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Effect of light stress and concentrations of nitrogen and carbon in the production of phytonutrients in the microalga *Scenedesmus obliquus* (Chlorophyceae, Chlorococcales)

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Running title: Phytonutrients production by *Scenedesmus obliquus*

Abstract
Under stress conditions microalgae produce phytonutrients. However, it is necessary to determine the environmental conditions that stimulate phytonutrient biosynthesis. We evaluate growth kinetic models and the influence of sodium nitrate and sodium acetate concentrations, as well as the irradiance in the production of total carotenoids, total phenols and proteins in culture of *Scenedesmus obliquus* (Turpin) Kützing. The microalga was cultivated in Arnon medium in bioreactors with a 16:8 light-darkness photoperiod. When the stationary phase was reached, sodium acetate (0, 2.5, 5 g/L) and sodium nitrate (0.27, 44.00, 87.73 g/L) were incorporated and the irradiance [36.7, 69.5, 102.3 µmol/(m^2s)] was modified to generate stress conditions. Growth was successfully predicted by the Gompertz model. The maximum content of protein was 4.92 mg/g dwc and of total phenols was 2.31 mg/g dwc using 2.5 g/L sodium acetate, 44 g/L sodium nitrate and irradiance of 65.9 µmol/(m^2s). The maximum production of total carotenoids was 374.30 mg β-carotene/g dwc with 5 g/L of sodium acetate, 0.27 g/L of sodium nitrate and irradiance of 65.9 µmol/(m^2s). The results showed that this microalga has potential application in the food industry in order to improve its nutritional and functional properties.

Keywords: Carotenoids; Phenols; Proteins; Growth kinetic models; *Scenedesmus obliquus*.

1. INTRODUCTION

Over the last decades, microalgae have been increasingly studied owing to their potential applications in the industry. Because of their great biodiversity, microalgae can produce a great amount of valuable compounds for biofuels, food supplementation, food, feed, pigments and pharmaceutical and cosmetic products (de Marchin et al., 2015; Tramontin et al., 2018). Microalgae are predominantly photosynthetic organisms that use solar energy to combine water, carbon dioxide, and inorganic nutrients to produce biomass rich proteins, vitamins, antioxidant, polysaccharides, polyunsaturated fatty acids and a range of secondary metabolites (Quevedo et al., 2008; Tramontin et al., 2018). Due to their phytonutrient content with beneficial properties for human health, certain microalgae are considered functional foods (Quevedo et al., 2008), which is of interest in the food industry.
Several studies have reported that under different stress conditions, the microalgae produce bioactive compounds as a cell-protecting mechanism (Dammak et al., 2018).

The adjustment of culture conditions to optimize the obtaining products of interest begins at laboratory scale with the definition of environmental requirements for each strain. Such conditions may be associated with optimum light intensity, concentrations of nitrogen and carbon sources, among others (Ho et al., 2015; Eze et al., 2018).

Some microalgae exhibit unique productivity and plasticity features: when grown under distinct sets of operating conditions, they may accumulate different products to high levels; hence, careful design and control of medium composition, temperature, pH, aeration, stirring and irradiance are recommended. Recently, many researchers have shown great interest in the study of the influence of the culture conditions on the production of certain particular analytes, such as proteins, carotenoids and phenolic compounds.

At present, a novel protein source is needed to satisfy increasing demand. Microalgae are considered as an important protein source. Among them *Dunaliella salina* can be mentioned, which can produce extremely high-quality protein for human nutrition at a stationary phase, using fluorescent tubes at the intensity of 55 µmol/(m^2s), regardless of the light regime (Sui et al., 2018).

On the other hand, the effect of the concentration of a nitrogen source on the cell growth and lutein content of microalga *Scenedesmus obliquus*, which was isolated from freshwater located in southern Taiwan, was reported. Maximum lutein content was obtained when the nitrogen concentration went from 8 to 1 mM, reaching a lutein content of 5.04 mg/g dry weight cells (dwc) (Ho et al., 2015).

Literature mentions that *Senedesmus* sp was isolated from the surface of rocks in Japan and various light conditions [0, 40, 120, and 200 µmol photons/(m^2s)] and salinity (0.3 M salts: NaCl, KCl $MgCl_2$, and $CaCl_2$) were studied to produce carotenoids, accumulating up to 34.2±3.8 mg/g dwc of total carotenoids (Aburai et al., 2015). *Scenedesmus obliquus* FSP-3 also achieved the highest lutein content (4.61 mg/g) and lutein productivity [4.35 mg/(L day)] in batch culture when using 8.0 mM calcium nitrate as the nitrogen source (Ho et al., 2015).

Some studies suggest that the production of phenolic compounds is related to lighting. Researchers reported the production of phenolic compounds of four species of marine microalgae (*Chaetoceros muelleri*, *Thalassiosira weissflogii*, *Dunaliella tertiolecta* y *Tetraselmis chuii*) under different lighting conditions. These found that the concentration of total phenols was different among microalgae species, but no significant differences were found regarding the lighting condition (Gómez et al., 2016). However, other authors suggest that the increase in phenolic compounds observed in *Cystoseira tamariscifolia* would be associated with its exposure to high illumination and UV radiation (Abdala-Díaz et al., 2014).

Microalgal biotechnology has a large potential to obtain phytonutrients or to obtain dehydrated microalgae to enrich certain foods with phytonutrients. Nevertheless, various authors have shown that to maximize the production of phytonutrients, it is not enough to produce biomass, but it is also necessary to determine the environmental conditions that stimulate phytonutrient biosynthesis within the cells (López-Elías et al., 2013). The key factor for their eventual economic feasibility is the possibility of operating large photobioreactors, able to handle biomass and metabolites to sufficiently high levels (Sánchez et al., 2008).

The main objective of this research was to study, in culture of *Scenedesmus obliquus*, the growth kinetic models, the influence of the concentrations of sodium nitrate and sodium acetate, as well as the irradiance in the production of total carotenoids, total phenols and proteins.

2. MATERIALS AND METHODS

2.1. *Microalgae and culture conditions*

The microalga used in this study was *Scenedesmus obliquus*, which was isolated from Embalse Salto Grande (Argentina), located in 30° 59’ 19.07’’ South latitude and 57° 54’ 19.24’’ West latitude (Jiménez-Veuthey et al., 2018).

The microalga was grown in bubble-columns (photobioreactor) inside culture chamber (MGC – 400H, China) at a temperature of 25 ± 1 °C, relative humidity of 65% and 16:8 light-darkness photoperiod. Fluorescent tubes of 36 W were used as a source of artificial light, positioned vertically 3 cm apart from each other and 4 cm apart from the cultures.

In each photobioreactor 200 mL of Arnon culture medium with supplemented nitrate and 50 mL of inoculum of strains of *Scenedesmus obliquus* were placed. The assembly was kept under stirring by injecting air at a rate of 0.2 v/v/min in order to maintain the homogeneous operating conditions and to avoid agglomeration of particles. The composition of the Arnon culture medium with nitrate was, in mg/L: 850 $NaNO_3$, 0.239 $NaVO_3$, 1.26 $Na_2MoO_4.2H_2O$, 2.86 H_3BO_3, 1.81 $MnCl_2.4\ H_2O$, 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 $FeSO_4.7H_2O$ y 174 K_2HPO_4.
Under these conditions, growth models were studied as detailed below.

2.2. *Stress conditions of the microalgae tests which were carried out*

Once the stationary phase was reached, the irradiance and the concentrations of nitrogen and carbon sources in the culture medium were modified. For this, 50 mL of the content of each photobioreactor was removed and 50 mL of sodium acetate and nitrate were incorporated, and the irradiance was adjusted as shown in Table 1. The final volume was maintained at 250 mL and the total culture time was 40 days. After each experience, the culture was centrifuged at 4400 g for 20 min (Boeco C-28, Alemania). After the supernatants were removed, the cell pellet was lyophilized at -90 °C (Heto Drywiner) until constant weight.

Table 1 Cultivation conditions for each assay.

Assay number	Nitrate sodium (g/L)	Acetate sodium (g/L)	Irradiance [µmol/(m^2s)]
1	0.27	2.5	69.5
2	44.00	2.5	69.5
3	87.73	2.5	69.5
4	44.00	0	69.5
5	44.00	2.5	69.5
6	44.00	5	69.5
7	44.00	2.5	36.7
8	44.00	2.5	69.5
9	44.00	2.5	102.3

2.3. *Growth models*

Initially the variation of the cell density of *Scenedesmus obliquus* was obtained as a function of the culture time during 25 days. Then three non-linear functions were used for the description the growth curves of *Scenedesmus obliquus* in Arnon culture medium with supplemented nitrate: Gompertz, Logistic and von Bertalanffy (Cayré et al., 2007; Castro et al., 2008; Vásquez-Villalobos et al, 2013; Koya and Goshu, 2013).
The mathematical expressions associated with the respective functions are:

$$N_t = N_0 + D\ e^{\{-e^{[-B(t-M)]}\}} \quad (1)$$

$$N_t = N_0 + \frac{D}{1+e^{-B(t-M)}} \quad (2)$$

$$N_t = N_0 + D\left\{1 - B\ e^{[-k(t-\mu)]}\right\}^3 \quad (3)$$

where:
N_t: Cell density at time t (cells/mL)
N_0: Initial cell density (inoculum) (cells/mL)
t: Cultivation time (day)
µ: Specific growth rate (1/day)
e = 2.7182
D: Difference between the maximum and initial population (cells/mL).
B: Represents the slope of the curve and describes the growth rate [(cells/mL)/day]
M: Time in which the growth rate is of greater magnitude.
k: Maximum absolute growth rate.

The maximum specific growth rate, $\mu_{máx}$ (1/day) was obtained as the tangent in the inflection point of the growth curve when the microalga grew exponentially. The lag time, λ (day), was defined as the x-axis intercept of this tangent; and the asymptote, D, [D=LN (N_{max}/N_0)], which is the maximum value reached, allowed to obtain the maximum population density (N_{max}, cells/mL) (Fig. 1) (Zwietering et al., 1990). Duplication time (DT) was calculated as LN(2)/μ_{max} (Quevedo et al., 2008).

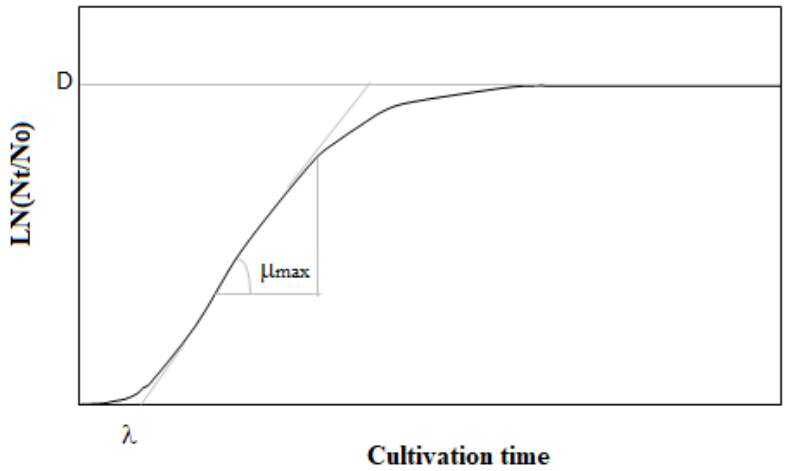

Fig 1. Growth curve

2.4. *Quality parameters*

Microalgal growth. Microalgal growth in the photobioreactors was monitored by counting cell numbers in a counting chamber Neubauer an inverted microscope (Leica, DMIL) (Castro et al., 2008).

Proteins. The proteins were quantified by a spectrophotometric method using UV-Vis spectrophotometer (Hach, DR600, Germany) and standard bovine serum albumin (BSA) (Arredondo-Vega and Voltolina, 2007). The measurements were made at 750 nm. The results are expressed in mg/g dwc.

Total carotenoids. The carotenoids were measured by spectrophotometric method using UV-Vis spectrophotometer (Hach, DR600, Germany) at 450 nm using standard β-carotene. The results are expressed in mg β-carotene/g dwc (Arredondo-Vega and Voltolina, 2007; Cerón et al., 2007).

Total phenols. The total polyphenolic content was determined according to the method of Folin–Ciocalteu (Copia et al., 2012). Absorbance was measured using a spectrophotometer (HACH DR600, USA) at 760 nm after addition of the Folin–Ciocalteu reagent. Gallic acid was used as a standard and the results are expressed in mg Gallic Acid Equivalents (GAE)/g dwc.

2.5. *Statistical analysis*

2.5.1. Models validation

Applying Matlab software (Matlab R2014a v. 8.3.0.532), the Gompertz and Logistic models were fitted against experimental data. Four statistical parameters were chosen to check the models validation: the determination coefficient (R^2), the sum of square error (SSE) and the root mean square error (RMSE) (Dammak et al., 2018).

2.5.2. Comparison of means

The experiment was performed in triplicate with results expressed as means ± standard deviations in figures. Significant differences were analyzed using software STATGRAPHICS (Statgraphics Centurion XVI v.16.1.1, USA), applying analysis of variance (ANOVA) and multiple range tests. Significant differences were defined at $P< 0.05$.

3. RESULTS AND DISCUSSION

3.1. *Growth curve and growth models*

Initial cell density of *Scenedesmus obliquus* was 5.1×10^6 cell/mL. Growth curve clearly showed the lag phase followed by an exponential phase and then by a stationary phase (Fig. 2).

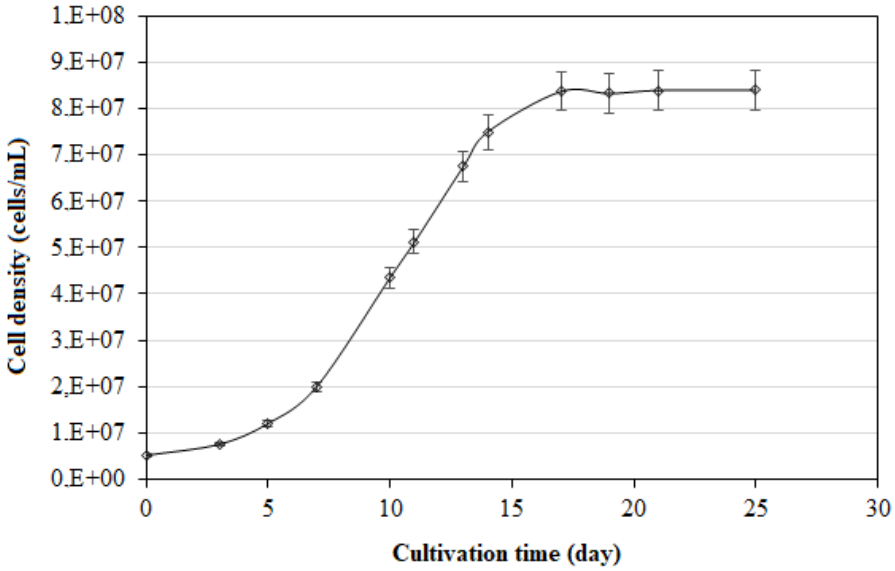

Fig. 2. Variation the cell density over cultivation time

Fig. 3 shows the adjustment of the Gompertz, Logistic and von Bertalanffy models from the experimental growth results of *Scenedesmus obliquus* in Arnon culture medium. At first glance it is observed that the models which best adjusted to the experimental results were the Gompertz and Logistic models. The von Bertalanffy model does not show a good fit to the experimental results.

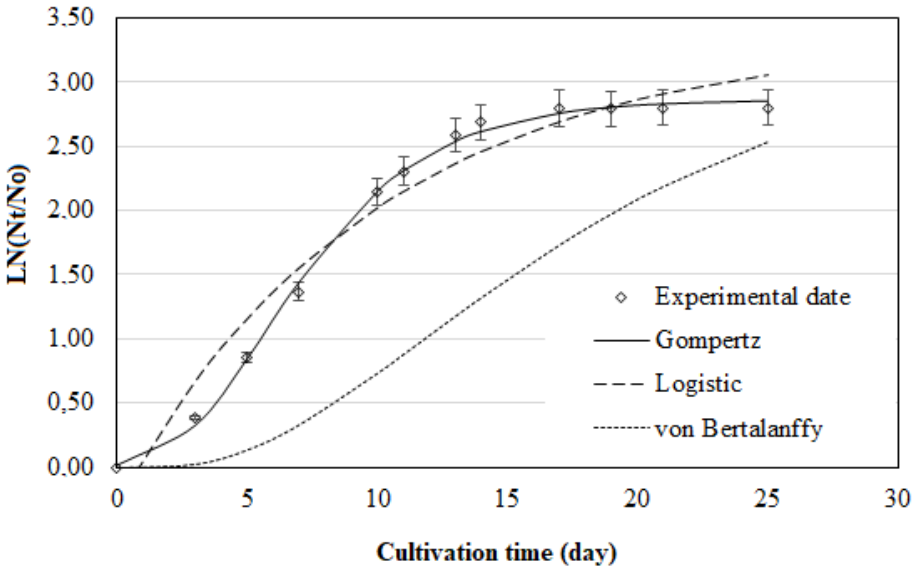

Fig. 3. Experimental growth curves of *Scenedesmus obliquus* and fit to Gompertz, Logistic and von Bertalanffy models in Arnon culture medium with supplemented nitrate. Symbols represent experimental data and lines represent the predicted date by models.

Table 2 summarize the predicted parameters and goodness of fit statistics obtained for the models studied. It is observed that the Gompertz model gave the highest value R^2 and the lowest values SSE and RMSE, so that it satisfactorily described microalgal growth; followed by the Logistic model. The von Bertalanffy model had the lowest value of R^2 and, as in Fig. 3 it is observed that as it did not fit the experimental data, the model was dismissed. Others authors also obtained good fitting for the *Tetraselmis* sp microalga growth kinetics with the Gompertz and Logistic models (Dammak et al., 2018).

Table 2 Parameters of growth kinetics and goodness of fit statistics obtained from the growth models applied to the experimental data of *Scenedesmus obliquus*.

Models	Gompertz	Logistic	von Bertalanffy
Kinetic parameters			
$\mu_{máx}$ (1/day)	0.26	0.26	-
λ (day)	2.0	0.5	-
D	2.87	3.35	-
$N_{máx}$ (cell/mL)	$9.00\ 10^7$	$1.45\ 10^8$	-
DT (day)	2.67	2.67	-
Statistical parameters			
R^2	0.9981	0.9571	0.6782
SSE	0.0224	0.5058	0.5058
RMSE	0.0449	0.2371	0.2514

The lag phase of 2 days calculated with the Gompertz model and 0.5 day with the Logistic model, would indicate an easy adaptation of the microalga *Scenedesmus obliquus* to the environmental conditions. These results being comparable to those found by researchers, who obtained λ= 1 day (Dammak et al., 2018).
Then, in the logarithmic growth phase, our results showed specific growth rate of 0.26/day, similar to that found in the literature (0.27/day) for the microalga *Scenedesmus* sp (Aburai et al., 2015); while the doubling time was 2.67 days. From day 14 of culture, a decrease in the growth rate of the microalgae was observed until the stationary phase was reached on day 17 where the number of viable cells remained constant.

3.2. *Microalga growth under stress conditions*

On day 18 of incubation sodium acetate and sodium nitrate were incorporated and irradiance was modified according to the assays indicated in Table 1. The growth of *Scenedesmus obliquus* under different cultivation conditions is shown in Fig. 4. Between days 18 and 24 of culture the cell density of *Scenedesmus obliquus* went from 8.3×10^7 cell/mL to 7.9×10^7 cell/mL, and then decreased rapidly, depending on cultivation conditions for each assay.

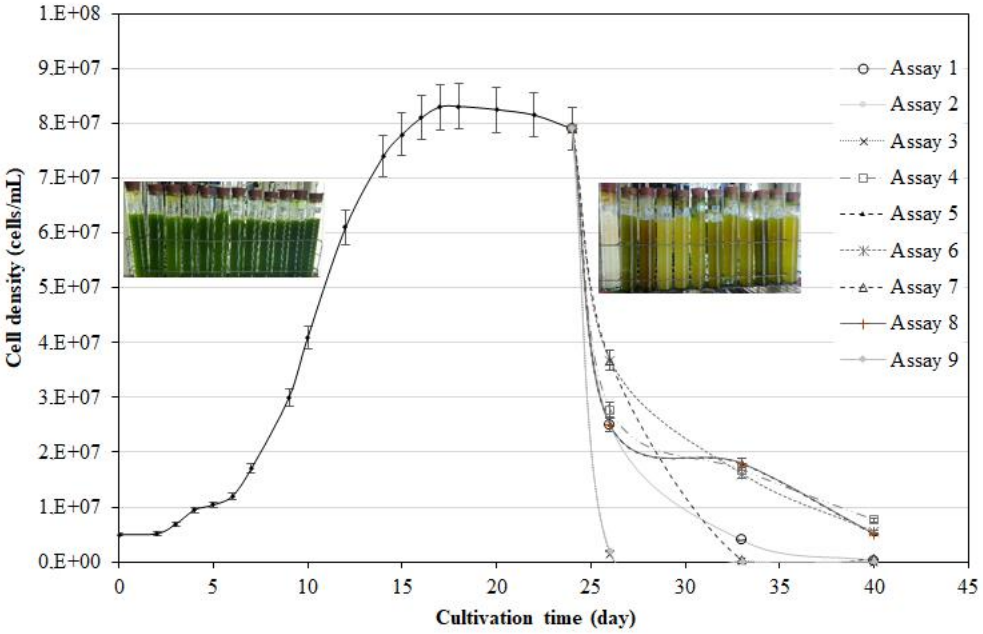

Fig. 4. Growth curve of the *Scenedesmus obliquus* modified under stress conditions.

Assay 3 [nitrate sodium = 87.73 g/L, acetate sodium = 2.5 g/L and irradiance 69.5 µmol/(m^2s)] and 9 [nitrate sodium = 44.00 g/L, acetate sodium = 2.5 g/L and irradiance 102.3 µmol/(m^2s)] were the ones that presented the death phase with the greatest slope, then the assay followed 1 [nitrate sodium = 0.27 g/L, acetate sodium = 2.5 g/L and irradiance 69.5 (µmol/(m^2s)] and 7 [nitrate sodium = 44.00 g/L, acetate sodium = 2.5 g/L and irradiance 36.7 µmol/(m^2s)]. The curves of assay 4 [nitrate sodium = 44.00 g/L, acetate sodium = 0 g/L and irradiance 69.5 µmol/(m^2s)], 2, 5 y 8 [nitrate sodium = 44.00 g/L, acetate sodium = 2.5 g/L and irradiance 69.5 µmol/(m^2s)] were similar. Finally, the assay whose death phase had the lowest slope was 6 [nitrate sodium = 44.00 g/L, acetate sodium = 5 g/L and irradiance 69.5 µmol/(m^2s)]. These results would indicate that cell death was higher at high sodium nitrate concentration and high irradiance, following by conditions with low sodium nitrate concentration and reduced irradiance. While cell death was slower at intermediate values of sodium nitrate and irradiance.
In the stationary and death phases, the microalga gradually modified the coloration from green to orange yellow and, in addition, protein concentration, total carotenoids and total phenols were modified. Under stress conditions, the amounts of proteins, total carotenoids and total phenols varied according to the culture conditions and cultivation time.

3.3. Protein production

At the beginning of the stress conditions, the protein content was 0.45 mg/g dwc and it increased as the cultivation time elapsed. The maximum obtained were 4.74 - 4.92 mg/g dwc. Fig. 5 shows the evolution of the protein content as a function of culture time by varying the culture conditions (concentrations of acetate and sodium nitrate and irradiance) one at a time.

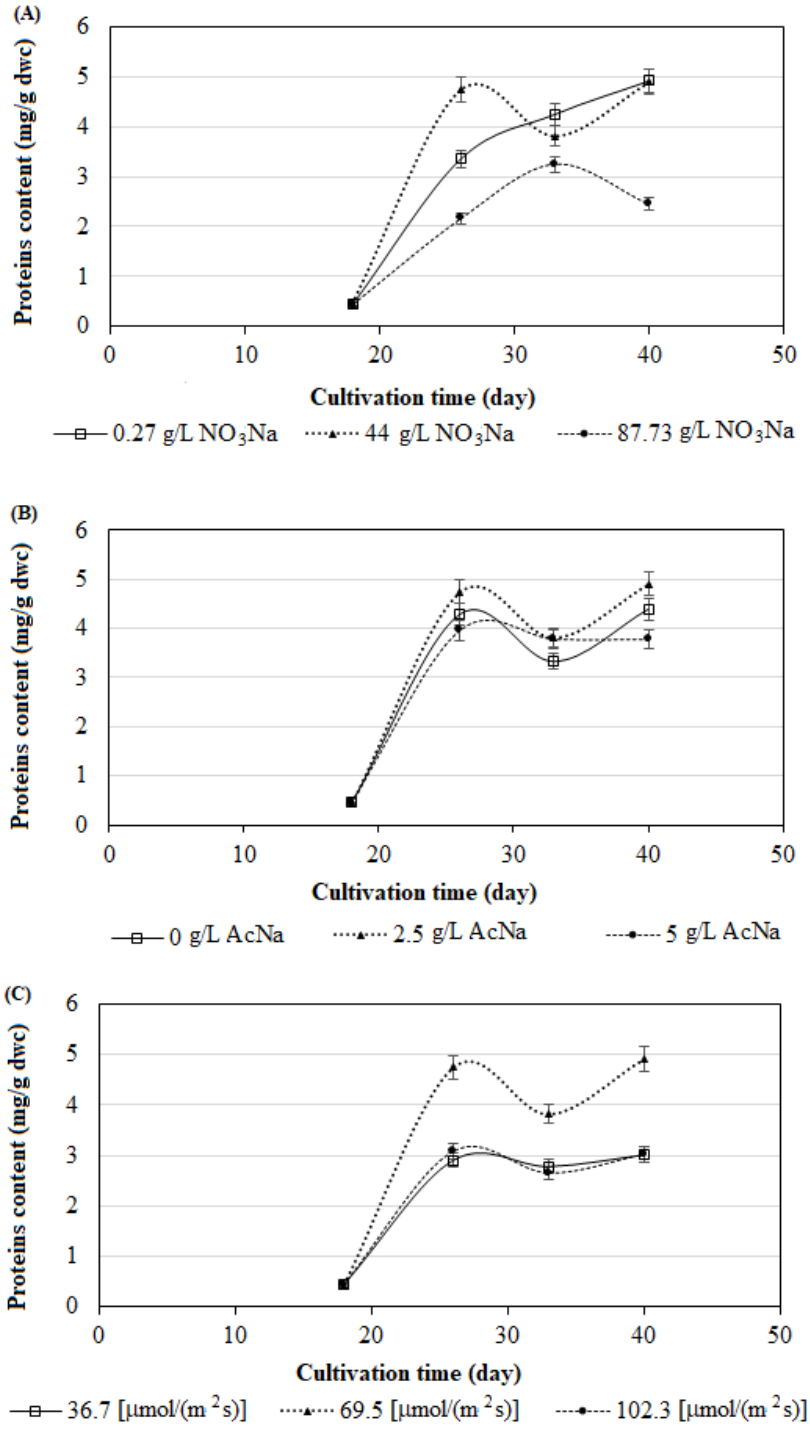

Fig. 5. Protein content of *Scenedesmus obliquus* as a function of cultivation time, varying: (A) concentration of sodium nitrate [sodium acetate= 2.5 g/L, irradiance= 69.5 µmol/(m^{2}s)], (B) concentration of sodium acetate [sodium nitrate= 44 g/L, Irradiance= 69.5 µmol/(m^{2}s)], (C) irradiance (sodium acetate= 2.5 g/L, sodium nitrate= 44 g/L).

In Fig. 5.A. can be seen that for constant conditions of sodium acetate (2.5 g/L) and irradiance [65.9 µmol/(m^2s)], a maximum protein was obtained with 44 g/L sodium nitrate at 26 days of cultivation time; while with 0.27 g/L sodium nitrate the maximum was obtained at 40 days of culture. At high concentrations of sodium nitrate (87.73 g/L) protein production was always lower. Therefore, it can be seen that the maximum protein content was obtained at 44 g/L of sodium nitrate, 2.5 g/L of sodium acetate concentration, and 65.9 µmol/(m^2s) of irradiance. In Fig. 5.B. is visualized that with 44 mg/L of sodium nitrate and 65.9 µmol/(m^2s) of irradiance, the differences in protein production for the different concentrations of sodium acetate are not as marked; observed at 16 days of culture a maximum in the production of proteins with 2.5 g/L of sodium acetate.
In Fig. 5.C. is clearly observed that with 65.9 µmol/(m^2s) of irradiance the protein production was higher, reaching a maximum at 26 days of culture; while for 36.7 µmol/(m^2s) and 102.3 µmol/(m^2s) irradiance the protein production was lower.
Researchers studied the impact of light regime (12-h/12-h light/dark) and growth phase on *Dunaliella salina* cultivation at 20 °C, pH 7.5 and a light intensity of 55 µmol/(m^2s) (Sui et al., 2018). The protein results achieved were higher to those obtained in the present investigation, probably due to the fact that the microalga was cultivated in Modified Johnson's medium at high concentration of salinity (2M). They obtained a maximum protein content of 800 mg/g dwc during the exponential phase, while in the stationary phase the protein concentration decreased to 480 mg/g dwc, contrary to the observations in the present investigation where the protein level increased from exponential phase to stationary phase, which was attributed to the addition of sodium nitrate to the culture medium and, hence, to the increase in the nitrogen availability, important element of the protein composition.

3.3. Total carotenoids production

Effects of sodium acetate and sodium nitrate concentrations and irradiance on the total carotenoids content are shown in Fig. 6. Under stress conditions, the content of total carotenoids in the microalga varied between 37.36-374.30 mg β-carotene/g dwc, reaching the highest concentrations at 26 day of cultivation.

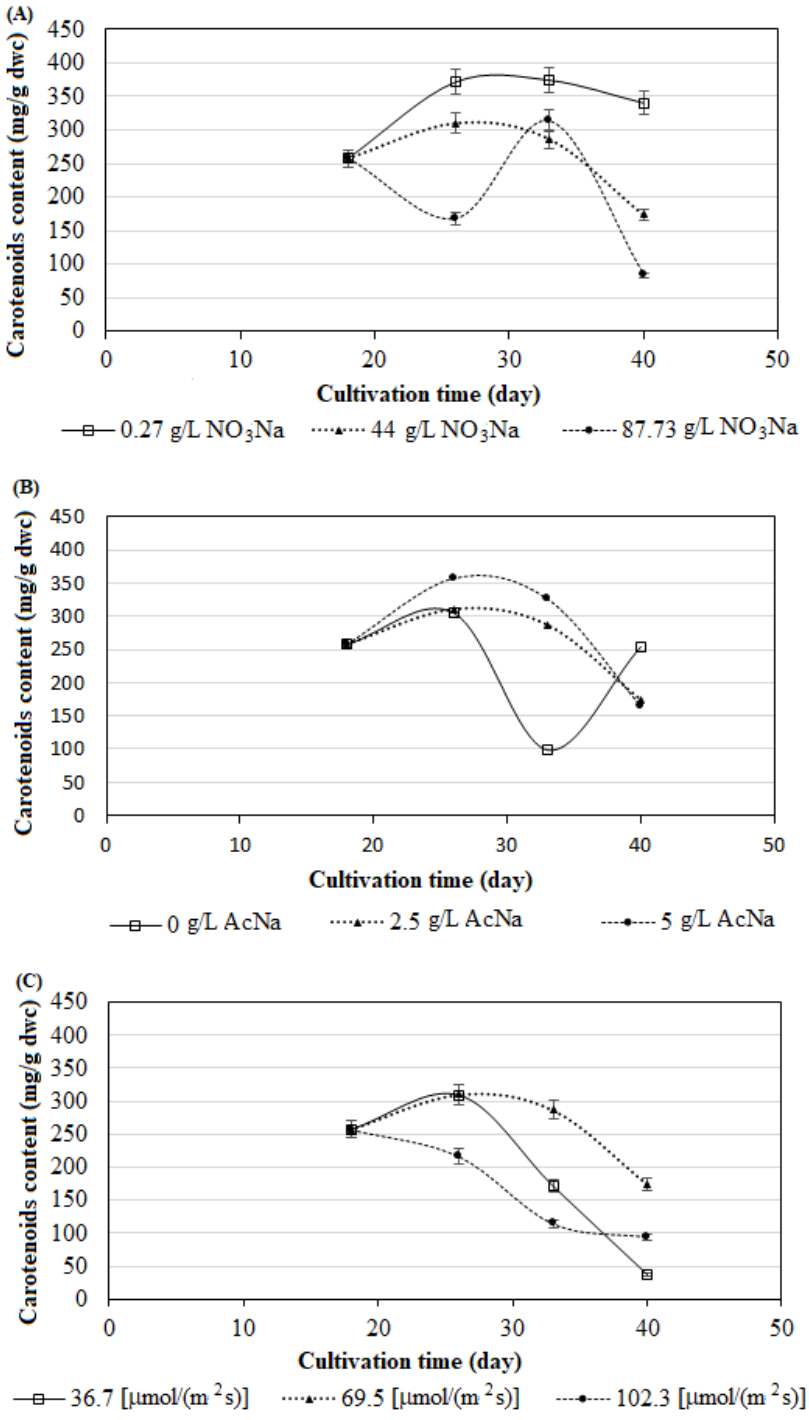

Fig. 6. Total carotenoids content of *Scenedesmus obliquus* as a function of cultivation time, varying: (A) concentration of sodium nitrate [sodium acetate= 2.5 g/L, irradiance= 69.5 µmol/(m^{2}s)], (B) concentration of sodium acetate [sodium nitrate= 44 g/L, Irradiance= 69.5 µmol/(m^{2}s)], (C) irradiance (sodium acetate= 2.5 g/L, sodium nitrate= 44 g/L).

Fig. 7. Total phenols content of *Scenedesmus obliquus* as a func
At a concentration of sodium acetate of 2.5 g/L and irradiance of 65.9 µmol/(m^2s) the maximum concentration of total carotenoids was with 0.27 g/L sodium nitrate (Fig 6.A); while when the concentration of sodium nitrate was kept constant in 44 g/L and the same irradiance value, the maximum total carotenoids was reached for a medium with 5 g/L sodium acetate (Fig. 7.B). As with protein production, the irradiance of 65.9 µmol/(m^2s) was it possible to obtain greater total carotenoids (Fig. 7.C).

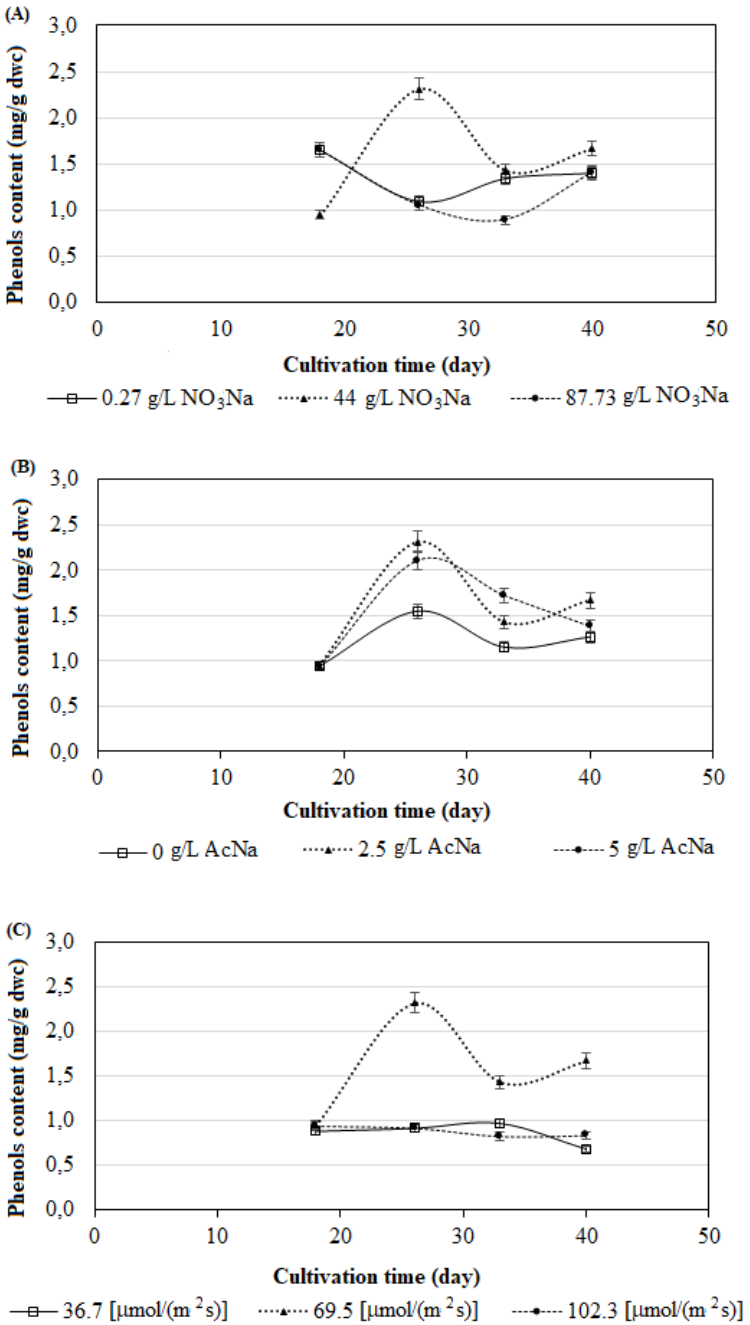

Fig. 7. Total phenols content of *Scenedesmus obliquus* as a function of cultivation time, varying: (A) concentration of sodium nitrate [sodium acetate= 2.5 g/L, irradiance= 69.5 µmol/(m^2s)], (B) concentration of sodium acetate [sodium nitrate= 44 g/L, Irradiance= µmol/(m^2s)], (C) irradiance (sodium acetate= 2.5 g/L, sodium nitrate= 44 g/L).

Therefore, the conditions that favored the synthesis of carotenoids were: concentrations of sodium nitrate and sodium acetate of 0.27 g/L and 5 g/L, respectively; and irradiance of 65.9 µmol/(m^2s).
Researchers obtained total carotenoid amounts of: 34.2±3.8 mg/g dwc [irradiance: 200 µmol photons/(m^2s)], 0.042 mg/g dwc [environmental culture conditions: salinity 40 ppt, irradiance 182 µmol photons/(m^2s) and pH 7] and 1203.9±98 µg/g dwc (with microalga *Auxenochlorella* spp, culture conditions: temperature 25-30 °C, pH 6-7, 2% sodium chloride concentration and cultivation time 5 days) (Aburai et al., 2015; Dammak et al., 2018; Asker and Awad, 2019). Interestingly, our studies achieved a total carotenoid content 9 times higher than those published by the authors mentioned.

3.4. Total phenols production

The stress conditions in the microalgal culture caused an increase in the concentration of total phenols, from 0.68 to 2.31 mg GAE/g dwc. In Fig. 7.A can be seen that by keeping the concentration of sodium acetate constant at 2.5 g/L and the irradiance at 69 µmol/(m^2s), the highest concentration of total carotenoids was obtained with 44 g/L sodium nitrate; while when the latter were kept constant two parameters in the values indicated above, the maximum concentration of total carotenoids was obtained with 2.5 g/L sodium acetate (Fig. 7.B). When sodium acetate was not incorporated, the carotenoid content was lower. In Fig. 7.C can be seen that only at irradiance of 69 µmol/(m^2s) biomass with a greater amount of phenolic compounds was obtained. Thus, the maximum total phenols was reached at 26 day, with 44 g/L sodium nitrate concentrations and 2.5 g/L sodium acetate concentrations and 65.9 µmol/(m^2s) irradiance.

The concentration of total phenols reported for *Dunaliella tertiolecta* culture was 1.54 mg GAE/g dwc and in culture of *Tetraselmis chuii*, 1.52 mg GAE/g dwc [standard culture medium f/2, irradiance 274,2 µmol/(m^2s)] (Gómez et al., 2016). In the present investigation, the concentration of phenolic compounds was 1.5 times higher than the one published by the researchers mentioned; probably because of the addition of sodium acetate to the culture medium and, hence, to the increase in the carbon source. However, with 5 g/L sodium acetate the amount of phenolic compounds was lower, therefore it was concluded that there is a ratio of carbon source in which the total phenols of the microalga stop increasing their value progressively, due to a possible saturation of the metabolic pathways.

CONCLUSIONS

Growth curve of *Scenedesmus obliquus* in Arnon culture medium with supplemented nitrate was successfully described using the Gompertz model, followed by the Logistic model. The specific growth rate was 0.26/day in the logarithmic growth phase and the doubling time of 2.67 days would indicate an easy adaptation of the microalga *Scenedesmus obliquus* to the environmental conditions. The maximum concentrations of phytonutrients were obtained after 26 days of culture, that is, 8 days after the start of the stationary phase and having induced stress conditions in the microalga with the incorporation of sodium acetate and sodium nitrate.
The maximum protein and total phenols values were 4.92 mg/g dwc and 2.31 mgGAE/g dwc, respectively; obtained at sodium acetate concentration of 2.5 g/L, sodium nitrate concentration of 44 g/L and irradiance of 65.9 µmol/(m^2s). This microalga yielded a maximum of total carotenoid of 374.30 mg β-carotene/g dwc at sodium acetate concentration of 5 g/L, sodium nitrate concentration of 0.27 g/L and irradiance of 65.9 µmol/(m^2s). Consequently, it required double amount of sodium acetate and a minimum amount of sodium nitrate, compared with that needed to produce the maximum protein and total phenols. Moreover, the maximum protein and carotenoid levels reached were 10 times higher than those obtained at the beginning of stress conditions, while those of total phenols were 3.4 times higher. Therefore, this species has a potential to be a carotenoid, protein and phenol source.

Acknowledgments
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NOMENCLATURE

λ - lag time
μ - specific growth rate
μ_{max} - maximum specific growth rate
AcNa - sodium acetate
B: represents the slope of the curve and describes the growth rate
BSA - bovine serum albumin
D: difference between the maximum and initial population
dwc - dry weight cells
e = 2.7182
GAE - gallic acid equivalents
k: maximum absolute growth rate
LN – natural logarithm
M: time in which the growth rate is of greater magnitude
N_0 - initial cell density (inoculum)
N_t – cell density at time t
R^2 - determination coefficient
RMSE - root mean square error
SSE - sum of square error
t: cultivation time

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