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Evaluation of Electro-Flocculation for Biomass Production of Marine Microalgae *Phaodactylum tricornutum*

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***Abstract*—**The commercial production of biodiesel using microalgae demands a high-energy input for harvesting biomass, making production economically unfeasible. Methods currently used involve mechanical, chemical, and biological procedures. In this work, a flocculation system is presented as a cost and energy effective process to increase biomass production of *Phaeodactylum tricornutum*. This diatom is the only species of the genus that present fast growth and lipid accumulation ability that are of great interest for biofuel production. The algae, selected from the Bank of Microalgae, Institute of Biology, Federal University of Bahia (Brazil), have been bred in tubular reactor with photoperiod of 12 h (clear/dark), providing luminance of about 35 μmol photons $m^{-2}s^{-1}$, and temperature of 22 °C. The medium used for growing cells was the Conway medium, with addition of silica. The seaweed growth curve was accompanied by cell count in Neubauer camera and by optical density in spectrophotometer, at 680 nm. The precipitation occurred at the end of the stationary phase of growth, 21 days after inoculation, using two methods: centrifugation at 5000 rpm for 5 min, and electro-flocculation at 19 EPD and 95 W. After precipitation, cells were frozen at -20 °C and, subsequently, lyophilized. Biomass obtained by electro-flocculation was approximately four times greater than the one achieved by centrifugation. The benefits of this method are that no addition of chemical flocculants is necessary and similar cultivation conditions can be used for the biodiesel production and pharmacological purposes. The results may contribute to improve biodiesel production costs using marine microalgae.

***Keywords*—**Biomass, diatom, flocculation, microalgae.

I. Introduction

THE emergent interest in the study of micro-organisms as microalgae, fungi and bacteria, is due to the essential importance of them as source of a wide variety of compounds used in different areas such as nutrition, human and animal health, wastewater treatment, energy production, and pharmaceutical industry [1]. In the production of biodiesel and ethanol, microalgae have a higher photosynthetic efficiency (4-7%) and increased productivity (390-700 bep), compared to sugarcane (2-3% and 210-250bep ha^{-1} $year^{-1}$), this being the only traditional culture with efficiency above 1% [2]. Microalgae present, therefore, great productive potential, compared to other plant sources.

Diatoms are a main group of microalgae with high biotechnological potential that has not been fully exploited. One of the reasons for that is the scarcity of available genetic tools. Very few works on genetic improvement of this species are related. The generation of an enhanced lipid producing strain (45-fold increase in triacylglycerol accumulation) through the disruption of the UDP-glucose pyrophosphorylase gene was achieved recently [3] and demonstrates the power of genome engineering to harness diatoms for biofuel production.

But, energy production is not the only application of this group. Marine eukaryotic microalgae are known to produce numerous useful molecules that have not been explored as it could for pharmacological purposes. One of the potentials, that have attracted little attention, is the search for novel antibiotic compounds. Cell lysates of *Phaeodactylum tricornutum*, have been reported to display antibacterial activity in vitro. Desbois et al. [4] used column chromatography and reversed-phase high-performance liquid chromatography, reporting the isolation of an antibacterial fatty acid from this species. Mass spectrometry and 1 H-nuclear magnetic resonance spectroscopy revealed it to be the polyunsaturated fatty acid, eicosapentaenoic acid (EPA). They showed that EPA is active against a range of both Gram-positive and Gram-negative bacteria, including MRSA, at micromolar concentrations. These results indicate promising application in the topical and systemic treatment of drug-resistant bacterial infections.

Regardless of the applications of the microalgae, the greater the amount of biomass achieved after cell growth, also greater is the extent of bio-products obtained. A major economic bottleneck for obtaining better results occurs during algal isolation. Different methods have been used for harvesting microalgae and producing algal biomass, and the most common are centrifugation, filtration, flotation, chemical and electro-flocculation. Despite some physical limitations, these methods are quite capable of separating the biomass from the surrounding media. The difficulty is the high cost, estimated to be about 25% of the total production [5]. Therefore, the success of microalgal biofuels depends on finding more efficient methods of biomass production, which varies among species due to cell size and morphology.

In the present study, biomass production of the marine microalgae *P. tricornutum* was evaluated using two methods of isolation: centrifugation and electro-flocculation. Algal

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growth was also analyzed in a tubular photo-bioreactor by cell count and absorbance, at regular intervals. This diatom is the only species of the genus that presents fast growth and lipid accumulation of great interest for biofuel production [6], and in addition to that, it produces different biomolecules of pharmacological interest [7], [4].

II. Methods

The microalgae *P. tricornutum* (Fig. 1) were grown in tubular reactor (Fig. 2) patent: Privilege of Innovation, *Tubular Vertical Photo-bioreactor to Produce Microalgae* [8], in triplicate, with photoperiod of 12 h (clear/dark), providing luminance of about 35 µmol photons m^{-2} s^{-1}, and temperature around 22 °C. This photo-bioreactor (Fig. 2) has total capacity of approximately 70 liters (7 for each cylinder). The species has been bred in Conway medium [9] added to silica (6.5 x 10^{-4} mM), with constant aeration and an increase of 2.5% of carbon gas during the light phase of the photoperiod, until the end of the stationary growth phase. To evaluate growth, two methods were used: daily cell count in Neubauer chamber and absorbance reading (680 nm) at the spectrophotometer (Shimatzu). Based on cell count and time of cultivation, it was estimated the growth rate during the logarithmic phase [10]. The calculation was made based on the equation: $\mu = \ln(Ny/Nx)/(ty - tx)$, where Ny and Nx are the numbers of cells per mL on the final day (ty) and starting day (tx) of the logarithmic growth phase, respectively. 21 days after seeding the cells, the microalgae reached the stationary phase, and then, followed algal isolation for obtaining the biomass. The total volume of medium (content of one cylinder or 7 liters) containing the microalgae was homogenized and divided in two aliquots: the first followed centrifugation at 5000rpm for 5 min (Eppendorf Centrifuge 5402), and the second, electro-flocculation at 19 EPD and 95 W for 15 min. After isolation, both pellets proceeded to lyophilization (Enterprize II Terroni) for 12 hours and the yield of biomass was compared between both isolation methods.

III. Results

The *P. tricornutum* is part of a collection from the Institute of Biology at Federal University of Bahia (UFBA), Iracema Nascimento Microalgae Bank (BMIN). The species was well adapted to the cultivation conditions described and presented regular growth and morphology (Fig. 1) when bred in a closed photo-bioreactor.

The growth rate (µ) obtained for this species, according to cell counts in Neubauer chamber, was 1.8±0.5 cells mL^{-1} day^{-1}, indicating that these algae can efficiently grow in a photo-bioreactor. The growth curve according to this evaluation method is shown in Fig. 3.

Cell growth was also evaluated by absorbance readings at 680 nm in a spectrophotometer and results are shown in Fig. 4. By both methods, the curve achieved a *plateau* at the 16th day, indicating the start of stationary phase, when accumulation of molecules with biotechnological interest starts [11], [12] for this species.

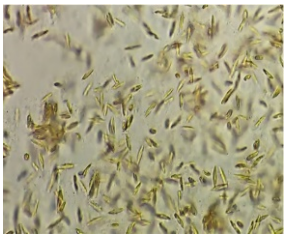

Fig. 1 *Phaeodactylum tricornutum* cultivation

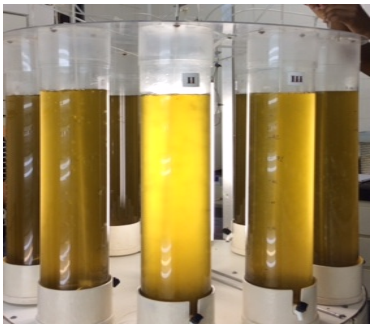

Fig. 2 Photo-bioreactor (patent: Privilege of Innovation, registration No. 0000221105500270)

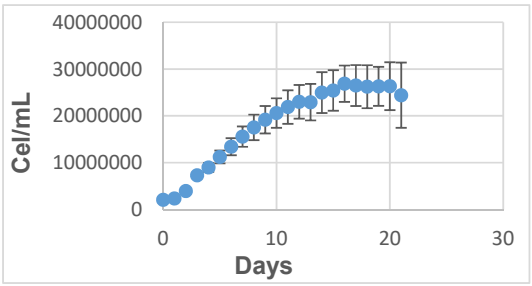

Fig. 3 Growth curve of *P. tricornutum* according to cell counts in Neubauer chamber

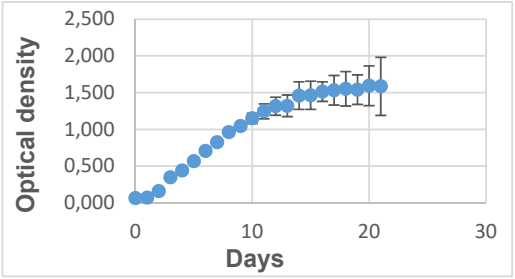

Fig. 4 Growth curve of *P. tricornutum* according to absorbance at 680 nm

Both methods used for analyzing microalgae growth (cell counts and absorbance at 680 nm) demonstrated to be appropriate for this species, and could be applied for calculating growth rates. After the end of the stationary phase (20th day after inoculation), the harvesting of biomass was performed.

Considering biomass separation, the electro-flocculation device (Fig. 5) constructed at the Laboratory of

Bioprospection and Biotechnology (LABBIOTEC) of Federal University of Bahia, was demonstrated to be very effective for *P. tricornutum*.

(a)

(b)

Fig. 5 Electro-flocculation device before (A) and after (B) isolation of microalgae

This is the first time that such isolation method and device were used for this diatom. Fig. 5 (a) shows the medium containing the microalgae after stationary growth phase, before electric discharge was applied. The cells are not separated, as can be seen by the dark brown color cultivation medium. After 15 min. of continuous electric discharge, the cells are completely isolated from the medium (Fig. 5 (b)), and biomass is totally separated.

Centrifugation method of algae isolation was also applied. This method is commonly used for obtaining biomass for different purposes. With the species used in this study, many hours and innumerous centrifugation cycles were necessary for isolating cells form the total volume of the cultivation medium. In large scale procedures, an industrial centrifuge would be necessary, with increased energy demand, which makes the method ineffective and expensive. Another negative aspect is the long time spent for isolation (3 hours compared to 15 min by electro-flocculation) resulting in a non-satisfactory amount of biomass yield. This step can be even longer depending on the culture volume and size of the microalgae, centrifuge rotors and tubes.

Between the two techniques compared for efficiency of harvesting, the electro-flocculation yielded significantly higher production of biomass (Fig. 6). Using centrifugation, it was obtained 1.14±0.24 g/L, whereas the electro-flocculation yielded 4.05±0.33 g/L.

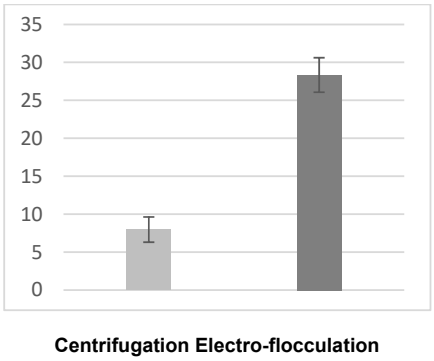

Fig. 6 Biomass production (g) chart by two algal isolation methods, for 7 liters of *P. tricornutum* cultivation medium

IV. Discussion

Among all algal isolation methods, electro-flocculation is a physical process that has the advantages of being simpler to operate and provides more predictable results. Contrasting with chemical flocculation, it does not introduce unnecessary anions in the growth medium, which can result in the lowering of pH [5].

It is well known that during different stages of growth, microalgae may suffer changes in the cell content or even on the morphology [11]. At stationary phase, cells tend to increase their reserves of lipids, proteins and carbohydrates, and reduce the rate of replication. This phase was achieved with P. tricornutum at the 16th day after algal inoculation, indicating the moment in which most of the molecules of interest start to be produced. The addition of carbon dioxide to the growth medium, previously shown [12] to increase microalgae biomass and metabolites production was also efficient for the studied species.

Even though the use electro-flocculation could be fairly effective, it is worth mentioning that the product from this treatment may be limited due to the presence of salts formed during the process. With the marine microalgae P. tricornutum tested in this study, no salt formation was observed, demonstrating that the selected species could be a good choice for obtaining high production of biomass, and consequently increased amounts of bioproducts.

There is still need to reduce the energy spent and water intake during the steps of cell growth and isolation for the commercialization of bioproducts obtained from microalgae. While electro-flocculation can improve biomass yield compared to other precipitation methods, additional procedures on this system can increase even more biomolecules production. An efficient electro-flocculation method for harvesting Dunaliella Salina integrated with local sand has been successfully applied [13]. The authors report that sand was advantageous for accelerating cell agglutination and sedimentation with this species. Besides that, the electrolytic hydroxides were necessary to link the sand with small flocs, forming larger and denser agglutinates. Biomass maximal recovery in this study improved approximately 3% (from 95.13 to 98.09%) during 4.5 min, and there was a

decrease in energy intake superior to 50%, compared to other electro-flocculation procedures. In addition to that, another advantage of this method is that the flocculated culture medium of *D. salina* could be recycled, and used in consecutive algal cultivation, which is a very cost saving aspect. It is not sure if this sand enhanced electro-flocculation (SEF) method would work with other species, but it certainly has a great potential for time and energy saving, added to a faster and more efficient harvest step.

Another innovative method of electro-flocculation was performed with Chlorella vulgaris using aluminium electrolysis to collect biomass. As flocculants were in-situ generated and gradually released, microalgae flocs formed in a snowballing mode, resulting in the compaction of large flocs [14]. When higher current density was applied, microalgae could be harvested more rapidly, although there was a trade-off between a higher energy use and more residual aluminium in the culture medium. There were two benefits of this flocculation method: the phosphate decrease in post-harvesting could improve nutrient removal in microalgae based wastewater treatment, while the ammonium increase may favor microalgae recovery for medium recycling. Also with Chlorella, an additional method was tested for algal isolation with an electro-coagulation-flotation (ECF) for biomass isolation by cell flotation and surface assemblage [15]. According to the author, the advantages of using an optimized ECF harvester over the traditional harvesting method involved the neutralization of microalgal surface charge by applying positively charged electrode plates to perform electro-coagulation-flotation of the microalgal biomass. The results obtained by this method serve as support for commercialization of a large-scale algal isolation device afterwards.

The use of electro-flocculation to a species with high potential for biomolecules production (including biodiesel) as *Phaeodactylum tricornutum* is of great importance since it may lead to economic advances in biomass harvest stage, one of the main factors for increasing production costs. In the present work, this method yielded approximately four times more biomass than the traditional method of centrifugation, proving its effectiveness.

Although this diatom may grow in the absence of silica, which is not possible for many microalgae, the addition of 6.5 x 10-4 mM of this micronutrient demonstrated to contribute for better biomass yield, when grown in the photo-bioreactor under tested conditions. While this strategy worked for improving harvest, sometimes micro or micronutrient stress are favorable for production of a few bioproducts. Chauton et al. [16] have shown that production of lipids and carbohydrates, abundant in diatoms, may increase in the restriction of the macronutrients nitrogen and phosphorus. According to the authors, nitrogen stress in *P. tricornutum* induces carbon integration to carbohydrates, which is good for fermentation and gas fabrication.

The results presented in this work greatly contribute for indicating an appropriate species and procedure for algal isolation that may contribute to pharmacological and energy purposes. Moreover, microalgae appear to be the only source of renewable [17] biodiesel that is capable of meeting the global demand for transport fuels.

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