Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v45i1.64996

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Antioxidant response of *Messastrum gracile* (Chlorophyceae) to light-induced stress

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ABSTRACT. The antioxidant enzymes activities during the *Messastrum gracile* growth period under two different light intensities were evaluated. The light intensities were designated as: control at 60 µmol photons m^{-2} s^{-1} and two cultivations under light-induced stress at 120 µmol photons m-2 s^{-1}, during 24 and 72 hours. The maximum cell density in all treatments was achieved on the 21st growth day with 140x105 cells mL^{-1} (control), 382x105 cells mL^{-1} (24 hours) and 236x105 cells mL^{-1} (72 hours). The antioxidant enzymes activities were more elevated in the control, however, GSH-Px, CAT and SOD activities were higher on the 7th growth day, while GPOX and GR activities were higher on the 28th and 21st growth day, respectively. The microalgae displayed lower MDA content, which indicates low stress levels. In terms of growth, biomass production and chlorophyll-*a*, the best results occurred under light-induced stress (120 µmol photons m-2 s^{-1}). Light-induced stress decreased antioxidant activity during *M. gracile* growth, and the results revealed a higher activity for glutathione peroxidase, catalase and superoxide dismutase.

Keywords: cell growth; CHU_{12}; enzymes activities; 120 µmol photons m^{-2} s^{-1}.

Received on September 12, 2022.
Accepted on July 21, 2023.

Introduction

The microalgae are important antioxidant sources, due to their vitamin composition, pigment and enzymes. Microalgae antioxidant may be used in health and cosmetics industries, for medical care, and in the aquaculture industry (Banskota, Sperker, Stefanova, Mcginn, & O'leary, 2019). The antioxidant power of microalgae is comparable, and even higher than the antioxidative activity of higher plants or fruits, and the variability is high. In that way, microalgae are highly promising as antioxidant providers with great potential (Sansone & Brunet, 2019). Microalgae produce reactive oxygen species (ROS) scavenging compounds such as phenols, vitamins (mainly C), pigments (carotenoids and chlorophylls) and enzymes, that are the main antioxidants (López-Hernandez et al., 2020).

The antioxidants play important roles by protecting the cells against oxidative damage (Pagels et al., 2021). Some of the antioxidants capable of neutralizing ROS, are the superoxide dismutase (SOD, EC 1.15.1.1) that acts as first line of defense and promotes the dismutation of superoxide anions (O2•-) generating H2O2 and O2, glutathione reductase (GR, EC 1.6.4.2), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), ascorbate peroxidase (APX, EC 1.11.1.11) and other peroxidases that acts against the H2O2 produced by SOD and/or other metabolism processes (Noctor & Foyer, 2016). Non-enzymatic mechanisms may also aid in the defense, including antioxidants such as the ascorbate (AsA) and the glutathione (GSH), in addition to vitamin and carotenoids, which protects the photosynthetic apparatus against oxidative damage (Gong & Bassi, 2016).

The microalgae have great potential for antioxidant production since the photosynthetic organisms are impaired by deleterious oxidative effects through the photosynthesis process in the natural environment and are also affected by the variation in light and oxygen in the water column. Thus, they depend on an effective antioxidant system to respond to the oxidative stress caused by the factors previously cited (Sansone & Brunet, 2019).

Light intensity is crucial to growth and development of photo-autotrophic organisms. It is the energy source for the photosynthesis process, which converts light, CO_2, water and nutrients to oxygen, and biomass

which is composed by vitamins, proteins, and antioxidants (Fontoura, Rolim, Farenzena, & Gutterres, 2017). The increase in light intensity during the microalgae growth can shift its development and may induce an enzymatic activity that interferes in the protein synthesis and the carotenoids production. However, excess light intensity may promote photo-inhibition (Córdova, Ruiz-Filippi, Fermoso, & Chamy, 2018). On the other hand, changes in light conditions allow the synthesis of specific compounds that change the overall efficiency of the organism through manipulation of metabolic pathways (Pagels et al., 2021). The light effect on antioxidants production, especially carotenoids, is known to be complex and species specific, however the production of secondary metabolites by microalgae is modulated by environmental conditions (Coulombier et al., 2020).

In tropical areas microalgae submitted to strong light variation must quickly adapt to the light excess or its limitation. The diversity of microalgae that contain antioxidant compounds which are capable of exert biological activities is not known yet, nevertheless they have phenolic compounds capable of minimizing oxidative stress (Shanab, Mostafa, Shaby, & Mahmoud, 2012).

The microalgae *Messastrum gracile* (Chlorophyceae), formerly identified as *Ankistrodesmus gracilis*, have been studied for its easy cultivation, fast growth rate and adaptation to indoor cultivation, growth in alternative culture media and can be used in the fish larvae feed (Sipaúba-Tavares & Braga, 2007). The purpose of the present report is to evaluate some *M. gracile* antioxidant enzymes under the influence of light-induced stress during the growth period. Current analyses evaluates whether: (1)- the use of light-induced stress (120 µmol photons m^{-2} s^{-1}) influences the *M. gracile* to further stimulate antioxidant activities; (2)- the biomass production is influenced by light-induced stress of 120 µmol photons m^{-2} s^{-1}; (3)- *M. gracile* had a potential to produce antioxidants enzymes.

Material and methods

Experimental conditions

Messastrum gracile (CCMA-UFScar 5) was cultured in CHU_{12} culture medium, at 22 ± 2°C, dissolved oxygen around 7.3 ± 0.1 mg L^{-1}, with illumination supplied with white LED lamps on each culture. The experiment started with 10 mL culture volume, that was transferred to a 250 mL culture volume at a density of 0.75 x 10^5 cells mL^{-1}. When culture reached the end of the exponential growth phase (7^{th} growth day), approximately 10 mL with 1.9 x 10^5 cells mL^{-1} density was transferred to a culture containing 2 L total volume. After the exponential growth phase *M. gracile* culture was transferred to 13 L volume sterilized recipients at a density of 4.4 x 10^5 cells mL^{-1}, where three treatments were set up: a control (60 µmol photons $m^{-2}s^{-1}$) and other two with light-induced stress (120 µmol photons $m^{-2}s^{-1}$) under two different stress time (24 and 72 hours) during the growth period. After this, each treatment was centrifuged, and a microalgae paste was used for antioxidants evaluation (Figure 1). Cell growth in each treatment was evaluated in triplicate (n=3), and samples were analyzed weekly (7, 14^{th}, 21^{st} and 28^{th} growth days) for growth and antioxidants evaluation and monitoring of other variables during experimental period.

Cell growth and chemical parameters

Cell density was monitored for 28 days, 1 mL aliquots were daily removed from the microalgae culture in triplicate. Minimum of 2x1 µL subsample was employed to quantify the cells density by a Neubauer hemocytometer. The growth rate (k) was obtained according to Guillard et al. (1973), and calculated as follows (Equation 1):

$$k = \frac{3.322 \times \log N_2}{t_2 - t_1 \qquad N_1} \quad (1)$$

where: k = divisions per day, t = time and N = number of cells, subscripts denote values at different periods. Total length of 50 specimens were determined with Leica DFC 295 microscope with image analysis system LAS core (LAS V3.8), and 40x micrometric objectives. Cell volume was calculated by average cell size using the most adequate geometrical form (two coupled cones) formula in the case of *M. gracile* (Hillebrand, Dürselen, Kirschtel, Pollingher, & Zohary, 1999). Doubling time (cell division time or generation time) was calculated from results obtained from growth rate, according (Guillard, 1973) (Equation 2):

$$DT = 1k^{-1} \quad (2)$$

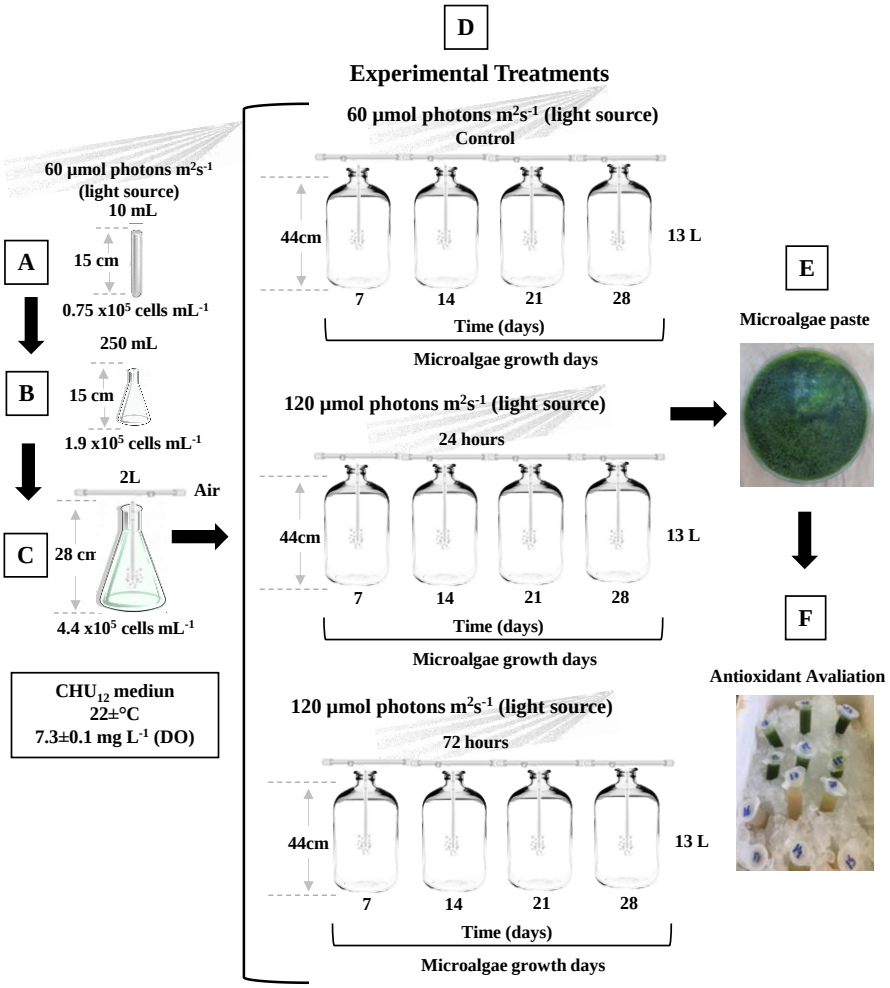

Figure 1. Diagram of *Messastrum gracile* microalgae cultured in CHU_{12} culture medium where: A= strain maintenance 10 mL; B= initial culture in 250 mL; C= culture in 2 L; D= experimental treatments in 13 L; E= microalgae paste and F= antioxidant assessment, with three treatments: a control under 60 µmol photons $m^{-2}s^{-1}$ and other two with light-induced stress under 120 µmol photons $m^{-2}s^{-1}$ during 24 and 72 hours.

where: DT = doubling time and $1k^{-1}$ = division per days. Total organic carbon (TOC) was calculated by regression following Rocha & Duncan (1985) (Equation 3):

$$TOC = 0.1204\ V^{1.051} \quad (3)$$

where: TOC = total organic carbon content in pg $cell^{-1}$ and V = cell volume.

Analytical procedure

Chemical parameters of the culture media were measured weekly. Dissolved oxygen, conductivity and pH of the culture medium was measured with YSI 556 MPS multi-sensor. Total inorganic nitrogen and total phosphorus was quantified by spectrophotometry following Koroleff (1976) and Golterman, Clymo, and Ohnstad (1978).

Procedure for antioxidants evaluation

Enzyme extraction

To perform the antioxidants extraction, 5 grams of *M. gracile* wet biomass (paste) were employed. This assessment type was designed in preliminary studies. Several protocols were tested for the antioxidants analysis. The chosen methodology was proposed by Fan et al. (2019) and consists in the microalgae samples centrifugation at 1,500 rpm for 10 minutes at 4°C. The supernatant was discarded, and 2.5 mL potassium phosphate buffer solution was added to the microalgae biomass remaining from the centrifugation. The procedure was performed three times for 20 seconds each. The samples were centrifuged again following the protocol proposed by Fan et al. (2019) at 13,800 rpm for 10 minutes at 4°C and the supernatant was stored at -80°C.

Lipid peroxidation

The lipid peroxidation was estimated by quantifying the content of reactive substances to thio barbituric acid reactive substance (TBARS), such as malondialdehyde (MDA). The MDA concentration expresses the organism's stress level as it reflects the extent of lipid peroxidation through the detection of aldehyde compounds. This concentration was measured using spectrophotometry (535 and 600 nm), with an extinction coefficient of 1.55 x 10^{-5} mol^{-1} cm^{-1} (Gratão et al., 2012).

Antioxidant analysis

Catalase evaluation (CAT, EC 1.11.1.6) was performed using a potassium phosphate buffer solution and H_2O_2. The catalase activity was determined by H_2O_2 decomposition in the presence of 25 µL protein extract from samples collected on the 7, 14th and 21st growth day and 50 µL samples from the 28th growth day (Azevedo, Alas, Smith, & Lea, 1998). Superoxide dismutase evaluation (SOD, EC 1.15.1.1) was performed with a 50 µL aliquot from the sample, sodium phosphate buffer solution, methionine, nitro blue tetrazolium (NBT), EDTA and riboflavin, for a total 5 mL, with reaction occurrence indicated by the blue formazan compound (Giannopolitis & Ries, 1977). Glutathione reductase evaluation (GR, EC 1.6.4.2) was performed with potassium phosphate buffer (pH 7.5) containing 1 mM, 5,5'-dithio-bis (2 nitrobenzoic acid), oxidized glutathione, NADPH and 25 µL extract. The GR activity was estimated by the oxidized glutathione reduction (Gomes-Junior et al., 2006). Guaiacol peroxidase (GPOX, EC 1.11.1.7) was determined by the phosphate-citrate buffer reaction, guaiacol 0.5% and 50 µL from the algal extract on the 7, 14th and 21st growth days and 30 µL from the 28th growth day (Matsuno & Uritani, 1972). Glutathione peroxidase (GSH-Px, EC 1.11.1.9) was determined using potassium phosphate buffer, EDTA, GR, GSH, sodium azide, NADPH and H_2O_2 (Flohé & Gunzler, 1984). All antioxidant enzymes were expressed as µmol min^{-1} mg^{-1} protein.

Statistical analysis

Biological microalgae data and culture medium parameters were determined by variance analysis (One-way, ANOVA) with the software Statistica 10. Tukey's test was applied when differences between treatments occurred ($p < 0.05$). Antioxidants analysis was performed using the software Agro Stat (v.1.1.0.694) (Barbosa & Maldonado Junior, 2015). Variance analysis (ANOVA) was calculated for each antioxidant analyzed and when significant differences were observed, means were compared using Tukey's post hoc test at 5% probability ($p < 0.05$). All experiments were carried out in triplicate. Data was expressed as mean ± SD.

Results

Cell growth, chemical and biological data

The maximum cell density in all treatments was achieved on the 21st growth day with 140 x10^5 cells mL^{-1} (control), 382 x10^5 cells mL^{-1} (24 hours stress) and 236 x10^5 cells mL^{-1} (72 hours stress). However, the light intensity promoted heavy influence in cell density. The light-induced stress treatment (120 µmol photons m^{-2} s^{-1}) obtained higher cell density than the control (60 µmol photons m^{-2} s^{-1}) in all treatments, except on the 14th growth day, when the 72 hours light-induced stress was similar to the control (128x10^5 cells mL^{-1} and 123x10^5 cells mL^{-1}, respectively) (Figure 2).

The cell density increase promoted an impact in biomass production, since the treatments under light-induced stress obtained higher biomass content (0.3 to 1.1 mg biomass $L^{-1}d^{-1}$) than the control (0.2 to 0.6 mg biomass $L^{-1}d^{-1}$). Doubling time was similar between control and light-induced stress, although a faster doubling time was observed on the 7th growth day under 72 hours light-induced stress (1 day). TOC was above 2.6±1 pg $cell^{-1}$ in all treatments and the highest levels were observed on the 7th growth day under 24 hours light-induced stress (7±1 pg $cell^{-1}$). In all treatments, k was higher on the 7th growth day and with highest rates at 120 µmol photons m^{-2} s^{-1} (k = 0.9). Cell volume was different in all treatments and the highest values were observed with 24 hours light-induced stress on the 7th growth day (43.7±21 $µm^3$). Total length was similar in all treatments ranging between 14.3±2 µm to 16.1±2 µm in control; 13.7 ±1 µm to 17±2 µm at 24 hours light-induced stress and 13.8±1 µm to 14.8±2 µm at 72 hours light-induced stress (Table 1). In the culture medium pH was alkaline above 9.1±0.04, except on the 7th growth day under 24 hours light-induced stress it was above 7.3 ±0.04, and conductivity was above 387 µScm^{-1} on the 7th growth day under 120 µmol photons m^{-2} s^{-1}. However, on the other evaluations, the conductivity was above 691 µScm^{-1} (7th growth day) under 72 hours

light-induced stress and the highest value was 868 µScm^{-1} under 120 µmol photons m^{-2} s^{-1} on the 28th growth day for both light-induced stress. Highest conductivity occurred due to high total inorganic nitrogen and total phosphorus contents in the culture medium (Table 1).

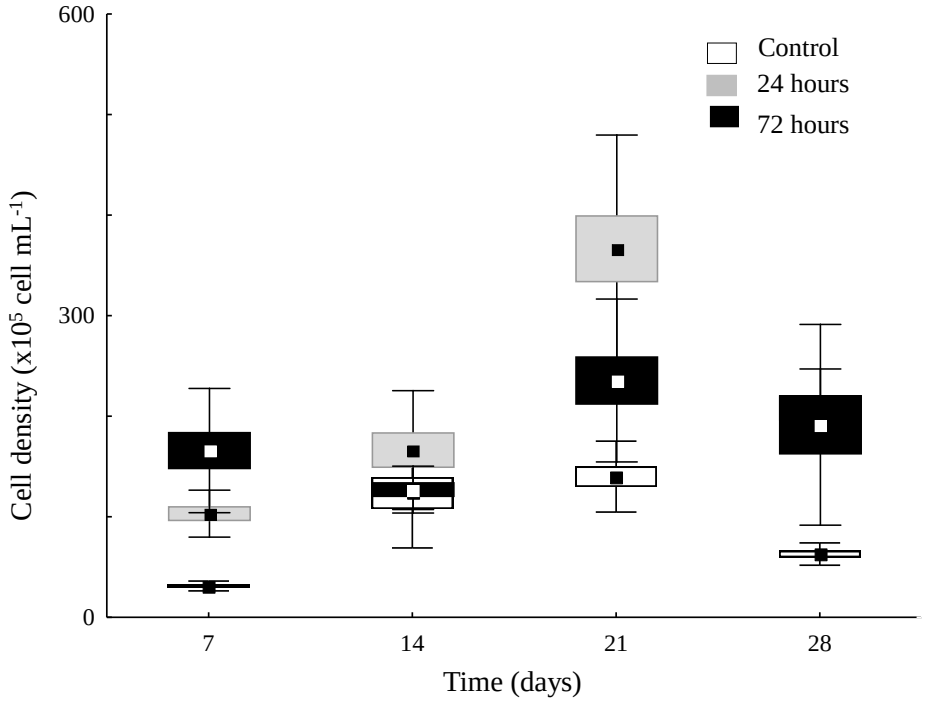

Figure 2. Boxplot of cell density of *Messastrum gracile* microalgae submitted to three light-intensity: a control under 60 µmol photons m^{-2}s^{-1} and light-induced stress (120 µmol photons m^{-2}s^{-1}) for 24 and 72 hours, at the 7, 14th, 21st and 28th growth days.

Table 1. Means and standard deviation variables (microalgae and culture medium) measured in the treatments: a control under 60 µmol photons m^{-2}s^{-1} and two other conditions light-induced stress under 120 µmol photons m^{-2}s^{-1} for 24 and 72 hours at the 7, 14th, 21st and 28th growth days.

Treatments	Days	Microalga						Culture Medium	
		k[1]	Bio[2]	CV[3]	TL[4]	TOC[5]	DT[6]	Cond[7]	pH
Control	7	0.6±0.02^a	0.2	26.8±14^b	14.3±2^a	4±1^b	1.7±0.05^d	791±15^a	9.9±0.2^a
	14	0.4±0.02^b	0.4	38.2±21^a	16.1±2^a	5.5±1^a	2.4±0.1^c	777±16^b	9.3±0.03^{bc}
	21	0.2±0.01^c	0.6	21±13^c	14.4±2^a	3.1±1^b	3.5±0.1^b	809±19^a	9.1±0.01^c
	28	0.1±0.03^d	0.3	35±8^a	14.3±2^a	3.9±1^b	4.4±0.1^a	818±20^a	9.6±0.1^b
Stress									
24 hours	7	0.9±0.03^a	0.3	43.7±21^a	17±2^a	7±1^a	1.1±0.03^c	387±3^c	7.3±0.04^b
	14	0.5±0.02^b	0.5	19.1±10^c	13.7±1^b	2.6±0.4^d	2.2±0.1^b	711±28^b	9.2±0.1^a
	21	0.3±0.01^c	1.0	31.9±15^b	15.5±2^{ab}	4.4±1^b	2.8±0.07^b	736±21^b	9.2±0.1^a
	28	0.2±0.01^d	0.6	15.9±15^c	14±2^b	3.7±07^c	4.3±0.1^a	868±12^a	9.3±0.3^a
72 hours	7	0.9±0.04^a	0.4	34.2±20^a	14.6±2^a	5.2±1.6^a	1.0±0.04^d	691±1^c	9.1±0.1^b
	14	0.4±0.01^b	0.3	34.7±19^a	14.8±2^a	5.4±1^a	2.3±0.05^c	794±16^b	9.1±0.04^b
	21	0.3±0.01^c	1.1	23.5±11^b	13.8±1^a	3.3±1^b	3.1±0.1^b	814±11^b	9.1±0.01^b
	28	0.2±0.01^c	0.6	22.9±12^b	14.5±2^a	3.1±1^b	4.4±0.2^a	868±12^a	9.5±0.2^a

[1]k = growth rate (divisions per day); [2]Bio = Biomass (mg biomass L^{-1} d^{-1}) [3]CV = cell volume (µm^3); [4]TL = total length (µm); [5]TOC = total organic carbon (pg cell^{-1}); [6]DT = doubling time (days); [7]Cond = conductivity (µScm^{-1}). Same letters in the row means that no significant differences occur ($p < 0.05$) in culture media and biological parameters for each treatment.

In general, total inorganic nitrogen and total phosphorus contents were elevated with values above 0.4 ±0.04 mgL^{-1} under 24 hours light-induced stress and 0.6 ±0.1 mgL^{-1} in the control, both on the 7th growth day (Figure 3).

The highest total inorganic nitrogen concentration and total phosphorus occurred at the control treatment with 4.5 ±0.6 mg L^{-1} and 2.5 ±0.2 mg L^{-1}, respectively. In the last *M. gracile* evaluation (28th growth day) total phosphorous was about three times as higher as the total inorganic nitrogen in all treatments. When under 24 hours light-induced stress total phosphorous was higher than total inorganic nitrogen during the whole experiment (Figure 3).

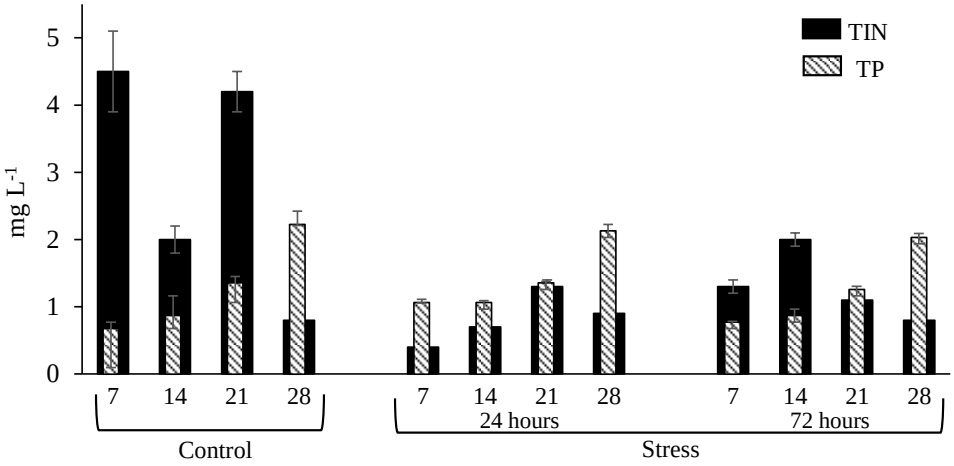

Figure 3. Total inorganic nitrogen (TIN) and total phosphorus (TP) contents from *Messastrum gracile* microalgae culture medium measured in the treatments: a control under 60 µmol photons $m^{-2}s^{-1}$ and two other conditions light-induced stress under 120 µmol photons $m^{-2}s^{-1}$ for 24 and 72 hours on the 7, 14th, 21st and 28th growth days.

Oxidative stress and antioxidant enzyme activities

MDA content was elevated in all treatments on the 28th growth day, with 1.93±0.0003 µmol mg^{-1} wet biomass in the control, 1.53±0.1 µmol mg^{-1} wet biomass under 24 hours light-induced stress and 1.24±0.1 µmol mg^{-1} wet biomass under 72 hours light-induced stress. Only in control the MDA content increased during the microalga growth. The MDA content on the 7, 14th and 21st growth days, were below 0.8±0.1 µmol mg^{-1} wet biomass, except in the control on the 21st growth day (1.37±0.06 µmol mg^{-1} wet biomass) in the control treatment (Figure 4).

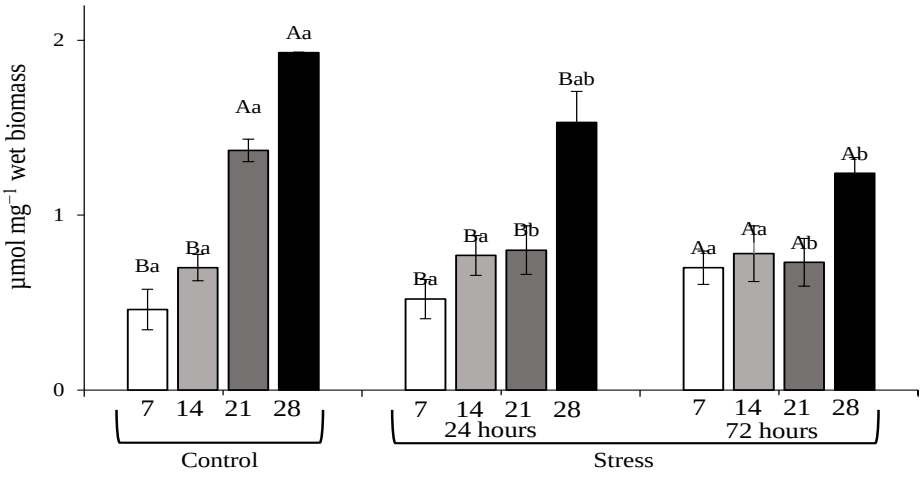

Figure 4. Malondialdehyde (MDA) content in *Messastrum gracile* microalgae measured in the treatments with light intensity: a control under 60 µmol photons $m^{-2}s^{-1}$ and other two light-induced stress under 120 µmol photons m^{-2} s^{-1} for 24 and 72 hours on the 7, 14th, 21st and 28th growth days. Different uppercase letters above columns indicate differences between treatments, while lowercase letters indicate days of growth with differences at $p < 0.05$.

In general, *M. gracile* showed high antioxidants enzymes activity in the control treatment, however the antioxidants activity decreased from the 7 to the 28th growth days, except GPOX that increased during cited interval. GSH-Px presented the highest antioxidant enzyme activity, being higher in control with 916.2±9.9 µmol min^{-1} mg^{-1} protein on the 7th growth day. Antioxidant enzyme activity for GSH-Px, CAT and SOD in light-induced stress was about 7 to 35 times lower than in the control treatment. The same did not happen for GR and GPOX activities, which remained below 3.64±0.4 µmol min^{-1} mg^{-1} protein and fluctuated throughout the microalga growth period. Only CAT had highest activities on the 7th growth day in all treatments with or without light-induced stress. Even under light-induce stress GSH-Px, CAT and SOD activities were higher, ranging from 1.48±0.8 µmol min^{-1} mg^{-1} protein (CAT) to 46.19±8 µmol min^{-1} mg^{-1} protein (GSH-Px), and GR and GPOX were below 2.8±0.1 µmol min^{-1} mg^{-1} protein (GR) (Figure 5).

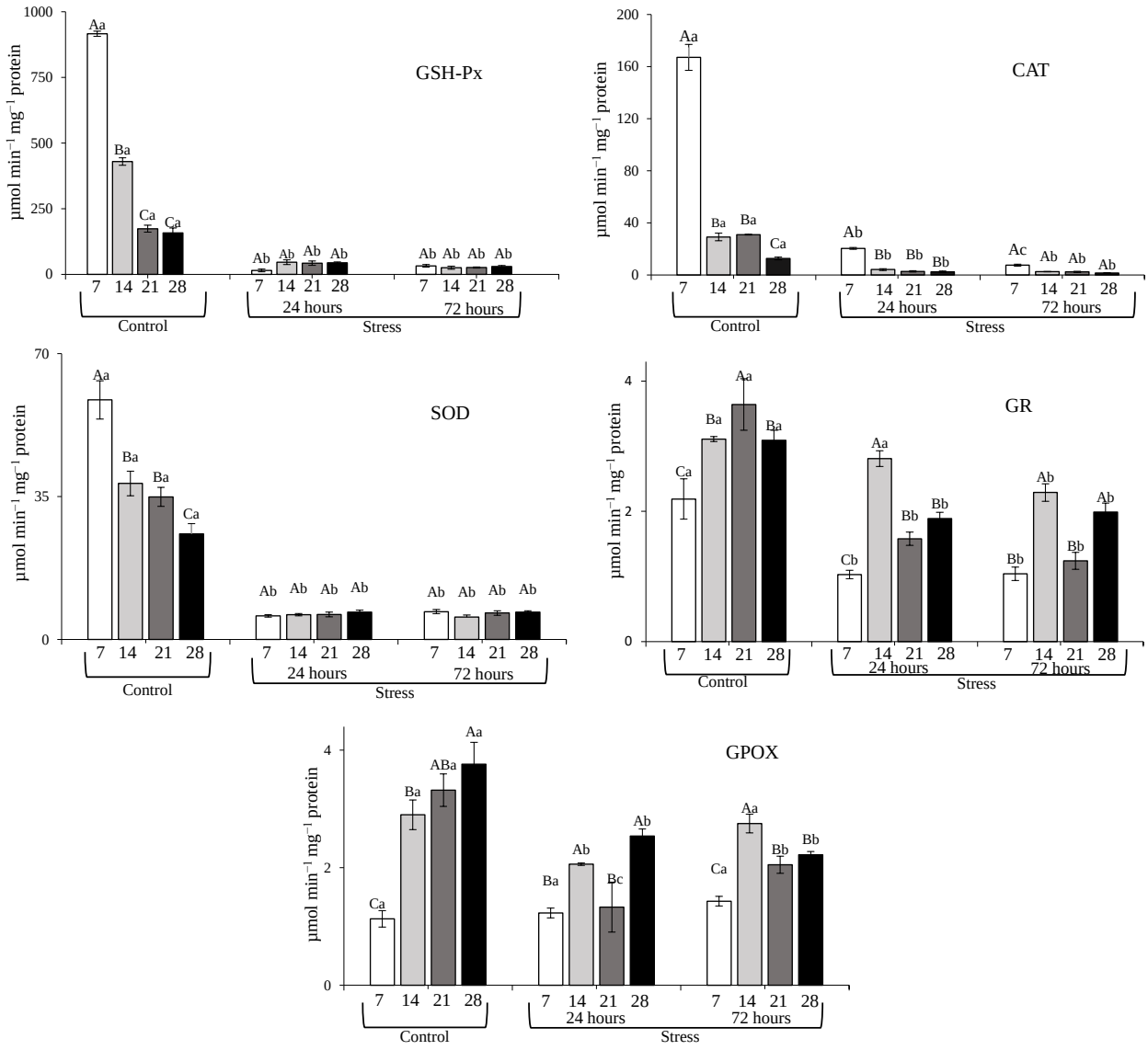

Figure 5. Antioxidant enzyme activity in *Messastrum gracile* microalgae measured in the treatments with light intensity: a control under 60 µmol photons $m^{-2}s^{-1}$ and two other light-induced stress under 120 µmol photons $m^{-2}s^{-1}$ for 24 and 72 hours on the 7, 14th, 21st and 28th growth days. Different uppercase letters above columns indicate differences between treatments while lowercase letters indicate days of growth with differences at $p < 0.05$.

Discussion

The results showed that light-induced stress (120 µmol photons m^{-2} s^{-1}) is not necessary to induce antioxidant enzyme activity by *M. gracile*, since high antioxidant enzymes activity (GSH-Px, CAT and GR) was observed at control (60 µmol photons m^{-2} s^{-1}) commonly employed for this microalga cultivation. However, they were lower during light-induced stress. SOD enzyme is the first defense line against ROS, since they target the first reactive species formed, the superoxide, which will be broken into hydrogen peroxide and water (Noctor & Foyer, 2016). Thus, SOD activity begins in the first days of the microalgae growth and decreases as the production of superoxide decreases during the microalgae growth. This was observed at control (60 µmol photons m^{-2} s^{-1}) with high antioxidant enzyme activity on the 7th growth day, however under light-induced stress (120 µmol photons m^{-2} s^{-1}) no decrease was observed, remaining similar throughout the algal growth days.

The antioxidant machinery involves enzymatic and nonenzymatic antioxidants. These extracts also prevent oxidative damage by efficiently neutralizing ROS, which includes catalase by the conversion of hydrogen peroxide in water and molecular oxygen (López-Hernandez et al., 2020). CAT, which is being produced mainly at the peroxisomes is activated by breaking hydrogen peroxide on the 7th growth day, decreasing its activity as most of the hydrogen peroxide is degraded.

The antioxidant enzyme activity is influenced by the culture medium components such as nitrogen. According to Abd El-Baky, Hussein, and El-Baroty (2020), the lower nitrogen concentration may supply an increase in pigment's concentrations due to increased light intensity promoting microalgae stress and increasing the production of carotenoids, which protects the cell against ROS produced as response to a stress condition. In a *M. gracile* cultivation in CHU12 culture medium, the authors observed high total inorganic nitrogen concentration of over 0.4±0.04 mg L^{-1} at the control treatment, in which all antioxidant enzymes activity were also elevated mainly GHS-Px, which was above 138±19 µmol min^{-1} mg^{-1} protein and may have influenced the low carotenoids content in the microalgae.

According to Abd El-Baky et al. (2020), low nitrogen concentration may promote pigment increase, due to microalga need for these compounds for growth. In general, *M. gracile* produces high levels of chlorophyll-*a* (Sipaúba-Tavares, Segali, Berchielle-Morais, & Scardoeli-Truzzi, 2017) and this fact may have directly influenced the antioxidants, since the pigments have derivative compounds bond to lipoproteins and play an important role as antioxidants during photosynthesis, associated with potential protection against lipid peroxidation, among others (López-Hernandez et al., 2020).

On the 28th growth day, all treatment obtained higher total phosphorus contents than total inorganic nitrogen. The same was observed by Sipaúba-Tavares et al. (2017) since the phosphorus is known to be removed by microalgae for biomass growth. Towards the end of the growth period, there is a tendency for the cells to die, leading to a change in the color of the culture medium from green to yellowish-green, and consequently, this compound can be released into the medium.

Many factors contribute to development and metabolic activities, such as pH levels that affect the growth of microalgae due to available carbon with alteration or disruption of cell membrane process. Ismael, El-Agouty, & Piercey-Narmore (2016) observed that the antioxidant enzyme activity of *Artrhospira platensis* was the highest at optimal growth conditions and overproduction of enzymes at high alkaline pH especially for SOD activity with 10.5 and growth at pH 9. In general, alkaline pH (> 9) was observed and the response of these factors were similar in *M. gracile* cultivation. Parameters such as pH, nutrients and oxygen are known to impact biochemical content of microalgae.

Irradiance seems to play a major role in pigment production and cell density since the highest mean cell density occurred at 120 µmol photons m-2 s^{-1} under 24 hours light-induced stress. The results showed that SOD, CAT and GSH-Px enzymes were effective antioxidants for *M. gracile* being even capable of minimizing the noxious effects of the light-induced stress.

In the last days of the algae growth, cell death begins to occur and, consequently, a higher production of ROS occurs and therefore higher oxidative stress. In a comparison between the light intensity regimes, the highest enzyme activity was observed in the cultivations with no light stress in all evaluations. New and safer antioxidants from natural sources are researched to prevent food oxidative deterioration and to minimize the oxidative damage in living cells.

Conclusion

Messastrum gracile had a great potential to produce antioxidant mainly GSH-Px, which presents remarkable antioxidant activity. For *M. gracile,* the production of antioxidant does not need light-induced stress due to the action of pigments on GSH-Px, CAT and SOD activities, mainly on the 7^{th} growth day, not being necessary a longer period to produce antioxidant enzymes. Moreover, the higher antioxidant enzymes were not associated with higher cell density. In terms of growth, high biomass production was reported with light-induced stress (120 µmol photons m^{-2} s^{-1}) where these enzymes had lower activities. When it comes to the antioxidant enzymes activity such as GSH-Px, CAT and SOD the intensity of the light can be 60 µmol photons m^{-2} s^{-1}. Thus, *M. gracile* microalgae may be an entry point in the obtention of natural antioxidants for employment in the market and research.

Acknowledgements

The authors would like to thank CAPES - *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* for the scholarship grant to the second author (17/11001-9) and CNPq - *Conselho Nacional de Desenvolvimento Científico e Tecnológico* for the third author (141586/2020-6).

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