ISOLATION AND CHARACTERIZATION OF MICROALGAE FROM SERGIPAN MANGROVES.

Tairan E. dos Santos[1,*] , Hannah M. S. Silveira[1], Fabiano R. F. Santos[1], Tárcio S. Santos[2], Marília R. Oliveira[3], Marcelo da C. Mendonça[1,2,3], Thiago R. Bjerk[1,3], Cláudio Dariva[1,3].

[1]Tiradentes University/Aracaju,Brazil.
[2] Sergipe Agricultural Development Company /Aracaju, Brazil.
[3]Institute of Technology and Research/Aracaju, Brazil.
** Endereço de e-mail do autor correspondente: tairaneutimio100@gmail.com*

ABSTRACT

Microalgae are single-celled photosynthetic organisms found in various aquatic environments, ranging from freshwater to hypersaline and wastewater. These microorganisms have a vital role in the ecosystem by converting CO_2 into O_2 through photosynthesis. Algal biomass contains valuable biocompounds with significant potential for utilization in biofuel production. This study aims to isolate diverse microalgae species obtained from Sergipe mangroves, assess their growth in a liquid medium with NPK, and analyze the compounds present in the algal biomass using Fourier-transform infrared spectroscopy (FTIR). The findings revealed the abundance of microorganisms, including microalgae, in the mangrove habitat, and the efficacy of the isolation techniques in obtaining uncontaminated strains. Moreover, the FTIR spectra displayed characteristic lipid bands in the microalgae biomass, suggesting that the isolated strains are suitable for biofuel production.

Keywords: Mangrove. Microalgae. Isolation. Biomass. Description.

1 INTRODUCTION

Microalgae are photosynthetic, microscopic, unicellular organisms found in various marine environments, including freshwater, brackish water (salt concentrations below 3.5%), saline waters (with 3.5% salt), hypersaline waters (greater than 3.5% salt), and wastewater[5]. These microorganisms play a crucial role in the ecosystem and environmental maintenance by capturing atmospheric CO_2 and converting it into O_2 through photosynthesis. While capable of thriving in diverse conditions, the control of water temperature, light intensity, pH levels, and nutrient availability is vital as these factors can influence algal growth. Bioreactors, which are closed systems, are indispensable for microalgae cultivation as they enable the precise control of cultivation parameters tailored to the needs of specific strains, thereby enhancing algal biomass production.

The high biomass yield of microalgae consists of a high concentration of lipids and carbohydrates, essential for bioenergy production. For this, the cultivation of this microorganism must occur under controlled conditions of pH, temperature, light, sugars, CO2, nitrogen, phosphate, and potassium, which can generate large quantities of lipids, proteins, and carbohydrates in a short period of time[3]. The organic compounds derived from microalgae biomass are used in different areas such as health, cosmetics, agriculture, and engineering, where they have great applicability in biofuel production. Currently, microalgae account for about 40% of global biomass for renewable energy production and a food source, also playing an important role in aquatic ecosystems by aiding in water purification.[4]

Given this, microalgae present fundamental characteristics and functionalities for the production of raw material that can be converted into biofuels, classifying this group as 3° generation energy. Therefore, the objective of this work is to isolate microalgae strains from Sergipe's mangroves and verify the possible compounds that make up the algal biomass to apply these compounds in biofuels.

2 MATERIAL & METHODS

2.1 Collection and Isolation of Microalgae

Soil samples from the mangrove were collected from five different points in the Treze de Julho neighborhood (Aracaju-SE), using sterile 50 mL plastic tubes. In the laboratory, the samples were mixed in a liquid medium with NPK fertilizer and placed in 500 mL Erlenmeyer flasks for the cultivation and growth of the species. For isolation, samples of microalgae cells were serially diluted (10^{-3}) in sterile NPK solution (3g/L) and inoculated on bacteriological agar plates with fertilizer under aseptic conditions. The plates were incubated in a B.O.D. incubator (25±2°C, 12h photoperiod, for 15 days). Subcultures on Petri dishes were repeated until pure cultures were obtained. Subsequently, the pure cultures of microalgae obtained were cultivated in a liquid medium containing NPK + Tetracycline antibiotic (1/100 mL), in a rotary orbital shaker (180 rpm) under constant white/cool illumination for 15 days. At the end of this procedure, the samples were observed under an optical microscope (40x) to confirm the purity of the samples.

2.2 Determination of Cell Density

Microalgae samples from growth in liquid medium were diluted in distilled water in 10 mL flasks and evaluated using a UV/VIS Spectrophotometer (SHIMADZU, UV-2600) at 682 nm. Dilutions were performed to obtain samples with absorbance values between 0.1 and 1.0. The cell concentration present in the diluted samples was also evaluated by direct counting using a Neubauer Chamber and an optical microscope at 40x magnification. The absorbance values and cell quantification of the samples were correlated to construct an analytical curve. Growth curves were prepared using the daily cell density from the average aliquots.

2.3 Fourier Transform Infrared Spectroscopy (FTIR)

The biomass of microalgae, produced in a liquid medium, was centrifuged (4000 rpm, 10 min) and dried by freeze-drying (-45°C, 140 µmHg) for 48 hours. The dried biomass was analyzed by FTIR (Agilent Cary 630) using transmission mode in the wave number range of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3 RESULTS & DISCUSSION

Samples collected adapted to the new culture medium (NPK), showing cellular development. Optical microscopy revealed different morphological structures of the cells, indicating the presence of more than one species of microalgae in the cultivation. However, only three strains of microalgae were isolated due to their ease of cellular development on solid medium (agar), as shown in Figure 1.

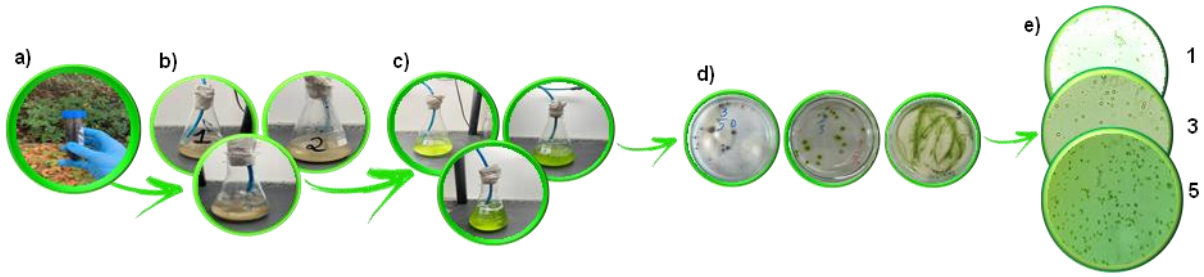

Figure 1: Scheme of microalgae cultivation: a) collection of mangrove soil; b) soil samples inoculated in NPK medium; c) microalgae species grown in NPK medium; d) purification process on solid medium; e) pure and isolated cultures, samples 1, 3, and 5.

Cell density analysis showed a similar profile for both isolated samples. The Lag phase was observed in the initial days of cultivation, ending by the fifth day. The phase of maximum cellular development occurred on the tenth day for both samples; however, the cell density followed the following order: samples 5 > 3 > 1. From the tenth day of cultivation, the samples entered the stationary and decline phases. Linear regression analysis of the samples resulted in the following equations: Sample 1: $y = 608949x - 2960.3$ ($R^2 = 0.99$); Sample 3: $y = 251785x + 50713$ ($R^2 = 0.99$); and Sample 5: $y = 354856x + 13789$ ($R^2 = 0.99$).

The FTIR spectra obtained in the analysis indicate the presence of functional groups such as hydroxyl (OH), carboxyl (COOH), amino (NH2), and other groups associated with organic compounds. Peaks in the region of 500-800 cm^{-1} suggest the presence of hydrocarbons from aliphatic groups. Absorption bands for carbohydrates and proteins in the region of 800-1800 cm^-1 indicate the presence of these biocompounds in the biomass. Additionally, distinct peaks for alkane groups at 2800-3100 cm^-1, aldehyde groups at 2695-2830 cm^{-1}, ester groups at 1700-1800 cm^{-1}, and carboxylic groups at 1330-1420 cm^{-1} were observed. For the identification and evaluation of lipid content by FTIR, two specific regions are considered: the methyl and methylene group vibrations at 2800-3600 cm^{-1} and the vibrational stretch of the ester bond at 1740 cm^{-1}. The high intensity peak in the region of 3200-3550 cm^{-1} indicates the presence of lipids in the biomass, specifically attributed to symmetric and asymmetric stretching vibrations of CH_2 groups. Spectra also showed vibrational bands in the range of 1500-1800 cm^{-1} and a stretching at 3500-4000 cm^{-1} belonging to the OH group, confirming the presence of fatty acids in the sample (Figure 2). The spectra observed in the analysis of the microalgae samples are similar to spectra reported in previous studies[1,2]. The difference in peak intensities is related to cell density; higher cell numbers in the culture medium lead to increased production of compounds such as lipids and proteins, thereby enhancing the intensity of peaks in certain regions. Consequently, the isolated samples exhibit the sequence 5 > 3 > 1 in terms of compound presence through FTIR analysis of algal biomass, which correlates with the sequence of cell density.

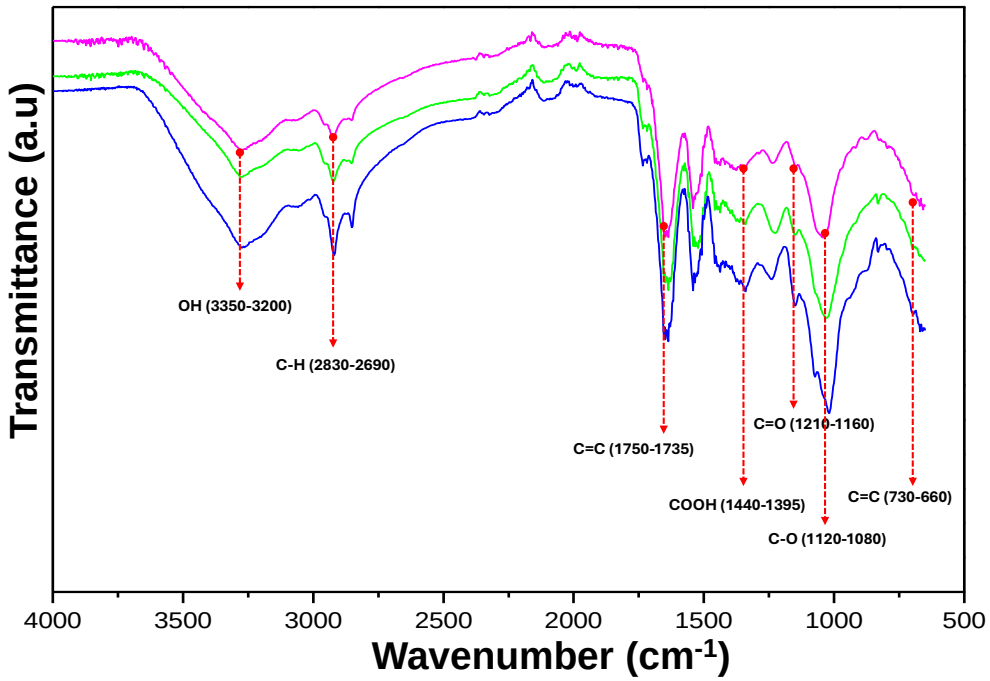

Figure 2: FTIR spectra of lyophilized biomass: (●) - sample 1; (●) - sample 3; (●) - sample 5.

4 CONCLUSION

The NPK culture medium proved effective for adapting strains from the mangrove soil of Sergipe. Among the various strains, three were isolated from contaminants through continuous plating, indicating the efficacy of this process in isolating different species of microalgae. FTIR spectra showed rapid screening of microalgae cells. Various peaks were observed indicating the presence of important functional groups associated with lipids, proteins, and carbohydrates, highlighting the high potential of these biomasses for biofuel production.

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