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AN ECO-FRIENDLY APPROACH USING LIGNOCELLULOSIC WASTE MATERIAL FROM LICURI AS A CARRIER FOR *Burkholderia cepacia* LIPASE IN HYDROESTERIFICATION

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ABSTRACT

The purpose of this research work is to evaluate the utilization of *Burkholderia cepacia* lipase (BCL) immobilized in licuri lignocellulosic waste (WLO) for the biotransformation of licuri oil via hydroesterification into high-value products. Esterification was achieved through sequential hydrolysis and esterification reaction of purified FFAs with butanol in a solvent-free environment. The efficiency of immobilization was demonstrated (≈95%). Furthermore, about 26% of the oil was hydrolyzed under pre-established reaction conditions after 120 minutes. In the esterification of purified FFAs, two different molar ratios and enzyme loadings were tested, resulting in the highest ester conversion (≈88%). The biocatalyst (WLO-BCL) exhibited partial activity in aqueous media, indicating potential for reaction optimization. On the other hand, it demonstrated high activity in non-aqueous media.

Keywords: Licuri oil. Enzyme. Immobilization. Fatty acid. Butyl Esters.

1 INTRODUCTION

The transition or integration of fossil fuels with vegetable oil derivatives is emerging as a promising alternative in biomass-based integrated production processes. One notable alternative is licuri, a palm tree from the Caatinga biome with high socio-economic relevance in northeastern Brazil. Its endosperm has a significant oil content (49%) [1,2], with a high content of medium-chain saturated fatty acids, such as lauric acid (42%), giving it desirable fluid-dynamic properties for industries [3,4].
The production of industrial esters from vegetable oils through enzymatic hydroesterification is an efficient and sustainable route [5,6]. Although it has several advantages, such as lower energy consumption and the ability to convert low-quality raw materials, its large-scale adoption faces challenges due to the cost of enzymes and low productivity [7]. Applying agro-industrial lignocellulose residues to immobilize enzymes offers an economic and ecological alternative to conventional supports [8,9]. The abundance of this waste makes it possible to explore its economic viability compared to high-cost options available on the market [10,11].In this context, this work proposes the application of Burkholderia cepacia lipase immobilized in licuri lignocellulosic waste in the biotransformation of licuri oil by hydroesterification into high-value products.

2 MATERIAL & METHODS

Materials

Licuri oil was provided by Cooperativa do Piemonte da Diamantina – COOPES, Capim Grosso countryside, Bahia, Brazil (11°23'15.5" S. 40°00'28.6" W). Commercial lipase used as a biocatalyst was *Burkholderia cepacia lipase* (BCL, code 534641), was used as obtained by Sigma Co, St. Louis, MO, USA, without further purification steps. N-butanol was purchased from Vetec Química, Sigma-Aldrich Brazil. Molecular sieve type 3 Å (form ball and size (0.3 nm) was purchased from Sigma-Aldrich Brazil. All chemical reagents were of analytical grade.

Enzyme immobilization

The lignocellulosic residue obtained after extracting Licuri oil (WLO) was dried in an oven to remove n-hexane. Afterwards, the material was ground to pass through 32- and 60-mesh sieves before being used to prepare of the support. Briefly, 1 g of support was immersed in 10 mL of n-hexane and kept at room temperature under stirring of 200 rpm for 15 min. Then, 10 mL of enzyme solution was added and stirred within 3 h at room temperature; afterwords, the material was left for 24 h at 4 ◦C. The hexane-aqueous interface was used to promote BCL interfacial activation [7–9]. The enzyme solution with an initial protein loading of 20 $mg_{protein}/g_{support}$ was prepared in sodium phosphate buffer (50 mM, pH 7.0). After the lipase immobilization, the biocatalyst was washed thoroughly with n-hexane and recovered by filtration under vacuum. Finally, the immobilized biocatalyst was dried in a desiccator for 48 h and stored at 4 ◦C. The immobilized protein loading was determined by the Bradford method and calculated according to Barbosa et al., [2].

Enzymatic hydroesterification reaction of Licuri oil

The hydroesterification reactions were conducted by the specifications outlined in Patent BR 10 2022 001442 6. The hydrolysis of Licuri oil was carried out according to the methodology described by Rodrigues et al. [12], with modifications. WLO - LBC were used as biocatalysts (10 mg $_{protein/reaction}$) for a period of 24 h in a system free of buffers and commercial emulsifiers. Thus, the purified fatty acids were washed with water (1:2) and dried via filtration through anhydrous Na_2SO_4. Productivity was determined according to the equation described by SILVA et al., [13].

After that, the esterification activities and butyl ester separation were obtained by the method of Rodrigues et al. [14], with modifications. The reactions were conducted in a solvent-free system containing 6 g of the reaction mixture (fatty acids from Licuri oil and butyl alcohol) at a 1:1 and 1:5 acid/alcohol molar ratio, and 10% w/w of molecular sieve. Once the free fatty acids had been fully solubilized in the reaction mixture, 5% or 10% w/w of biocatalysts were added to the reaction and incubated in an orbital shaker at the fixed experimental conditions for 180 min. After establishing these conditions, the kinetic process was studied for 300 min. The acid conversion percentage and enzymatic productivity (µmol/h.mg protein) were determined in accordance with the methodologies proposed.

3 RESULTS & DISCUSSION

The residue obtained through the extraction of licuri oil (WLO) was used in immobilization by physical adsorption to support the BCL enzyme. The immobilized biocatalyst (WLO) was prepared with an initial loading of 20 mg of protein per gram of enzyme, reaching an immobilization yield of 95.82%, around 19.16 ± 0.08 mg/$g_{protein}$. The enzymatic hydrolysis of Licuri oil produced free fatty acids (FFA). The hydrolysis was conducted using WLO - BCL in stirred tank reactors with Licuri oil and distilled water. The choice of biocatalyst was based on previous studies aimed at producing FFA from licuri oil using different lipases, including BCL in its free form, achieving approximately 50% conversion in 90 min, without the addition of buffers [1]. In Figure 1A, the degree of hydrolysis was shown for 60, 90, and 120 min. The increase in time leads maximum values of 26.39% and 17151.68 µmol h^{-1} g^{-}, leading to an increase in conversion and productivity (Figure 1B), suggesting that WLO-BCL has potential and is effective in biotransforming vegetable oils with a high concentration of lauric acid.

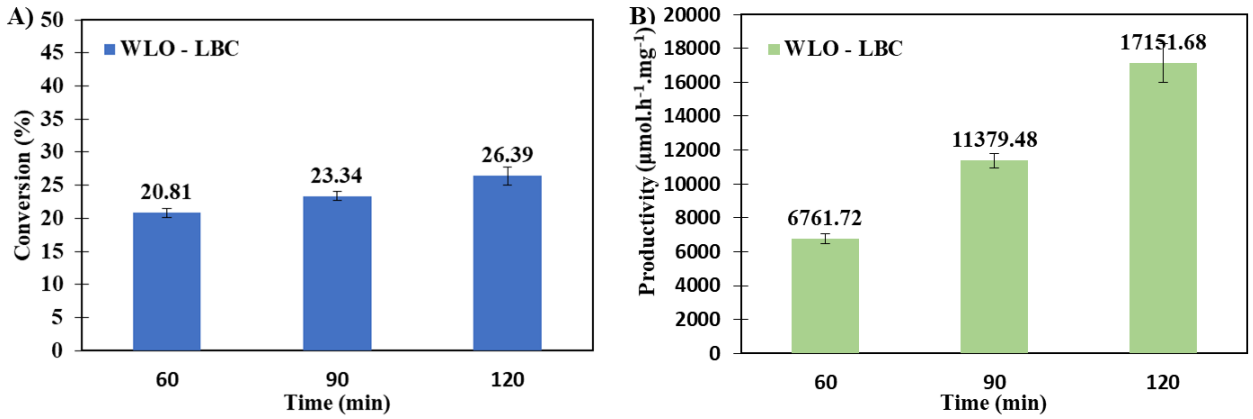

Figure 1 A) Effect of time on conversion (B) and productivity in the hydrolysis of licuri oil using WLO-LBC.

The conversion of the fatty acids from licuri oil following hydrolysis was obtained through an esterification reaction with butanol, utilizing the same WLO-BCL biocatalyst, with two conditions: equimolar and an excess of alcohol (1:5) and with 5% e 10% of enzyme loading in 180 min. After establishing these conditions, the process kinetic observed for 300 minutes. Figure 2A depicts the esterification process to produce butyl esters. The highest conversion (88.24 ± 0.63%) was obtained with a greater dilution (1:5) allowing enrichment of esterification regardless of the enzyme load. As illustrated in Figure 2B, the kinetics reached the maximum conversion rate in 120 minutes of reaction time, during which the maximum degree of hydrolysis was verified and the highest productivity was observed, totaling 583.48 ± 21.96 µmol h^{-1} g^{-1}. The data presented are more satisfactory when using the WLO-BCL than those reported in other studies in the literature, such as that by Rodrigues et al. (2024) [14], in which BCL in free form required 24 hours to produce approximately 89% of butyl esters.

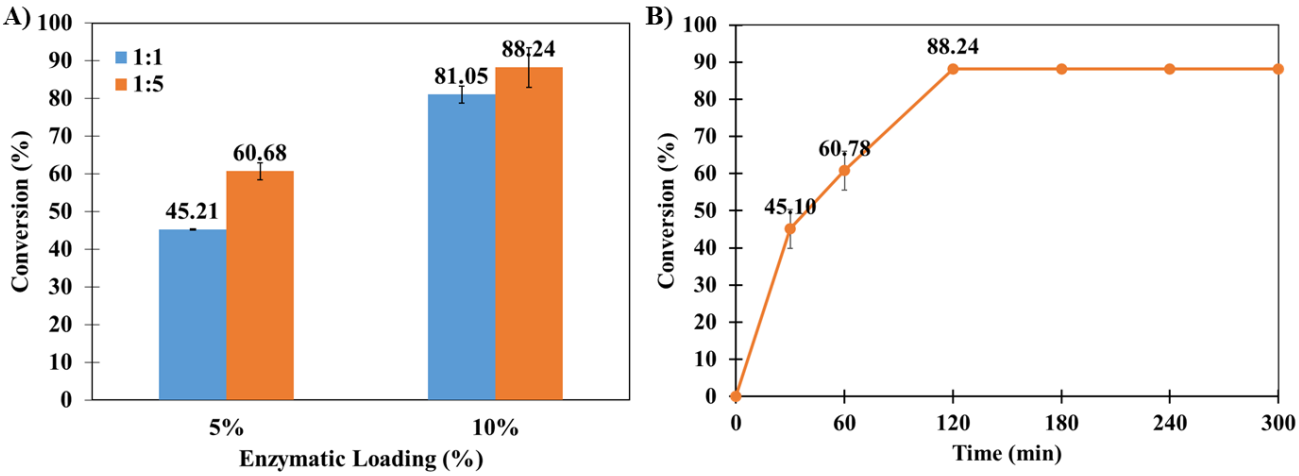

Figure 2 A) Enzymatic loading and molar ratio amount, B) effect of time on conversion.

4 CONCLUSION

In this work, a promising and eco-friendly approach was suggested for producing butyl esters via hydroesterification of licuri oil utilizing *Burkholderia cepacia* lipase (BCL) immobilized in licuri lignocellulosic waste (WLO). This biocatalyst, featuring low-cost support and high activity in non-aqueous solutions, exhibited partial activity in hydrolysis, suggesting potential for reaction optimization. This sequential process reached approximately 88% conversion into butyl esters (biolubricants) was achieved after 2 hours of reaction, demonstrating its attractiveness for obtaining industrially relevant compounds.

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