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STRATEGIES FOR PIGMENT EXTRACTION FROM MICROALGAE: EVALUATION OF EXTRACTING SOLVENTS AND DIFFERENT MOISTURE LEVELS

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ABSTRACT

The present work aimed to evaluate the influence of biomass moisture, as well as the type of solvent used in the extraction of biopigments from the microalgae *Dunaliella salina*. Initially, the microalgae was cultivated for 7 days in 10L photobioreactors. After this period, the microalgal biomass was recovered and subjected to the spray dryer drying process under the following conditions: 130 ºC, pump flow rate of 0.25 L h^{-1}, and air flow rate of 1.4 m^3 min^{-1}. The dried biomass from the spray dryer had a moisture content of 2.53% and 5.88%, which were subjected to pigment extraction and compared with the microalgal biomass obtained from centrifugation with a moisture content of 72.99%. For pigment extraction, tests were conducted using 0.3 g of biomass with 20 mL of extracting solvent, ethanol and acetone, in order to determine the best solvent and biomass moisture for obtaining the highest quantification of biopigments. From the results obtained, it was found that ethanol was the best solvent for the extraction of chlorophyll a and b, while acetone was the best for the extraction of total carotenoids. Furthermore, the spray dryer dried biomass with the lowest moisture content (2.53%) provided the extraction of the highest amount of pigments.

Keywords: Microalgae. Spray Dryer. Pigments. Solvents. Moisture.

1 INTRODUCTION

Microalgae belong to an extremely diverse class of microorganisms, which exhibit a highly varied chemical composition. These microorganisms are of great industrial interest due to their biomass production rate, which stores various high-value bioproducts such as antioxidants, biopolymers, carbohydrates, lipids, pigments, polysaccharides, proteins, and vitamins [1]. Among all classes of bioproducts obtained from microalgae, pigments have stood out due to their relevance in various industrial sectors, such as their use in the cosmetic industry and as natural dyes in the food industry, due to their high stabilizing action [2]. Carotenoids constitute the largest class of natural pigments, serving as precursors to vitamin A and mainly used as colorants and antioxidant agents. Additionally, along with chlorophylls, they are considered high-value pigments. Similarly, chlorophylls also function as antioxidants, owing to their ability to interrupt the peroxidation chain reaction [3]. Furthermore, pigments have the ability to prevent degenerative diseases, making them competitive in the development of various costly products, such as medicines [4].

Some factors that can influence pigment extraction from microalgal biomass include the biomass drying method, moisture content, solvent nature and its proportion. Drying processes are important for maintaining the quality of bioproducts present in dehydrated biomass and for making the quantification of extracted pigments more efficient, as well as optimizing the process. Additionally, pigments are susceptible to oxidation due to their properties, and therefore, when the objective is to extract these high-value compounds, drying methods should ensure higher yields, stability during processing, and long-term storage [5]. Moreover, the extraction efficiency depends on the organic solvent's ability to solubilize the pigments without altering their structure [6].

Thus, the present work aimed to quantify the pigments extracted from *Dunaliella salina* microalgal biomass, as well as to study the influence of biomass moisture content and the use of different solvents in pigment extractions.

2 MATERIAL & METHODS

The present work used biomass from the marine microalgae *Dunaliella salina*, obtained through *Banco de Algas Marinhas do Instituto Oceanográfico da Universidade de São Paulo*. The topics below describe the methodologies applied to this study.

Cultivation of microalgae: All cultivation steps, including inoculum preparation, were conducted using Guillard f/2 culture medium. Cylindrical bubble column photobioreactors were employed in the microalgae cultivation, with a diameter of 15 cm, height of 1 m, and useful volume of 10 L. The photobioreactor was maintained at 25°C, using 10% inoculum and an air compressor with 75 W power and 100 L min^{-1} capacity (BOyu ACQ 007) connected by latex tubes to promote system aeration. Biomass harvesting was carried out by adding the flocculating agent $Al_2(SO_4)_3$, in a proportion of 2 mL L^{-1}.

Biomass drying: The biomass was dried using a spray dryer (LabMaq, model MSD 0.5), operating under the following conditions: 130°C, air pump flow rate of 1.4 m^3 min^{-1}, and fixed pump flow rate of 0.25 L h^{-1}. The optimal drying conditions were established in previous studies conducted by the research group. After drying was completed, the powdered samples were weighed on an analytical balance (Shimadzu, AUY220). To perform a comparative study of pigment extraction from biomass with different

moisture contents, the spray dryer dried biomass, with moisture contents of 2.53% and 5.88%, was compared with biomass recovered by other methods, with a moisture content of 72.99%.

Pigment extraction: Pigment extractions from microalgal biomass were carried out using 20 mL of extracting solvents (ethanol and acetone), employing 0.3 g biomass with different moisture contents of 2.53%, 5.88%, and 72.99%. For this purpose, a probe ultrasound (Model-UP200S, Hielscher-Ultrasound Technology), with a power of 200 W and a frequency of 24 kHz, was used. Finally, the samples were centrifuged for 5 minutes, and the supernatant was analyzed in a spectrophotometer (Lambda Bio-AppliedBiosystems, Foster City, CA, USA). Based on the absorbance values, the pigment quantification was performed [7].

3 RESULTS & DISCUSSION

The microalgal biomass, subjected to the spray dryer drying process, according to the previously described conditions, was obtained with a dry microalgal biomass yield of 58.44%. After drying, the biomass was subjected to the extraction process with two extracting solvents, acetone and ethanol, aiming to obtain the best solvent for complete pigment extraction. Figure 1 presents the results obtained.

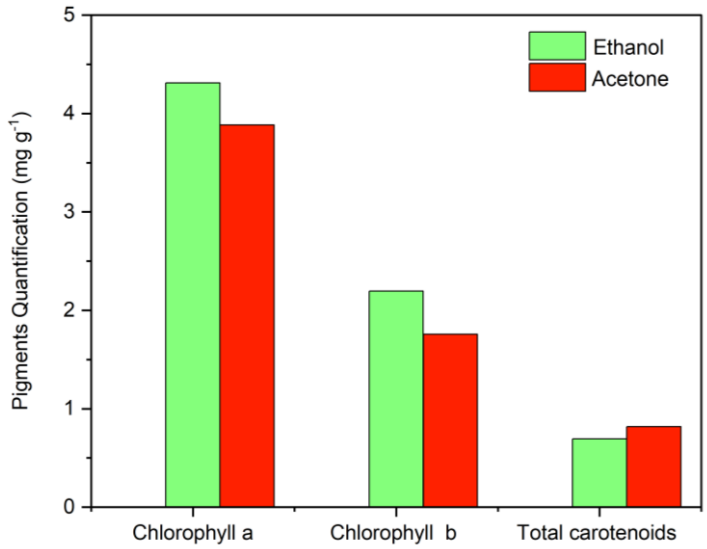

Figure 1: Influence of solvent on pigment extraction

As observed from the data in Figure 1, ethanol showed greater efficiency in recovering chlorophylls from the microalgal biomass, being 10.98% more efficient for chlorophyll a and 24.85% for chlorophyll b. Regarding carotenoid recovery, acetone proved to be the most efficient solvent, extracting 17.89% more than ethanol.

The difference in quantification observed previously occurred due to the chemical interactions between the pigments and their respective solvents. Since the bonds between chlorophyll molecules are very fragile, they break easily upon contact with organic solvents. Moreover, the hydrophilic/hydrophobic nature and polarity of the pigment directly influence the choice of the best solvent for its extraction. Ethanol and acetone have different polarities, with ethanol being more polar than acetone. Thus, among these solvents, ethanol is more effective in the complete extraction of chlorophylls [8], as evident from the results obtained in Figure 1.

On the other hand, carotenoids are predominantly composed of hydrocarbons and are soluble in lipids and organic solvents such as acetone and ethanol. They exhibit better solubility in less polar solvents; thus, acetone is more efficient in the extraction of total carotenoids due to its lower polarity compared to ethanol [9].

After the tests with different solvents, the biomass dried by spray dryer with moisture contents of 2.53% and 5.88% and the biomass obtained by centrifugation, with a moisture of 72.99%, were subjected to the pigments extraction process for comparative purposes, as shown in Figure 2. It is important to highlight that this test was done only with ethanol, since it allowed a more efficient extraction of two of the three pigments of interest in the work.

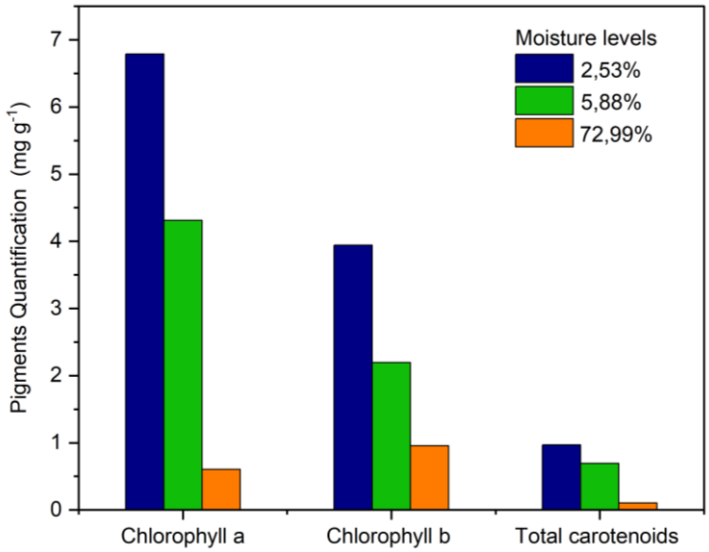

Figure 2: Influence of moisture on pigment extraction and quantification

The significant difference in pigment quantification using the different moisture contents, as seen in Figure 2, is due to the contact surface with the solvent. The drier the biomass, the larger the contact surface with the solvent, resulting in a more complete and efficient extraction. Moreover, the higher the moisture content of the biomass, the greater is the amount of water, and the solvent used, due to its polarity, tends to interact more with water than with the pigments intended to be extracted, reducing the extraction efficiency of these compounds.

Thus, from the analysis of Figure 2, it is noted that the lower the moisture content in the biomass (2.53%), the greater was the efficiency of pigment extraction, in the order of 6.79, 3.94, and 0.97 mg g^{-1} of chlorophyll a, chlorophyll b, and total carotenoids, respectively. Conversely, the biomass with a moisture content of 72.99% provided the lowest efficiency of pigment extraction, with 0.60, 0.96, and 0.10 mg g^{-1} of chlorophyll a, b, and total carotenoids, respectively.

4 CONCLUSION

Based on the experiments conducted and the results obtained, it can be concluded that the choice of solvent and the moisture content of the microalgal biomass significantly influence the extraction of pigments. It was observed that ethanol is the ideal extracting solvent for chlorophylls, while acetone is the ideal extracting solvent for total carotenoids, due to the different polarities of the solvents and the extracted pigments Additionally, it was also observed that microalgal biomass with lower moisture content exhibits greater efficiency in pigment extraction. From the biomass with the lowest tested moisture content (2,53%), it was possible to obtain 6.79 mg g^{-1} of chlorophyll a, 3.94 mg g^{-1} of chlorophyll b, and 0.97 mg g^{-1} of total carotenoids.

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