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Research paper

Synthesis of butyl esters via ultrasound-assisted transesterification of macaúba (*Acrocomia aculeata*) acid oil using a biomass-derived fermented solid as biocatalyst

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ABSTRACT

Enzymatic synthesis of fatty acid esters from macaúba (*Acrocomia aculeata*) acid oil was studied in a solvent-free system using a fermented and dry babassu (*Orbygnia oleifera*) cake with lipase activity from *Rhizomucor miehei*, named solid enzymatic preparation (SEP). The reactions were carried out under the influence of ultrasound irradiation and using different alcohols. Higher ester yields were obtained using butanol as acyl acceptor. An experimental design was used to evaluate the effects of SEP concentration (2–12 U g⁻¹ based on oil mass), butanol to oil molar ratio (3:1–12:1) and amount of water (0–10% based on oil mass) on the reaction yield. Fatty acid butyl ester (FABE) content around 80% was achieved after 70 h with a substrate molar ratio of 5.47:1, water content of 10% as oil mass and amount of biocatalyst of 12 U g⁻¹ as oil mass, at 40 °C. It was possible to obtain high yields using a low-cost biocatalyst, produced using an agricultural waste, and low-cost raw-material, making biocatalytic route competitive compared to chemical route.

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1. Introduction

The conventional alkaline route for biodiesel production has negative aspects such as complex downstream processes (in order to remove the alkaline catalyst from the product) [1,2] and requirements of rigorous feedstock specifications with low contents of water and free fatty acids (FFA), which contributes for the high biodiesel cost [3]. In this context, the use of alternative and cheaper raw materials, as well as catalysts, is a key point towards economic competitiveness of this biofuel.

Macaúba (*Acrocomia aculeata*) is a native palm from South American tropical forests and can be found in southern Mexico, Brazil, Paraguay, Bolivia and Argentina [4]. Its productivity reaches between 1500 and 5000 kg of oil per hectare per year and the pulp presents from 46 to 78 wt% oil, which in the current post-harvesting conditions, generally has a high acid value, making it inappropriate for use as food or feedstock in conventional biodiesel production process [5,6]. The exploration of this alternative non-edible oil crop for biodiesel production appears as a very promising alternative source [5–7]. However, there are few technical reports in the literature about the use of this oil as feedstock for esters production [4,6,8–10].

Moreover, lipase-catalyzed process has some benefits in relation to the alkaline route including high selectivity, possibility of using cheaper raw materials with high FFA contents and easy recovery of glycerol and biocatalysts [11]. In order to make the enzymatic biodiesel more cost-competitive, the cost of the biocatalyst can be reduced by direct use of solid enzymatic preparations (SEP), obtained by solid-state fermentation (SSF) [8,12–15].

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Table 1
Processes variables and their levels used in the CCD.

Variables	Name	Coded Levels				
		−1.68	−1	0	1	1.68
X ₁	Substrate molar ratio (butanol:oil)	3:1	4.8:1	7.5:1	10.2:1	12:1
X ₂	Amount of water (% based on oil mass)	0	2.02	5	7.98	10
X ₃	Amount of SEP (U g ^{−1} based on oil mass)	2	4.02	7	9.98	12

The use of ultrasound mixing in transesterification reactions for biodiesel production has been employed as a novel technology, which enhances mass transfer characteristics leading to an increase in the reactions rates [16–18]. Ultrasound is a sound wave with a frequency higher than that the human ear can perceive and it is considered a “green” technology because of its high efficiency, its economic performance and low instrumental requirements [19]. Generally, ultrasound is used for processes intensification because its chemical and physical effects [20,21]. The physical effects are through cavitation bubbles near the phase boundary between the alcohol and oil, which contributes to effective solution mixing, disruption of immiscible liquid layers and promotion of mass transfer at the liquid–solid interfaces [16,22,23]. Chemical effects are due to the generation of chemical of free radicals. In the case of biodiesel reaction, which is limited by mass transfer, physical effects are probable to be controlling [24]. Moreover, ultrasonic irradiation avoids the generation of water or glycerin phases inside the catalyst, since the dispersion occurs also inside the particle, which does not occur with conventional stirring. Specifically for enzymes, the ultrasound has shown increases on their stability and catalytic activity [4].

Although esterification and transesterification catalyzed by lipases under ultrasound mixing have been reported in the literature, the number of studies on this subject is still scarce. The majority of works has been conducted using commercial lipases as biocatalysts [17,18,23,25,26]. Yu et al. [17] used ultrasonic irradiation associated with vibration and obtained a fatty acid methyl ester (FAME) yield of 96% in 4 h using Novozym 435 in transesterification of refined soybean oil at 40 °C.

In a previous work, our group has reported the use of SEP with lipase activity from *Rhizomucor miehei* as biocatalyst in esterification of FFA from macaúba acid oil [8]. In the present study, we explored the ester production from macaúba acid oil by simultaneous enzymatic transesterification and esterification reactions, under the influence of ultrasound irradiation. The reactions were carried out in a solvent-free system using a low-cost raw material (macaúba acid oil) and biocatalyst (SEP). The effect of reaction conditions on the yield of fatty acid butyl esters was investigated and the reuse of the biocatalyst after the reactions was also attempted. To the best of our knowledge, this is the first report on ester production from macaúba pulp oil through enzymatic esterification/transesterification under ultrasound irradiation, using a SEP and acid oil as low-cost biocatalyst and raw material, respectively.

2. Materials and methods

2.1. Raw material and reagents

The acid oil from macaúba (*Acrocomia aculeata*) pulp was obtained from PETROVASF (Montes Claros, Brazil). The acidity of the macaúba oil was 9.5–10 wt%. The macaúba oil presented the following composition in fatty acids (wt%): 67.3% oleic acid (C18:1), 13.6% palmitic acid (C16:0), 12.1% linoleic acid (C18:2), 2.6% stearic acid (C18:0), 2.25% palmitoleic acid (C16:1), 1.55% linolenic acid (C18:3n3) and 0.6% lauric acid (C12:0). The average molar mass of macaúba oil, calculated from the oil composition, was 870.5 g mol^{−1}. The babassu (*Orbignya oleifera*) cake, a solid residue

from the babassu oil industry, was kindly provided by Tobasa S.A. (Tocantinópolis, Brazil). All other reagents were of analytical grade.

2.2. Biocatalyst production

The fermented solid was obtained by SSF of babassu cake, using the mesophilic strain of *Rhizomucor miehei* (IDAC accession number 071113-01). Fermentations were carried out according to Agueiras et al. [8], during 72 h. The fermented solids were lyophilized until moisture content of less than 3 wt%, stored at 4 °C until use and named “dry fermented solid” or “solid enzymatic preparation” (SEP).

2.3. Enzyme activity determination

Hydrolytic activity of the biocatalyst was measured using *p*-nitrophenyl laurate (pNPL) as substrate according to Gutarra et al. [27]. After fermentation and lyophilization, enzymes were extracted with phosphate buffer (0.1 mol L^{−1}, pH 7.0) as described by Gombert et al. [28]. The supernatant was used for hydrolytic activity determination. One unit (U) of hydrolytic activity was defined as the amount of enzyme that catalyzes the release 1 μmol of *p*-nitrophenol per minute under the assay conditions. The hydrolytic activity of the SEP used in this work was 43 U g^{−1}.

2.4. Ultrasound equipment setup

The equipment used in all experiments was an ultrasonic bath (Unique Inc., model USC 2800A, Brazil). The equipment presents the capacity volume of 9.5 L with the following dimensions: 300 × 240 × 150 mm (length × width × height). Two disc transducers were placed at the bottom of the reactor. The ultrasonic frequency was 40 kHz and the total ultrasonic power 220 W. Additionally, the equipment has temperature control.

2.5. Ultrasound-assisted transesterification

The transesterification reactions were carried out in 25 mL Erlenmeyer flasks, containing 1 g of oil and appropriated amounts of alcohol, water and SEP content according to the experimental design. All reactions were carried out in the ultrasonic bath described in the Section 2.4 at 45 °C.

After reaction completion, 5 mL of distilled water were added, followed by centrifugation (2500g, 5 min, 4 °C). The upper phase, containing esters, was analyzed by gas chromatography (Shimadzu, model GC-17A) equipped with a flame ionization detector (FID) and DB1 capillary column (30 m × 0.25 mm id × 0.25 mm; J&W Scientific). The injector temperature was 300 °C, split ratio = 1:30 and the FID detector temperature was 310 °C. The carrier gas used was nitrogen at a flow of 1.0 mL min^{−1}. The chromatographic conditions were: initial column temperature of 50 °C, heating rate of 10 °C min^{−1} reaching a final temperature of 310 °C. The amount of sample injected was 1 μL, and total time of the analysis was 30 min.

Methyl heptadecanoate, which was used as an internal standard, was mixed with heptane to prepare a stock solution. The sample was accurately weighted, and added to the internal standard stock solution. A standard FAEE (Fatty Acid Ethyl Esters) mix (C4–C24)

Table 2
Effect of different alcohols in SEP-catalyzed transesterification of macaúba pulp oil under ultrasound system.

Alcohol	Ester yield (%)
Methanol	4.70 ± 0.51
Ethanol	9.90 ± 0.86
Propanol	11.33 ± 0.72
Butanol	34.59 ± 1.97

The results are the average of three experiments ± standard deviation.

from Supelco was used to identify the peaks at different retention times and to correct the peak area using the response factors of the compound. The ester content was calculated using the compensated normalization method with internal standardization, based on the European standard DIN EN 14103 [29].

2.6. Effect of different alcohols

In order to evaluate the effect of different alcohols on the reaction, methanol, ethanol, isopropanol and *n*-butanol were tested. Apart from ethanol, which was 96% (v/v), all other alcohols were of analytical grade. Macaúba oil, alcohol (molar ratio 6:1 alcohol:oil), and SEP (12 U g⁻¹, by oil mass) were loaded into the reaction vessel, and placed in the ultrasonic system at 45 °C for 24 h.

2.7. Experimental design

A central composite design (CCD) was employed to evaluate the effects of three variables in the transesterification reaction. The variables and their coded and uncoded values are presented in Table 1, whereas in Table 3 are shown the 17 treatments obtained for the three variables, each at five levels. The design was constructed of eight factorial points, six axial points (two axial points on the axis of design variable), and three replications at the central point. In each case, the ester yield was determined after 24 h of reaction. The second-order polynomial equation for the variables is as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \quad (1)$$

Where *Y* is the response variable, β_0 the constant, β_i , β_{ij} , β_{ii} were the coefficients for the linear, quadratic, and for the interaction effects, respectively, and X_i and X_j the coded level of variables x_i and x_j . The above quadratic equation was used to plot surfaces for all variables.

2.8. Statistical analysis

The experimental design and analysis of results were carried out using Statistica 12.0 (Statsoft, USA). The significance of the regression coefficients and the associated probabilities, $p(t)$, was determined by Student's *t*-test; the second order model equation significance was determined using the Fisher's *F*-test. Statistical analysis of the model was performed as analysis of variance (ANOVA). The variance explained by model was given by the multiple determination coefficients, R^2 . For each variable, the quadratic models were represented as contour plots (2D).

2.9. Reuse of the SEP

In order to verify the operational stability of SEP, repeated batches were performed under optimal condition. After each batch, SEP was separated from the reaction medium by vacuum filtration using a sintered glass funnel. The biocatalyst was washed 3-times with 5 vols of hexane or isopropanol, and solvents were eliminated

Table 3
Experimental design and results of the CCD.

Treatment	X ₁	X ₂	X ₃	FABE (%)
1	-1	-1	-1	37.13
2	-1	-1	1	45.34
3	-1	1	-1	20.47
4	-1	1	1	48.35
5	1	-1	-1	22.46
6	1	-1	1	28.70
7	1	1	-1	5.09
8	1	1	1	27.10
9	-1.68	0	0	54.93
10	1.68	0	0	19.21
11	0	-1.68	0	20.57
12	0	1.68	0	27.76
13	0	0	-1.68	8.09
14	0	0	1.68	38.78
15	0	0	0	30.49
16	0	0	0	28.62
17	0	0	0	27.05

Table 4
Statistical analysis of CCD.

Variable	Effect	Standard error	p-value
Mean	28.64 ^a	0.99	0.0011
Linear			
X ₁	-18.75 ^a	0.93	0.0024
X ₂	-3.01	0.93	0.0837
X ₃	16.98 ^a	0.93	0.0029
Quadratic			
X ₁ X ₁	6.42 ^a	1.02	0.0245
X ₂ X ₂	-2.71	1.02	0.1178
X ₃ X ₃	-3.23	1.02	0.0875
Interactions			
X ₁ X ₂	-1.32	1.21	0.3885
X ₂ X ₃	-1.96	1.21	0.2481
X ₂ X ₃	8.85 ^a	1.21	0.0183

^a Statistically significant at 95% confidence level.

by incubation at 40 °C for 4 h. A control experiment was carried out without solvent washing.

3. Results and discussion

3.1. Selection of the best alcohol for transesterification

In ultrasonic-assisted reactions, the reactor geometry is an important factor, because the placement of reactants or the glass reactor in the bath can affect the distribution in the cavitation activity [30,31]. In the present work, the cavitation distribution was not assessed. However, the results were the mean of three independent assays randomly distributed in the bath to avoid possible local effects of cavitation activity. The influence of the type of alcohol in transesterification reactions was evaluated using methanol, ethanol (96% v/v), isopropanol and *n*-butanol. Apart from ethanol, all alcohols were of analytical grade. As can be seen in Table 2, the ester yields were higher with the increase of carbon chain of the alcohol. An ester yield of 34.6% was attained using butanol as acceptor. These results were expected since short chain alcohol can interact with the water molecules necessary for the maintenance of the native structure of the lipase causing its inactivation [32–34]. Additionally, using the commercial preparation from *R. miehei* (Lipozyme RM-IM), Rodrigues et al. [35] also observed that increasing alcohol chain the ester yield increased for soybean, sunflower and rice oils. Alcohols with more than three carbons are more soluble in the vegetable oil and, therefore less harmful to the enzyme [33,34]. In view of these results, butanol was chosen to

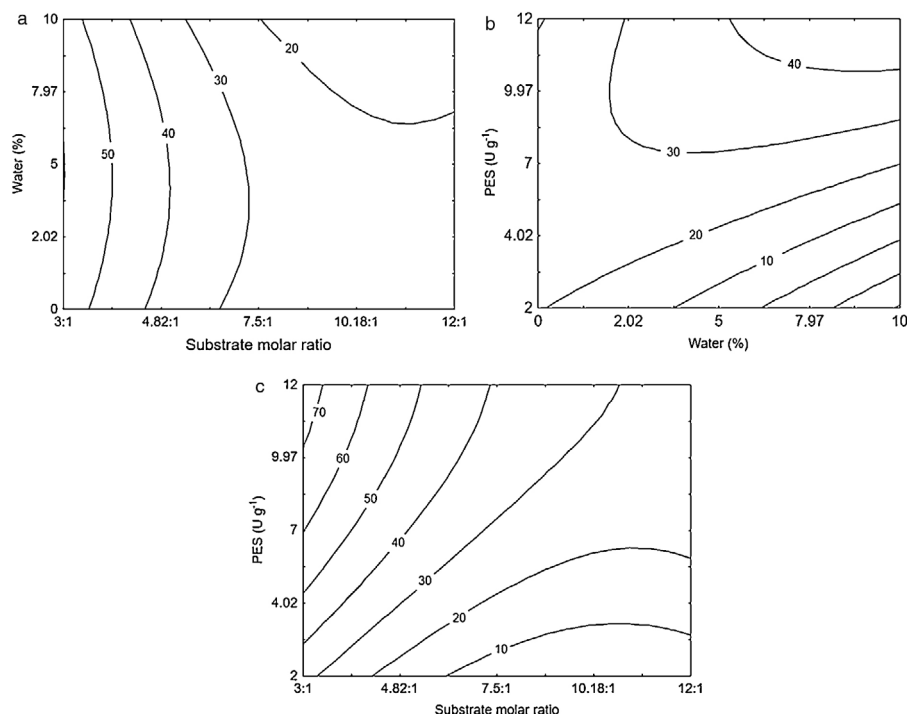


Fig. 1. Contour plots of transesterification reaction. (A) amount of water versus substrate molar ratio; (B) SEP content versus amount of water; (C) SEP content versus substrate molar ratio. The numbers inside the contour plots indicate the ester yields (%) under the tested reaction conditions. The missing variable was fixed at the central point.

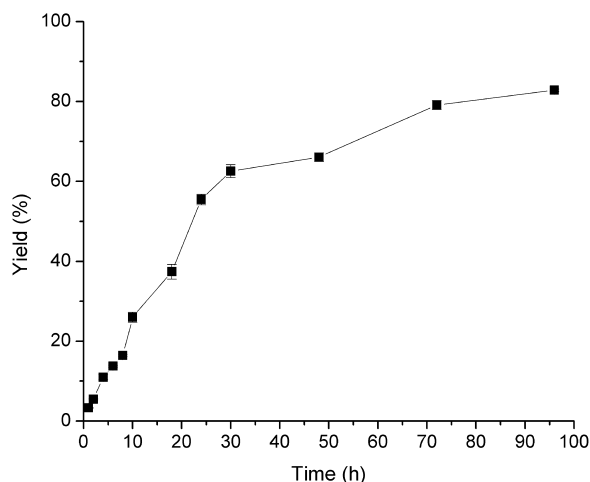


Fig. 2. Time course of SEP-catalyzed transesterification of macaúba pulp oil. Reaction conditions: substrate molar ratio 5.47:1 butanol:oil; water content of 10% as oil mass and amount of SEP of 12 U g⁻¹ as oil mass.

better investigate the effects of some parameters in ultrasound-assisted transesterification of macaúba oil catalyzed by SEP.

3.2. Optimization of transesterification reaction

3.2.1. Model fitting and ANOVA

Experimental data for synthesis of esters from macaúba pulp oil and butanol are given in Table 3. Among the various treatments, the highest ester yield (54.93%) was obtained in treatment 9 (3:1 butanol:oil, 5% of added water, SEP content of 7 U g⁻¹). The lower ester yield (5.09%) was obtained in experiment 7 at substrate molar ratio of 10.2:1, added water of 7.9% and SEP content of 4.02 U g⁻¹.

The experimental data were adjusted to the proposed model described in Eq. (1) and the second-order polynomial model to transesterification reaction is presented in Eq. (2).

$$Y = 28.64 - 9.37X_1 + 3.21X_1^2 + 8.49X_3 + 4.42X_2X_3 \quad (2)$$

Where Y is the ester yield for transesterification reaction, and X_1 , X_2 , and X_3 are the coded values of substrate molar ratio, water content and SEP content, respectively.

Fisher's statistical test for analysis of variance (ANOVA) showed computed F -values of 10.47 ($p = 0.0071$), statistically significant. The determination coefficient ($R^2 = 0.94$) and the correlation coefficient ($R = 0.87$) suggest a satisfactory representation of the process model and good correlation between the experimental results and the theoretical values predicted by the model equation.

3.2.2. Effects of parameters on the transesterification rates

Table 4 presents the linear, quadratic, and the interaction effects of the variables substrate molar ratio (X_1), initial water content (X_2), and SEP content (X_3) on the ester yield of the enzymatic transesterification. Linear effects are the most important, and represent the average change in the response changing each variable from level -1 to 1 . If the effect is positive, the change represents an increase in the response, and the contrary when the effect is negative. Substrate molar ratio showed the greatest linear negative effect, which indicates that an increase in the level of this variable affects negatively the yield. The stoichiometric relation is 3:1 alcohol:oil, however, an excess in the alcohol content is usually required to improve the system homogeneity and especially to displace the reaction equilibrium to product formation avoiding reversible reaction [1,36]. Initial water content showed linear and quadratic positive effects. This parameter plays an important role in lipase-catalyzed reactions and must be optimized for different types of biocatalyst. Lipase activity generally depends on the interfacial area, so these enzymes need a minimum water content to maintain its active conformation and catalyze the reactions [11]. Some works have reported that the addition of water in the reaction

medium increases the available interfacial area, and hence, helps to maintain lipase activity [36–38]. However, an excess of water might lead to a rise in oil hydrolysis in transesterification process. The optimum water content keeps the hydrolysis at the minimum level and ensures the active conformation of the biocatalyst [37].

SEP content showed a positive effect, indicating that highest ester yields were obtained at highest levels of this variable. However, this increase was directly influenced by other variables as can be seen in Table 4. Batistella et al. [23] also observed that enzyme concentration exerted a significant positive effect while soybean oil to ethanol molar ratio had a negative influence on FAEE yield of reactions catalyzed by Novozym 435. It is recognized that an increase in enzyme concentration leads to an increase in reaction yield, especially at shorter times (i.e., initial reaction rates). However, in the present study, this increase was observed at higher reaction times (24 h). This can be explained due to the lower activity of synthesis for the non-commercial preparation, as compared to commercial immobilized preparations, which presents a higher superficial specific activity. For commercial immobilized lipases, the equilibrium is reached in shorter times.

The relationship between reaction variables and response can be better understood by examining the contour plots generated from the predicted models, presented in Fig. 1.

3.2.3. Optimal conditions for ester synthesis and model validation

The optimal conditions for ester synthesis were determined by the response desirability profile calculated using the software Statistica 12.0. The optimal values of each variable were obtained for the desired response that, in this work, was the maximal ester yield after 24 h of reaction. The results were: substrate molar ratio of 5.47:1; water content of 10% as oil mass and 12 U g^{-1} of SEP, relative to oil mass. Under these conditions, the theoretical value for the ester yield predicted by the model was 64.3%. Experimental validation of the proposed model was carried out under optimized conditions with three repetitions and the average ester yield was $55.5 \pm 1.32\%$, showing a satisfactory correlation between experimental results and the statistical predicted by the model.

3.3. Time course of enzymatic reactions

The time course for SEP-catalyzed transesterification of macaúba pulp oil is presented in Fig. 2. A FASE yield of 79.12% was attained after 72 h under optimum conditions. Reaction time achieved in this work is too long and not recommend for scale-up process. The operating parameters such as power dissipation, frequency of irradiation and type of the reactor can affect the processing rates. Optimization of all these parameters is necessary step for the development of large-scale efficient sonochemical reactors for biodiesel synthesis [24].

Literature has reported ultrasound-assisted reactions as a mean to reduce reaction times, by enhancement in mass transfer process and increase in reaction rates. Jahdavi and Gogate [39] verified that for ultrasound-assisted enzymatic conversion of non-edible oil for methyl esters, the reaction time was significantly reduced from 20 h for conventional stirring to about 7.5 h in the presence of ultrasound. Gharat and Rhatod [25] studied the production of biodiesel from waste cooking oil using dimethyl carbonate as acyl acceptor through ultrasound-assisted transesterification catalyzed by Novozym 435. Ultrasound irradiation enhanced the enzyme-catalyzed conversion from 38.7% to 57.68% compared to stirring, using 10 wt% of lipase, after 4 h. A conversion of 86.6% was achieved under ultrasound irradiation coupled with stirring. Michelin et al. [4] used macaúba oil as raw material to produce FAEE under the influence of ultrasound irradiation. Yield of 70% was obtained after 30 min. However, it is important to note that these authors worked with a high concentration of an immobilized commercial biocatalyst

(20 wt% of Novozym 435), which is very expensive (\$2510/Kg) [40], contrary to our biocatalyst produced by SSF. The concentration of SEP used was relatively high (4.6–27 wt%). However, our biocatalyst was produced using an agro-industrial waste. A preliminary economic analysis performed by our group using the Super Pro Designer V. 8.5 software showed that the production prices of the fermented and dry babassu cake was \$567.54 per ton. Furthermore, it was also reported that the unitary cost for lipase preparation are three-times higher for submerged fermentation than for SSF [41]. Moreover, an advantage of ultrasound-assisted processes is the reduction of the energy required for the process, since ultrasound requires one-third to half the energy consumed by mechanical agitation [42]. Thus, with these preliminary results it is expected that the costs of our process could be reduced with the use of the biocatalysts prepared in laboratory compared to the use of commercial lipases.

Although a high ester yield has been obtained at longer reaction times, it is essential to note that the effect of ultrasound on lipases varies according to the type of biocatalyst. It is important to remark that preliminary tests showed that ultrasound improved the reaction rate. Compared to mechanical stirring, the ester yield under ultrasound was always higher in 24 h for the 4 alcohols tested (4.4 and 4.7% for methanol, 9.9 and 13.8% for ethanol, 9.9 and 11.3% for propanol and 30.6 and 34.6% for butanol, respectively for mechanical stirring and ultrasound), representing an increase of 7, 40, 14 and 14%, respectively for methanol, ethanol, propanol and butanol. As mentioned before, most of the studies have investigated the effects of such system using commercial immobilized biocatalysts, particularly Novozym 435, and just a few studies about the effect of the ultrasound under the enzyme activities are related in the literature. The true potential of ultrasound is not well known, due to the lack of knowledge of the molecular effects of the ultrasound on enzymes and living cells. In fact, contradictory results of activation/deactivation have been presented [4]. The effects of the ultrasound in our non-commercial biocatalyst, which consists of the lipase adsorbed onto the solid support used for SSF, have never been studied. In this way, a better comprehension of the effect of the ultrasound on the biocatalyst properties is necessary to develop more efficient systems with high productivity. Ultrasound reactor configuration, operating parameters, should be well-studied for reaction time reduction, increase in the reaction yield, use of less forcing conditions (temperature and pressure) and increase the effectiveness of the catalyst used in the reaction [20,21].

3.4. Repeated batches of transesterification for butyl esters synthesis

In order to check the viability of reuses of the biocatalyst, seven batches were performed under the optimal conditions defined in the statistical optimization. At the end of each batch the biocatalyst was washed with hexane or isopropanol. SEP reuse without washing between the cycles was also investigated. The results of the repeated batches are presented in Fig. 3, with their relative ester yield considering the first batch as 100%. It can be seen that the relative ester yield obtained without previous washing was 40% in the second batch.

Some studies have also reported a drastic reduction on enzyme activity without the previous washing of the immobilized lipase before its reuse [35,43]. It can be explained due to a deposition of the glycerol by-product over the pores of catalyst, which blocks the access of substrates to active sites of lipase [44]. This problem can be avoided with previous washing of the lipase before its use in another batch, using polar solvents such as ethanol and acetone. Hydrophobic solvents, such as hexane can be also used to wash the biocatalyst, since they can remove non-polar components (sub-

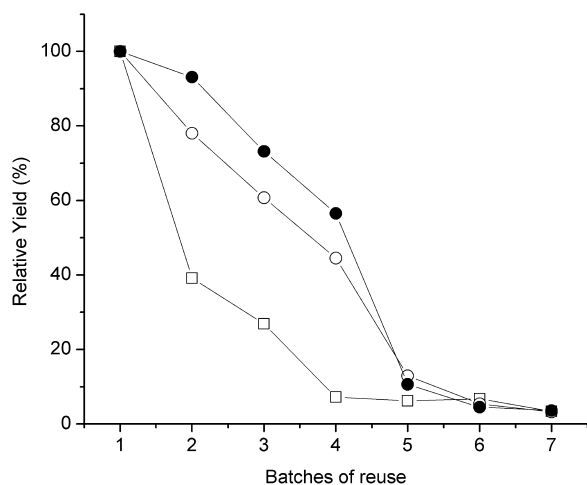


Fig. 3. Biocatalyst stability over repeated batches of reuse. Without treatment (□); hexane wash (○) and isopropanol wash (●). All reactions were carried out at the optimal conditions.

strates and products) that may be adsorbed on the enzyme, which preserves enzyme activity [35,45].

In the present work, two solvents were used to wash lipase before its reuse: isopropanol ($\log P = 0.26$) that is a hydrophilic solvent and hexane ($\log P = 3.5$). These solvents were chosen due to previous results obtained in the literature using these solvents for lipase washing [35,45,46]. It was possible to reuse the biocatalyst during four cycles, for the two solvents with relative ester yield above 60 and 40% for hexane and isopropyl alcohol after the fourth batch, respectively.

Similar results were obtained by our and other groups, where biocatalyst washing with hydrophobic solvents caused higher retention of lipase activity than that obtained when washing with the polar solvents and without washing control [4,35,45]. Michelin et al. [4] used macaúba oil as raw material to produce FAEE under the influence of ultrasound irradiation and using Novozym 435 as biocatalyst. The enzyme was washed with hexane before each batch and could be reused five times. Hexane was also used for wash of the immobilized lipases Novozym 435 and Lipozyme RM IM in ethanolysis of soybean oil [23]. Novozym 435 could be reused during seven batches, while for Lipozyme RM IM it was observed a drastic reduction on both ester yield and lipase activity, after the third cycle. However, it is important to note that these authors used high concentration of commercial immobilized lipases (20–27 wt%), which can limit the economic viability of the process. The results of the present work stand out from the literature since uses a low-cost biocatalyst (SEP).

4. Conclusions

This work demonstrated for the first time the possibility of using a biomass-derived fermented solid (SEP) in ultrasound-assisted system to convert macaúba acid oil into alkyl esters for biofuel purposes. The reaction parameters were optimized and a butyl ester yield above 60% was attained after 24 h using this alternative low-cost and non-edible oil. The assessment of biocatalyst stability over repeated batches of reuse was also carried. This low-cost biocatalyst kept around 55% of its original activity after four reaction cycles, after washing with hexane. Nevertheless, further studies regarding the behavior of several types of biocatalysts in ultrasound systems are necessary in order to better understand the effect of the ultrasound on the enzyme properties and to develop more efficient systems. Experiments comprising detailed investigations of

transesterification reaction kinetics are underway by our working group.

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