $NaNO_3$/L, to 14, 45 g N/L; while the concentration of 5.02 g CH_3COONa/L is equivalent to 1.47 g C/L.

For each test, the microalgae were cultivated as mentioned before in Allen & Arnon culture medium. Reached the stationary phase, at 15 day, the culture conditions were modified as shown in Table 1. Thus, a total of 14 assays were performed, each one for triplicated.

Table 1 Experimental design for studies of nitrogen and carbon sources and saline stress

Assay	Nitrogen source (g/L)	Carbon source (g/L)	Irradiance µmol/(m^2 s)	ClNa (g/L)	Component of interest obtained
1	$NaNO_3$: 0.69	CH_3COONa: 5.02	54.71	0	Total carotenoids and Total proteins
2	$CO(NH_2)_2$: 0.24	CH_3COONa: 5.02	54.71	0	
3	$NaNO_3$: 87.73	CH_3COONa: 5.02	45.04	0	Total phenols
4	$CO(NH_2)_2$: 31.00	CH_3COONa: 5.02	45.04	0	
5	$CO(NH_2)_2$: 0.24	CH_3COONa: 5.02	54.71	0	Total carotenoids and Total proteins
6	$CO(NH_2)_2$: 0.24	$C_6H_{12}O_6$: 3.68	54.71	0	
7	$CO(NH_2)_2$: 31.00	CH_3COONa: 5.02	45.04	0	Total phenols
8	$CO(NH_2)_2$: 31.00	$C_6H_{12}O_6$: 3.68	45.04	0	
9	$CO(NH_2)_2$: 0.24	$C_6H_{12}O_6$: 3.68	54.71	0	Total carotenoids
10	$CO(NH_2)_2$: 0.24	$C_6H_{12}O_6$: 3.68	54.71	17.55	
11	$CO(NH_2)_2$: 0.24	CH_3COONa: 5.02	54.71	0	Total proteins
12	$CO(NH_2)_2$: 0.24	CH_3COONa: 5.02	54.71	17.55	
13	$CO(NH_2)_2$: 31.00	$C_6H_{12}O_6$: 3.68	45.04	0	Total phenols
14	$CO(NH_2)_2$: 31.00	$C_6H_{12}O_6$: 3.68	45.04	17.55	

2.2 Effect of the nitrogen source on the production of bioactive components

In these trials, 2 nitrogen sources were compared: $NaNO_3$ and $CO(NH_2)_2$, using a nitrogen concentration of 0.11 g/L. To study the nitrogen source that allowed obtaining the highest concentration of total carotenoids and total proteins, assays 1 and 2 were performed (Table 1), while for total phenols the assays 3 and 4 were carried out.

Periodically, the number of cells and total carotenoids, total proteins and total phenols concentration were analyzed. After these experiences, our team continued working with the nitrogen source that allowed obtaining the highest concentration of the bioactive components studied.

2.3 Effect of the carbon source on the bioactive components production

Table 1 show the assays 5 and 6, which were carried out to evaluate the carbon source which enables obtaining of highest total carotenoids and total proteins concentrations; while the experiment 7 and 8 were made for total phenols.

After these assays, in the culture mediums, the carbon source that allowed obtaining the highest concentration of bioactive component was utilized.

2.4 Effect of saline stress

The saline stress was researched in the production of total carotenoids, total proteins and total phenols, carrying out a total of 6 experiments.

According to the previous results of the tests, nitrogen and carbon sources were incorporated into photobioreactors, once that the stationary phase was reached (assays 9, 11 and 13).

Assays 10, 12 and 14 were performed in the same way as the previous tests but 17.55 g ClNa/L were added, with the objective of causing saline stress in microalgae cells.

As in previous assays, the number of cells and the bioactive components concentrations were periodically evaluated.

Thus, a culture medium was developed for microalgae *Scenedesmus obliquus* (IOAC081F) with the objective of maximizing the total carotenoids, total proteins and total phenols production.

2.5 Obtaining dehydrated biomass

Separation of microalgae from the culture medium was carried out by centrifugation at 4000 rpm during 20 min (Boeco C-28, Alemania). Microalgae biomass was collected, washed with distilled water and lyophilized at -90 °C (Heto Drywiner) until constant weight. In dehydrated microalgae, total carotenoids, total proteins and total phenols were quantified.

2.6 Analytical methods

Number of cells: Microalgae growth in the photobioreactors was monitored counting cell numbers in a Neubauer chamber in an inverted microscope (Leica, DMIL) [13, 14].

Total carotenoids: Were measured using UV-Vis spectrophotometer (Hach, DR600, Germany) at 450 nm employing standard β-carotene [15, 16]. The results were expressed as mg β-carotene/g dry weight content (dwc).

Total proteins: Were quantified by spectrophotometric method using UV-Vis spectrophotometer (Hach, DR600, Germany) at 750 nm [16]. Standard bovine serum albumin (BSA) was used and the results were expressed in mg BSA/g_{dwc}.

Total phenols: Were determined by reduction of the Folin-Ciocalteu reagent, according to the developed method by Copia et al., [14]. The measure was at 760 nm wavelength in an UV-Visible spectrophotometer (HACH DR600, Germany). Gallic acid was used as standard and the results were expressed in mg gallic acid equivalents (GAE)/g_{dwc}.

2.7 Statistical analysis

Mean values and standard deviations were calculated. Error bars in figures correspond to standard deviations. All measurements were performed by triplicates. Differences among mean values were determined using analysis of variance (ANOVA) and Fisher's least significant differences procedure (LSD) at a confidence level of 95% ($P < 0.05$). The software STATGRAPHICS Centurion XVI v.16.1.1, USA. Was used.

3 Results and discussion

Microalgae were cultivated in Allen & Arnon culture medium until the stationary phase was reached, which corresponded to 15th d cultivation time. Then, the culture medium was modified according to the tests indicated in Table 1, in agreement with assays of nitrogen and carbon sources and saline stress. The total cultivation period was 40 d.

3.1 Total carotenoids

Two types of nitrogen sources were studied: $NaNO_3$ (assay 1) and $CO(NH_2)_2$ (assay 2).

Figure 1 show total carotenoids concentration of the different nitrogen sources. *Scenedesmus obliquus* accumulated more total carotenoids with 0.24 g $CO(NH_2)_2$/L than with 0.69 g $NaNO_3$/L.

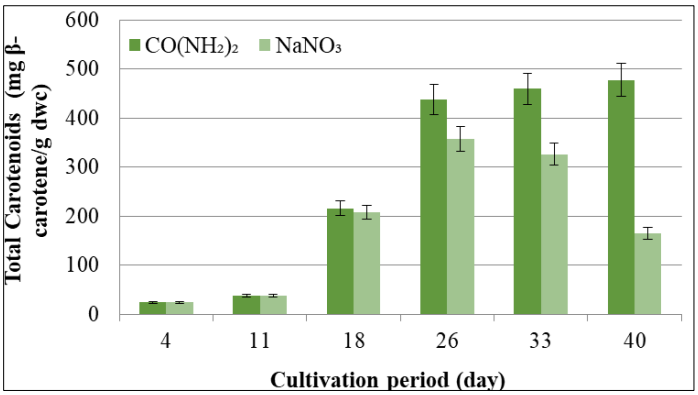

Figure 1 Effect of nitrogen source on total carotenoids content

When nitrogen source was $NaNO_3$ the total carotenoids concentration increased reaching a maximum of 356.98 ± 17.60 mg β-carotene/g_{dwc} in the 26th d and then decreased. Instead, when the nitrogen source was $CO(NH_2)_2$ the concentration increased from 23.92 ± 1.22 to 477.28 ± 22.45mg β-carotene/g_{dwc}. No significant differences were observed in the concentrations for these bioactive components at 26, 33 and 40 d; being the average and the standard deviation, of the registered concentrations in those days, of 457.82 ± 20.15 mg β-carotene/g_{dwc}. Consequently, in the case of total carotenoids, microalgae cultivation can be interrupted at 26th d. At that time of cultivation, when $CO(NH_2)_2$ was used as a nitrogen source, 23 % more of total carotenoids were obtained than when $NaNO_3$ was used.

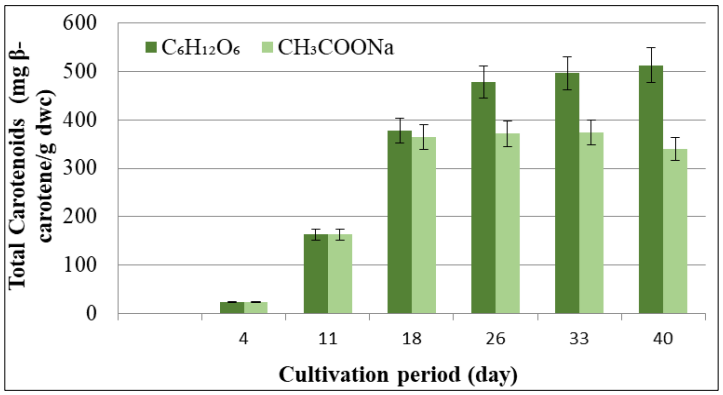

Figure 2 Effect of carbon source on total carotenoids content

Furthermore, we carried out experiments where 2 carbon sources were tested: CH_3COONa (assay 5) and $C_6H_{12}O_6$ (assay 6). In both experiments, $CO(NH_2)_2$ was used as nitrogen source at a concentration of 0.24 g/L.

At 15th d of cultivation 5.02 g CH_3COONa/L was incorporated as carbon source. No significant increasing in total carotenoids content was observed, being the average between the days 18 and 40 of 362.84 ± 15.40 mg β-carotene/g_{dwc} (Figure 2). Nevertheless, when 3.68 g $C_6H_{12}O_6$/L were added, the total carotenoids increased by 24 %, reaching in the last 14 d of cultivation a concentration of 495.82 ± 17.63 mg β-carotene/g_{dwc}.

Since no significant differences were observed in total carotenoids contents between days 26 and 40, the culture could be interrupted at 26 d.

Once the most suitable nitrogen and carbon sources were selected to obtain total carotenoids, new tests were carried out with the hypothesis that a saline stress in the microalgae culture might cause an increase in such components. When

the microalgae culture in the Allen & Arnon medium reached the stationary phase, 0.24 g $CO(NH_2)_2$/L and 3.68 $C_6H_{12}O_6$/L were added and then the assay continued until 40th d (assay 9). Assay 10 realized under the same conditions but with an addition of 17.55 g NaCl/L.

Figure 3 shows that saline stress affected negatively the accumulation of total carotenoids, obtaining 40 % less of these components on day 26 and 128 % less on day 40.

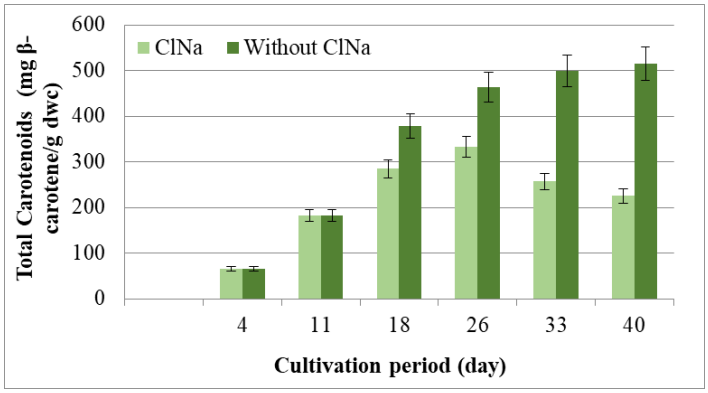

Figure 3 Effect of saline stress on total carotenoids content

The results showed an increase of 24% in total carotenoid production when $C_6H_{12}O_6$ was incorporated as a carbon source. This could be because, in microalgae cells, $C_6H_{12}O_6$ is transformed into pyruvate, a precursor for the synthesis of carotene through glycolysis pathway [5]. However, Kong et al. [17] obtained different results. When these authors added $C_6H_{12}O_6$ to the culture medium, they got a decrease in levels of carotenoids produced by *Chlorella vulgaris*. In change, when they incorporated 2 g CH_3COONa/L an increase in carotenoids was accomplished.

It was previously pointed out that the incorporation of 17.55 g NaCl/L did not favor the production of total carotenoids. On the contrary, Abdul-Adel et al. [10] obtained, with saline stress, a reduction of chlorophyll, phycocyanin, allophycocynin, and phycobiliproteins and an induction of carotenoids. These authors worked whit *Spirulina platensis* in Zarrouk culture medium with 10 g/L NaCl and 1.182 mg/L carotenoids were obtained. Also, Annamalai et al. [18] achieved higher concentrations of carotenoids when cultures of *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were subjected to a saline stress of 50 mM NaCl. In these conditions between 3.08 and 3.90 mg/L of carotenoids were reached. Moreover, Hashemi et al. [19] got the highest carotene production (4.85 µg β-carotene/mg_{dwc}), in *Dunaliella salina* culture medium with 2.5 mol/L of salinity.

3.2 Total proteins

In these tests, $NaNO_3$ (assay 1) and $CO(NH_2)_2$ (assay 2) were evaluated as nitrogen sources, using 5.02 g CH_3COONa/L as carbon source.

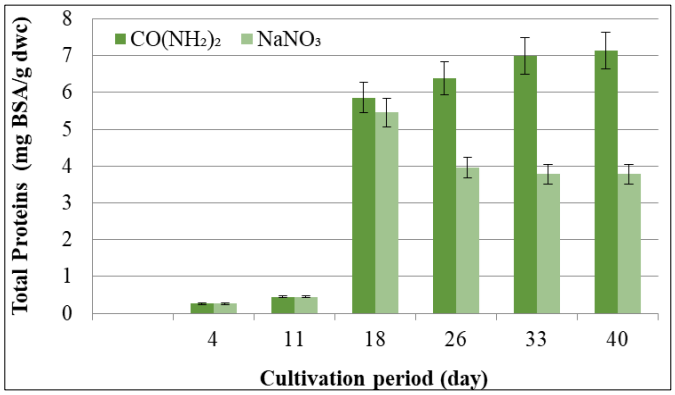

Figure 4 Effect of nitrogen source on total proteins content

The nitrogen source that favoured the accumulation of total proteins, as well as for total carotenoids, was 0.24 g $CO(NH_2)_2$/L. In Figure 4, the concentration increase of these bioactive components is showed. Otherwise, the addition of 0.69 g $NaNO_3$/L did not improve the accumulation of total proteins.

For both nitrogen sources, no significant differences in total proteins were observed measured at 26, 33 and 40 days; being the averages of 6.84 ± 0.40 mg BSA/g_{dwc} and 3.80 ± 0.10 mg BSA/g_{dwc} when $CO(NH_2)_2$ and $NaNO_3$ were used, respectively. While with $CO(NH_2)_2$ 80 % more of total proteins were obtained.

In assays 5 and 6, carbon sources (5.02 g CH_3COONa/L and 3.68 g $C_6H_{12}O_6$/L) were studied. In these test 0.24 g $CO(NH_2)_2$/L was utilized as nitrogen source.

Since day 26, significant differences in the total proteins content were observed in the cultures with different carbon sources. When CH_3COONa was used, there was an increase in the concentration of these bioactive components, while when $C_6H_{12}O_6$ was used, the concentration of total proteins decreased (Figure 5). At the 40th d of cultivation, the tests with CH_3COONa showed a 77 % higher content of total proteins

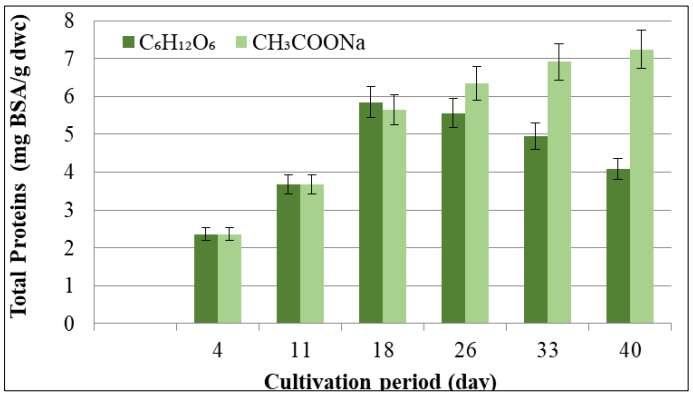

Figure 5 Effect of carbon source on total proteins content

In culture media with CH_3COONa the range of total proteins was between 2.36 ± 0.11 to 7.25 ± 0.34 mg BSA/g_{dwc}. Significant differences were observed in the means of total proteins on days 26 and 40. However, between days 33 and 40 and, 26 and 33 no significant differences were observed.

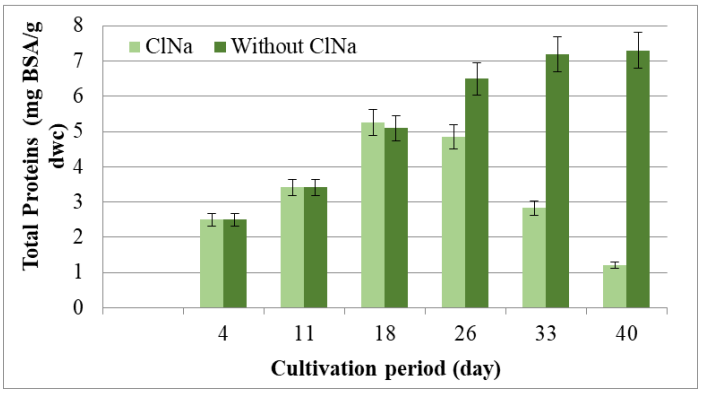

Figure 6 Effect of saline stress on total proteins content

Figure 6 presents the assays where NaCl was incorporated (assay 14). The initial concentration was 2.50 mg BSA/g_{dwc} and then it reached the maximum of 5.26 mg BSA/g_{dwc} at 18th d. At the end of the test, the total proteins concentration decreased at 1.21 ± 0.07 mg BSA/$g_{dwc.}$

We noticed an extremely negative effect, on the accumulation of total proteins, caused by saline stress since 26th d.

In lack of ClNa, the content of total proteins increased. No significant differences were observed in means between days 26, 33 and 40. The average value was of 6.99 ± 0.44 mg BSA/g_{dwc}.

As regards studies of nitrogen sources, López-Elías et al. [20] examined protein production in *Chaetoceros muelleri* culture using two nitrogen sources, $CO(NH_2)_2$ and NO_3^-. They obtained, as in the present investigation, higher total proteins concentrations when $CO(NH_2)_2$ was used as the nitrogen source. These results were attributed to the fact that the oxidation state of N in $CO(NH_2)_2$ is -3 and in $NaNO_3$ +5. Highly oxidized nitrogen must be reduced to -3, which is how it is found in proteins. Firstly, NO_3^- is reduced to NO_2^- in the plasma membrane. Secondly, a fraction of this compound is reduced to NH_4^+ in chloroplasts. Finally, nitrogen is incorporated into organic molecules [21].

Using $C_6H_{12}O_6$ as a carbon source, the protein content decreased; probably due to a degradation of intracellular proteins [22]. When CH_3COONa was employed, 40 % more proteins were observed at day 33.

The lower concentrations in total proteins, with the incorporation of NaCl, was attributed to a decrease in the microalgal biomass content, caused by saline stress [9]. Researchers Annamalai et al. [18] obtained whit *Chlorella vulgaris* and *Chlamydomonas reinhardtii* 7-8 times greater protein concentrations (14 µg/mL and 17 µg/mL) in saline stress conditions. In contrast, Ermis and Altınbaş [9] observed, in a mixed microalgal culture of *Chlorella vulgaris* and *Scenedesmus armatus*, that protein content decreased while increasing the NaCl concentrations.

3.3 Total phenols

As for bioactive components total carotenoids and total proteins, tests were carried out for total phenols with 87.73 g $NaNO_3$/L (assay 3) and 31.0 g $CO(NH_2)_2$/L (assay 4). The carbon source was 5.02 g CH_3COONa/L. The evolution of total phenols as a function cultivation period can be seen in Figure 7. The initial concentration was 0.32 mg GAE/g_{dwc}. Total phenols concentration did not change significantly with $NaNO_3$ as nitrogen source, while with $CO(NH_2)_2$ the content increased significantly, reaching a concentration of 5.44 ± 0.04 mg GAE/g_{dwc} in the last 7 d. This value was 296 % higher than the one obtained in the assay where $NaNO_3$ was used.

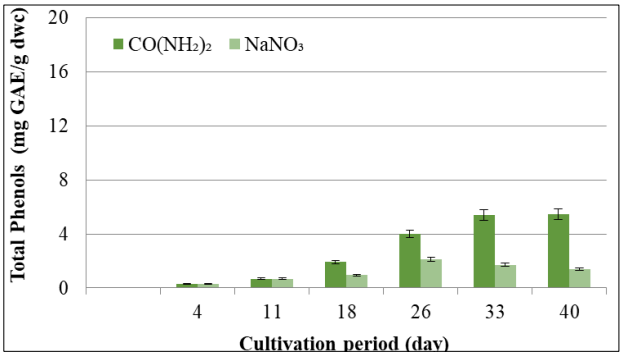

Figure 7 Effect of nitrogen source on total phenols content

Once the stationary phase was reached in the carbon source assays, the culture medium conditions were modified as indicated for assays 7 (5.02 g CH_3COONa/L) and 8 (3.68 g $C_6H_{12}O_6$/L). In both assays, 31.0 g $CO(NH_2)_2$/L were utilized as nitrogen source. Figure 8 shows that with CH_3COONa total phenols decreased, while with $C_6H_{12}O_6$ these compounds increased rapidly from 2.90 ± 0.15 to 16.78 ± 0.54 mg GAE/g_{dwc}

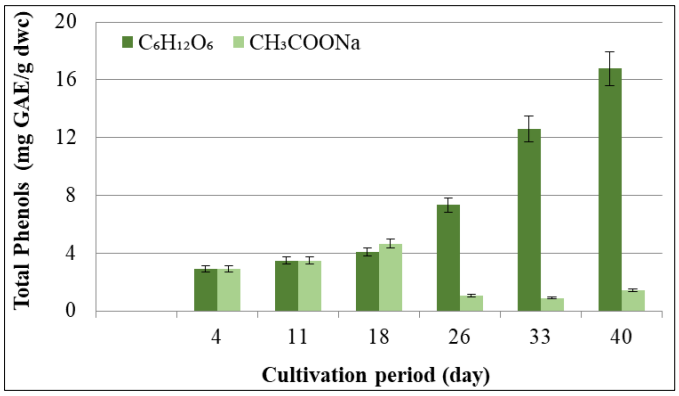

Figure 8 Effect of carbon source on total phenols content

Last, the effect of saline stress on the accumulation of total phenols was evaluated. In assay 13, 31.0 g $CO(NH_2)_2$/L and 3.68 g $C_6H_{12}O_6$/L were utilized as nitrogen and carbon sources, respectively. Assay 14 was carried out under the same conditions, but 17.55 g ClNa/L was added (Figure 9).

Statistical analysis of tests between the cultures with and without NaCl, for the same culture period, indicated significant differences between the means of total phenols for days 26 and 40; but, not for the rest of the days.

Therefore, the incorporation of ClNa into the culture medium not improved the accumulation of total phenols. At 40th d, total phenols content in the culture medium without ClNa was 64 % higher than in the medium with ClNa.

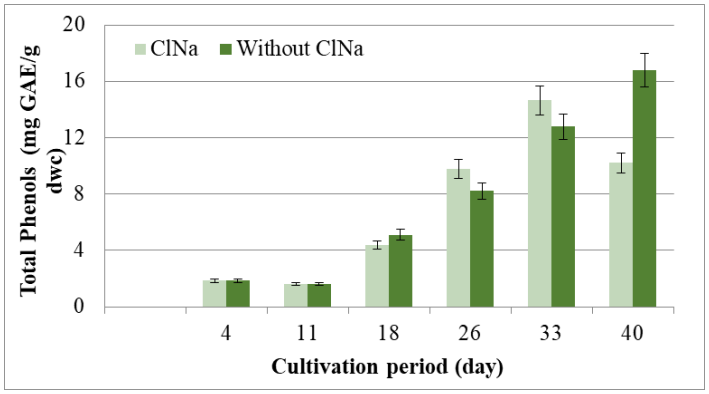

Figure 9 Effect of saline stress on total phenols content

Effect of saline stress on the accumulation of total phenols depends on the microalgae. In one hand, Annamalai et al. [18] realized studies that showed the response of saline stress in *Chlorella vulgaris* and *Chalamydomonas reinhardtii*. They observed that the phenols content increased up to 33 ± 0.7 µg/mL at low salinity (50mM ClNa) in *Chorella vulgaris*, while that at higher concentrations, phenols decreased. On the other hand, as regards *Chalamydomonas reinhardtii* a decrease in phenols was observed at all ClNa concentrations.

Nevertheless, difficulties were found to compare the total phenols of this work with those published by other authors, due to the different operating conditions and the culture medium composition.

4 Conclusion

Results of this study showed that the *Scenedesmus obliquus* microalgae can use NO_3Na and $CO(NH_2)_2$ as nitrogen source and CH_3COONa and $C_6H_{12}O_6$ as ~~a~~ carbon source to produce total carotenoids, total proteins and total phenols. However, $CO(NH_2)_2$ was the best nitrogen source to improve the accumulation of all the bioactive compound of interest. When the concentration was 0.24 g/L, the production of total carotenoids and total proteins was improved especially between days 26 and 40 of culture. While, with 31.00 g $CO(NH_2)_2$/L the concentration of total phenols improved, particularly from day 33 of culture. When 3.36 g $C_6H_{12}O_6$/L was used as ~~a~~ carbon source, a higher concentration of total carotenoids and total phenols was obtained, especially the cultivation time of 26 - 40 d and 40 d; respectively. Furthermore, the highest amount of total proteins was obtained when the carbon source was 5.02 g CH_3COONa/L since 26th day of culture. When microalgae *Scenedesmus obliquus* was exposed to saline stress, a decrease in the bioactive components was observed. Maximum concentration of total carotenoids was 513.20 ± 13.21 mg β-carotene/g_{dwc}, total proteins 7.25 ± 0.34 mg BSA/g_{dwc}, and total phenols 16.78 ± 0.54 mg GAE/g_{dwc}. To conclude is possible to modify the culture medium composition of *Scenedesmus obliquus* microalgae with the objective of achieve an increase in the bioactive components concentrations of industrial interest.

Compliance with ethical standards

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Disclosure of conflict of interest

Luz Marina Zapata, Mariana Jiménez-Veuthey and Natalia Agustina Sacks and Gina Fiorella Vezzosi-Zoto declare that they have no conflict of interest.

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