EVALUATION OF EXTRACTION EFFICIENCY IN *Mucor circinelloides* BIOMASS DIRECT TRANSESTERIFICATION

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ABSTRACT

The growing global demand for energy drives a search for new energy sources that can supply the market without causing environmental impacts and competing with the food industry. In this sense, biorefineries have been excelled as promising alternatives, using oils of microbial origin for biofuel production, however microbial biodiesel production is still an expensive process, which hinder its implementation in industrial sector. Thus, this work aimed to investigate *Mucor circinelloides* URM 4182 lipid extraction by ethanol microwave-assisted extraction and direct transesterification (simultaneous extraction/reaction). Analysis were performed by FTIR and LIBS after the procedures and the results indicated that both approaches are efficient in lipid recovery. Therefore, it can be concluded that direct transesterification process is an interesting alternative for microbial biodiesel production implementation in biorefineries by diminishing process steps and consequently the global costs.

Keywords: *Mucor circinelloides*. Biorefinery. Biodiesel. Lipids extraction.

1 INTRODUCTION

Global energy demand is expected to increase by 28% by 2040 according to the Energy Information Administration (EIA)[1]. This growth is alarming considering that renewable energy infrastructure is not yet ready to replace the growing volume of energy demand from hydrocarbons, on which the world has relied. Additionally, there is the geopolitical responsibility established in the Paris Climate Agreement in which participating countries committed to: limit global warming to less than 2°C in the next 15 years, peak CO_2 emissions between 2020 and 2030, achieve net-zero emissions by 2050, and work towards negative emissions thereafter[2].In this regard, the development of integrated processes from renewable materials that promote increased global energy security without competing with the food chain and without generating waste is crucial[3].

The implementation of biorefineries emerges as a promising alternative in the production of bioenergy and other high-value-added products from renewable materials and agro-industrial by-products[4].Vegetable oils, for example, are the most employed lipid source in biofuel generation. According to data from the National Petroleum Agency (ANP) of 2023, biodiesel production in Brazil relies primarily on vegetable oils, accounting for over 80% of production[5].This highlight one of the challenges encountered in biofuel manufacturing: competition with the food industry, reflecting a potential economic imbalance due to high demand. Furthermore, regarding the predominant use of vegetable oils in biofuel production, research indicates that these resources may not be sufficient to meet future global demand. Therefore, biodiesel production from microbial oils and biological waste emerges as a promising and innovative research field[6].

The lipid composition of oleaginous fungi and yeasts consists mainly of triglycerides, primarily from the C:16 and C:18 series of fatty acids, resembling the composition of vegetable oils, providing suitable properties for biofuel. Additionally, they present extremely attractive advantages, such as high growth rate and lipid productivity, with the capacity to accumulate between 20 and 70% of lipids in dry mass, making them more efficient in terms of biofuel production than oilseeds. Other advantages include the enhancement of new technological routes and the lower need for labor and climatic influence, which reduces development costs. Within the Zygomycetes class, fungi of the genus Mucor are strong candidates for converting a wide range of substrates into biomass with high oil content.

Concurrently, the circular bioeconomy is an interdisciplinary principle that integrates essential aspects of sustainability, circular economy, and bioeconomy. This contemporary economic development model aims to decouple economic growth from fossil fuel dependence by providing biomass-based raw materials and reusing by-products and waste as energy sources[7]. In this context, most agricultural residues are lignocellulosic and rich in carbohydrates, facilitating microbial growth. Microorganisms and microbial products can be used in biorecycling and the production of value-added products, such as biomolecules[8].

In situ or direct transesterification have been demonstrate as an efficient approach for integrated microbial biodiesel production merging extraction and reaction steps into one, collaborating for the industrial implementation feasibility reducing the global process costs[9].

Thus, the present study aimed to evaluate the lipid extraction efficiency in the direct transesterification comparing with separated step of microwave-assisted extraction of *Mucor circinelloides* URM 4182.

2 MATERIAL & METHODS

Cultivation was conducted in a BioFlo®/CelliGen® 115 Bioreactor 1 L model with a volume of 700 mL of medium conducted aerobically (1.5 vvm air and 250 rpm), at pH 4.5, 26 °C for 120 h. Culture medium was based in glucose (40 g L^{-1}) and minor growth factors. After cultivations, biomass was harvested by filtration and submitted to lipid extraction or direct transesterification.

Extractions were carried out in three cycles of 1 hour each in a microwave reactor (Discover/University-Wave Model, Cem Corporation) at 60 ºC with a maximum microwave power of 100 W. Extraction was performed using 0.5 g of fungal biomass (dry basis) suspended in 50 mL of 96% ethanol used as a solvent.

Direct transesterification for ethyl ester synthesis was carried out in a pressurized stainless-steel reactor (Parr Series 5500 Reactor) with magnetic stirring (300 rpm) at 200 °C for 6 h using alumina-supported heteropolyacid catalyst (HPMo/Al2O3).

Residual biomass after extraction and after direct transesterification were washed with distilled water and submitted to Laser Induced Breakdown Spectroscopy (LIBS) and Fourier Transform Infrared spectroscopy (FTIR) analysis.

LIBS was performed by a single pulse and the setup consisted of a laser (Brilliant, Quantel, USA) operating at the second harmonic at 532 nm, with maximum pulse energy of 180 mJ, duration of 4 ns and a frequency of 10 Hz. An ARYELLE 400-Butterfly spectrometer (LTB Laser technik, Germany) operating at 175–340 nm and 275–770 nm equipped with an ICCD camera was used. The acquisition delay time was 200 ns and the ICCD gate width was set at 10 µs. A total of 60 spectra per sample were acquired from different regions of the samples with the accumulation of 5 pulses from each laser. The CI - 247.85 nm emission line was identified according to the NIST database. FTIR (Spectrum GX FTIR spectrometer, Perkin Elmer, MA, USA) of the samples was obtained from the accumulation of a total of 32 scans at a range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}, and potassium bromide (KBr) was used as a matrix.

3 RESULTS & DISCUSSION

FTIR analysis is indicated in Figure 1. The FTIR spectra of the samples showed similar profiles, with the reduction or disappearance of bands in the region of 2855 to 3010 cm^{-1}, corresponding to C-H, C=O, and C-O-C vibrations in esters. The band at 1740 cm^{-1}, associated with C=O stretching in esters, was not observed after extraction and direct transesterification, indicating the efficiency in extracting mono-, di, and triacylglycerols. The absence of the band at 3010 cm^{-1}, related to =C-H in unsaturated fatty acids, confirms the complete extraction of these compounds in both cases. These results demonstrate the effectiveness of extraction during direct transesterification, presenting similar characteristics in FTIR analysis.

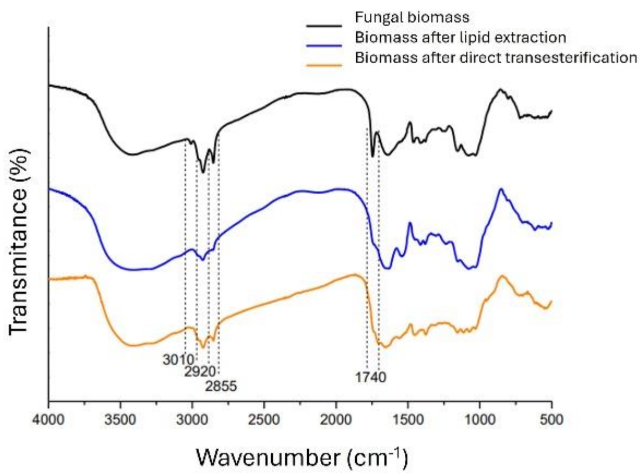

Figure 1 - FTIR analysis of unmodified M. circinelloides biomass, after microwave extraction, and after direct transesterification.

In Figure 2, we have the average LIBS spectrum (60 spectra) in the UV region showing the CI emission line at 247.85 nm. The intensity of the LIBS signal is generally proportional to the concentration of the element in the sample. The result demonstrated that the samples have different intensity in the analysis probably related to the presence of residual lipidic compounds. The analysis indicated that both extraction and direct transesterification were able to cause a different response, showing less intensity in the sample after direct transesterification.

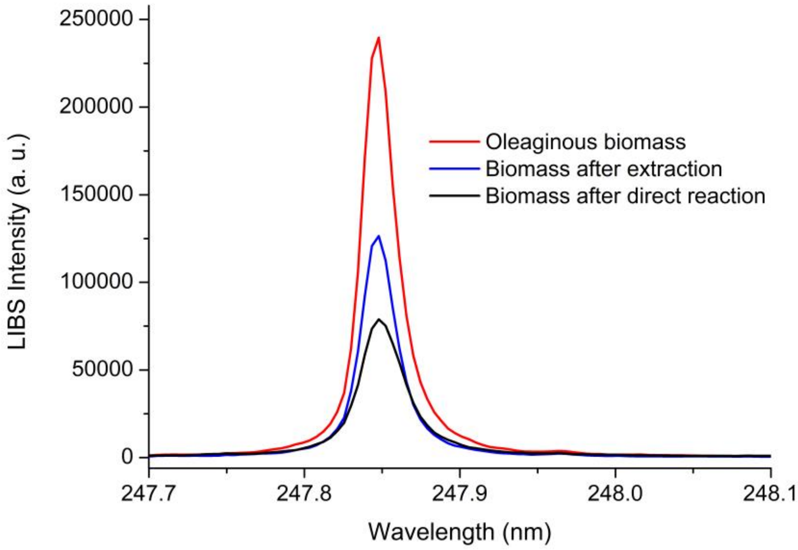

Figure 2 Averaged LIBS spectra in the UV range. The CI - 247.85 nm emission line correspond to the measurament on unmodified M. circinelloides biomass (red line), after microwave extraction (blue line), and after direct transesterification (black line).

4 CONCLUSION

The results obtained indicated that direct transesterification presents extraction efficiency similar to the separated extracted process, showing that most of lipids were recovered from fungal biomass and the integrated extraction/reaction process is an interesting alternative for microbial biodiesel production implementation in biorefineries.

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