INFLUENCE OF PRE-TREATMENT OF *Dunaliella salina* BIOMASS ON PIGMENT EXTRACTION

Izabela Q. Silva[1], Cláudia M. L. L. Texeira[2], Lucielen O. Santos [1]*

[1] *School of Chemistry and Food, Federal University of Rio Grande, Laboratory of Biotechnology, Rio Grande, Brazil.*
[2] *National Institute of technology microalgae biotechnology laboratory, Rio de Janeiro, Brazil.*
** Corresponding author's email address: santoslucielen@gmail.com*

ABSTRACT

The pigment production by microalgae has been of increasing interest in recent years. Dunaliella salina is known to synthesize a variety of pigments, including carotenoids and chlorophylls. However, pigments are produced intracellularly and, one of the challenges that affects the extraction yield of these pigments is the cell wall rupture stage. In this context, the aim of this study was to evaluate two pre-treating biomass methods, C1 (centrifugation, drying for 24 h and freezing for 24 h) and F1 (filtration through a glass membrane and freezing for 24 h). Biomass was obtained from cultivations was carried out in 500 mL Erlenmeyer flasks with 300 mL of saline medium. The extraction process was carried out with acetone and quantification was performed by a spectrophotometer. Filtering and freezing process was better for pigment extraction, reaching maximum concentrations of chlorophyll-a (10.97 mg L^{-1}), chlorophyll-b (2.84 mg L^{-1}) and carotenoids (6.65 mg L^{-1}).

Keywords: Bioprocess. Extraction. Biomass.

1 INTRODUCTION

Dunaliella salina, a unicellular halophilic microalga, has become known for sustainable and natural pigments production in industrial sector. Due to its versatility, it may synthesize a wide range, including carotenoids and chlorophyll, which makes it a valuable resource with broad applications [1].

Dunaliella salina cells are oval biflalgates and are characterized by the absence of a polysaccharide cell wall, allowing it to adaptability to environmental changes and promoting their development in a wide range of salinity levels. The less rigid cell wall makes it possible transcriptional adjustments and simplifies the extraction of substances of interest, such as pigments [2, 3].

The extraction efficiency of pigments produced by microalgae depends on cell wall rupture process [4,5]. Therefore, different biomass pre-treatment methods have been studied to effectively disrupt the cell wall and thus increase the extraction yield of these pigments. Thus, the aim of this study was to evaluate two different methods of pre-treatment *Dunaliella salina* biomass: C1 and F1.

2 MATERIAL AND METHODS

Microalgae used was *Dunaliella salina,* isolated in São Pedro da Aldeia - RJ and belonging to INT strain bank. Biomass was obtained from cultivation in 500 mL-erlenmeyer flasks with 300 mL of the saline medium described by Ben-amotz et al. (2) and incubated for 15 days at 25°C and irradiance of 93 $\mu mol_{photons}$ m^2 s^{-1}. Aliquots of 10 mL were collected at the end of cultivation to pigment extraction.

Two biomass pre-treatments were evaluated (FIGURE 1). In C1 dry biomass is obtained by subjecting the culture medium to centrifugation (4000 rpm for 10 min) followed by a 24-hour drying period at 35°C and frozen at -18°C for 24 h, as described by Costa et al. (7). In F1 wet biomass is obtained through process of glass membrane filtration (pore size 0.7 - 1.4 µm) and frozen for 24 h, as described by Strickland and Parsons (8).

Extraction process was carried out with 5 mL of acetone and stirring. The extract was centrifuged, and pigments were quantified by a spectrophotometer at different wavelengths (nm): 470, 644.8 and 661.6, according to Lichtenthaler (9) with Equations 1, 2 and 3 Responses were evaluated by the analysis test t student used to compare means ($p \leq 0.05$).

$$\text{Chlorophyll-a} = 11.24 \cdot A_{661.6} - 2.04 \cdot A_{644.8} \cdot c \quad (1)$$

$$\text{Chlorophyll-b} = 20.13 \cdot A_{644.8} - 4.19 \cdot A_{661.6} \cdot c \quad (2)$$

$$VCC = \frac{1000 \cdot A_{470} - [\text{Clorofila a}] - 63.12 \cdot [\text{Clorofila b}]}{214} \cdot \frac{v_{acetone}}{v_{filtered}} \quad (3)$$

Where: [Chlorophyll-a] is concentration of chlorophyll in the extract in μg g^{-1}, [Chlorophyll-b] is concentration of chlorophyll b in the extract in μg g^{-1}, [VCC] is volumetric carotenoid concentration in μg L^{-1}, $v_{acetone}$ is the volume of acetone used in the extraction (mL) and $V_{filtared}$ is the filtered volume of cell suspension (mL).

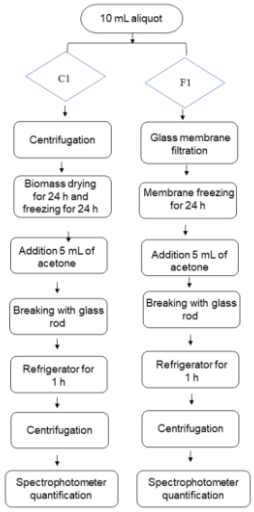


Figure 1 Pigment extraction process evaluating two biomass pre-treatments.

3 RESULTS & DISCUSSION

Membrane filtration and freezing (F1) emerged as the optimal pre-treatment method for preserving concentrations of chlorophyll-a and carotenoid, by comparison with method C1, which employes centrifugation, drying and freezing. These results are likely linked to the maintenance of the biomass in a wet stage throughout the F1 method. and according to Rammuni et al., (10) during the freezing process ice crystals were formed, which improved the process of cell rupture and consequently the release of pigments. On the other hand, using dry biomass in the extraction process did not favor the extraction of pigments.

Asevedo et al. (6) evaluated the influence of wet and dry biomass on carotenoid extraction from *Dunaliella salina* using *deep eutectic solvent* and 83 % recovery was achieved using wet biomass, while 66 % was achieved using dry biomass. According to D'Alessandro and Antoniosi Filho (11) pigments are sensitive to temperature, which may lead to changes in molecular conformation of pigments, affecting their ability to absorb light and transfer energy.

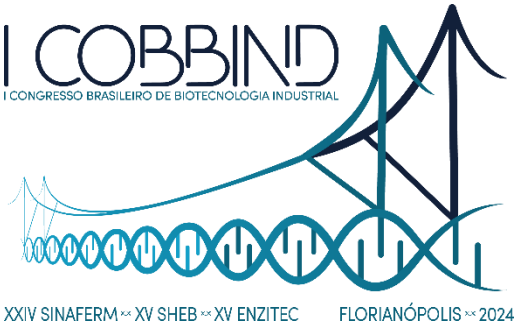
Pigments are also sensitive to the presence of oxygen. Prolonged exposure to oxygen may trigger oxidation processes resulting in changes in color, loss of bioavailability and impairment of their antioxidant and photoprotective functions [12].

Table 1 Pigment concentration of *Dunaliella salina* with different pre-treatments

Pigment (mg L^{-1})	C1	F1
Chlorophyll-a	$7.27 \pm 0,03^{b}$	10.97 ± 1.10^{a}
Chlorophyll-b	$2.18 \pm 0,13^{b}$	2.84 ± 0.24^{b}
Carotenoids	$3.25 \pm 0,28^{b}$	6.65 ± 0.84^{a}

*Means ± standard deviations (n=3). Different letters in the same line correspond to significant difference among the assays by the t test ($p<0,05$)

Therefore, given the relatively less rigid cell wall of this microalga species, the drying process may potentially weaken the cell structure, thereby increasing the surface area available for water evaporation, accelerating dehydration process and potentially increasing pigment loss. Also, the centrifugal force applied during centrifugation could be strong enough to cause physical damage to cell structure, including the cell wall, resulting in cell ruptures and subsequent loss of metabolites [13]. Thus, filtering and freezing biomass proved to be more promising approach for pigment extraction.



4 CONCLUSION

The most efficient pre-treatment for pigment extraction was to filter biomass and freeze it for 24 h, which resulted in a 33% higher concentration of chlorophyll-a and 48% higher concentration of carotenoids by comparison with drying and freezing treatment.

REFERENCES

[1] BARBOSA, M., INÁCIO, L. G., AFONSO, C., MARANHÃO, P. 2023. The microalga *Dunaliella* and its applications: a review. Appl. Psychol. 4 (1). 99–120.

[2] BEN-AMOTZ, A., SHAISH, A., & AVRON, M. 1991. The Biotechnology of Cultivating *Dunaliella* for Production of fl-Carotene Rich Algae. Bioresour. Technol. 38.

[3] HE, Q., LIN, Y., TAN, H., ZHOU, Y., WEN, Y., GAN, J., LI, R., & ZHANG, Q. (2020). Transcriptomic profiles of Dunaliella salina in response to hypersaline stress. BMC Genom. 21 (1). 1–17.

[4] GEBREGZIABHER, B. S., ZHANG, S., QI, J., AZAM, M., GHOSH, S., FENG, Y., HUAI, Y., LI, J., LI, B., & SUN, J. 2021. Simultaneous determination of carotenoids and chlorophylls by the hplc-uv-vis method in soybean seeds. Agron., 11 (4), 758.

[5] SOLOMONOVA, E. S., SHOMAN, N. Y., AKIMOV, A. I., & RYLKOVA, O. A. 2022. Ecotoxicological Aspects of the Influence of Ionic and Nano Copper on Structural and Functional Characteristics of *Dunaliella salina* (Teod.). Russ. J. Plant Physiol. 69 (5). 1–11.

[6] ASEVEDO, E. A., CHAGAS, B. M. E., OLIVEIRA, S. D., SANTOS, E. S. 2023. Recovery of lipids and carotenoids from *Dunaliella salina* microalgae using deep eutectic solvents. Algal Res. *69*.

[7] COSTA, S. S., PERES, B. P., MACHADO, B. R., COSTA, J. A. V., SANTOS, L. O. 2020. Increased lipid synthesis in the culture of *Chlorella homosphaera* with magnetic fields application. Bioresour. Technol, *315*.

[8] STRICKLAND, J. D. H., & PARSONS, T. R. 1972. A practical handbook of seawater analysis. Fisheries Research Board of CANADA, Bulletin.167.

[9] LICHTENTHALER, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Method enzymol. 148. 350–382.

[10] RAMMUNI, M. N., ARIYADASA, T. U., NIMARSHANA, P. H. V., & ATTALAGE, R. A. 2019. Comparative assessment on the extraction of carotenoids from microalgal sources: Astaxanthin from *H. pluvialis* and β-carotene from *Dunaliella. salina*. Food Chem. 277. 128–134.

[11] D'ALESSANDRO, E. B., & ANTONIOSI FILHO, N. R. (2016). Concepts and studies on lipid and pigments of microalgae: A review. Renew. Sustain. Energy Rev. 58. 832–841.

[12] XI, Y., KONG, F., & CHI, Z. 2021. ROS Induce β-Carotene Biosynthesis Caused by Changes of Photosynthesis Efficiency and Energy Metabolism in *Dunaliella salina* Under Stress Conditions. Front. bioeng. biotechnol. 8.

[13] PAGELS, F., PEREIRA, R. N., VICENTE, A. A., GUEDES, A. C. (2021). Extraction of pigments from microalgae and cyanobacteria-a review on current methodologies. Appl. Sci. 11 (11).

ACKNOWLEDGEMENTS

This study was funded in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance code 001. The authors would also like to thank FAPERGS and CNPq for their financial support.