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LIPASE-CATALYZED ETHANOLYSIS OF *Jatropha curcas* L. OIL ASSISTED BY ULTRASONICATION

L. A. Lerin[1], D. Remonatto[2], T. M. M. Pereira[2], M. C. Zenevicz[2], A. Valério[2], J. V. Oliveira[2] and D. de Oliveira[2*]

[1]Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Ferrara, CAP: 44121, Ferrara, Italy.
[2]Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, 88040-900, Brazil.
Phone: +55 48 3721 2515; Fax: +55 48 3721 9687
E-mail: debora.oliveira@ufsc.br

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Abstract – Transesterification of non-edible oils using immobilized lipase is a promising process for biodiesel production. Thus, this study aimed to evaluate the enzymatic transesterification of the non-edible *Jatropha curcas* oil for FAEE production under ultrasound irradiation in a solvent-free system. The effects of enzyme concentration, water concentration, molar ratio of ethanol to oil and ultrasound power on the FAEE conversion have been evaluated. The results show that enzyme concentration and irradiation power have a positive significant effect on FAEE conversion, where an increase in these variables leads to higher conversions. Conversion above 54% of FAEE was achieved with 1.5 hours of reaction time using ultrasound irradiation, reducing reaction time by at least 3 times, when compared with the same experimental conditions without ultrasound irradiation. Results showed that ultrasound can improve reaction conversion mainly by enhancing the mass transfer between the constituents of the reactions.

Keywords: Lipase; *Jatropha curcas*; transesterification; ethanol; ultrasonication.

INTRODUCTION

In order to increase the commercial competitiveness of biodiesel, to ensure the availability of oil and to improve the use of the regional resources, it is expected that alternative vegetable non-edible oil crops will increase their participation as feedstock for biodiesel production (Bergmann *et al*., 2013; Hama and Kondo, 2013). Therefore, it is necessary to find new feedstock sources suitable for biodiesel production, which would not drain the edible vegetable oil supply and that would be capable of growing in marginal lands with minimum agricultural inputs (Araújo *et al*., 2014).

Jatropha curcas L. (1753), belonging to the *Euphorbiaceae* family, is seen as one of the most appropriate renewable alternative sources for biodiesel production in terms of availability and cost (Makkar *et al*., 2009; Rashid *et al*., 2010). The *Jatropha* seed is toxic, an advantage as its use for biodiesel production does not compete with the food chain market. *Jatropha* is native to Mexico and Central America and distributed in Latin America, Africa, India and South East Asia (Pandey *et al*., 2012; Gubitz *et al*., 1999). The plant is well adapted to arid and semi-arid climatic conditions and can grow in different land types, including marginal, degraded and contaminated lands (Openshaw, 2000; Foidl *et al.*, 1996).

Some potential attributes of biodiesel production from *Jatropha* include energy security, revitalizing marginal and degraded lands and alleviating rural poverty through employment and sustainable biofuel production (Abhilash

*To whom correspondence should be addressed

et al., 2013; Ariza-Montobbio *et al*., 2010; Kant and Wu, 2011; Edrisia *et al*., 2015). Furthermore, the use of ethanol instead of methanol is interesting due to the possibility of producing a fully renewable biodiesel, producing fatty acid ethyl ester (FAEE). According to Shah and Gupta (2007), for a sustainable technology, ethanol for biodiesel preparation would be derived from fermentation of sugars, which in turn would be obtained from plant starches/celluloses.

Lipase-catalyzed transesterification of feedstock oil has been considered as one of the most promising techniques for biodiesel production, a mixture of fatty acid alkyl esters (Zhao *et al*., 2015). The catalytic ability of lipase has shown high purity of products and less wastewater generation and mild reaction conditions are required (Guldhe *et al*., 2015). Different lipases have been used in biodiesel production, among which immobilized lipase shows great potential for industrial application due to high stability with regard to temperature and chemicals. This enables easy handling, recovery and recycling of the biocatalyst, and hence lowering the costs (Ranjbakhsh *et al*, 2012; Yang *et al*., 2013; Kalantari *et al*., 2013).

Processes of production of biodiesel face various problems related to lower rates of synthesis typically attributed to mass transfer limitations due to the heterogeneous nature of the reaction system, and the requirement of high molar ratio (alcohol to oil) as transesterification itself is a reversible reaction. Both of these facts result in high operating cost and energy consumption and hence low production efficiency for the biodiesel production (Maddikeri *et al*., 2012). Thus, there is a need to develop sustainable process intensification technology for biodiesel processing from non-edible oil sources with an objective to improve the mixing, mass and heat transfer between two liquid phases in the transesterification reaction and, consequently, reduce the cost of the process. Among alternative technologies, the use of microwave and ultrasound as energy sources have presented potential results (Gole and Gogate, 2012a; Jadhav and Gogate, 2014). Recent developments in sonochemistry (Veljkovic *et al*., 2012; Deshmane *et al*., 2008) attracted great attention with the use of an ultrasound bath as a new, efficient mixing tool, based on emulsification of immiscible liquids (reactants) by microturbulence, which is generated by the implosion of cavitation bubbles (Andrade-Tacca *et al*., 2014; Gebicka and Gekicki, 1997). Hence, to overcome the drawbacks of the conventional stirring method, an ultrasound bath is considered to be green methodology due to its high efficiency and mild reaction conditions (Waghmare *et al*., 2015). Transesterification reactions depend on the basic parameters such as molar ratio, catalyst concentration, and reaction temperature. In addition, the parameters based on the sonochemical reactors such as power dissipation, frequency of irradiation and type of the reactor also affect the reaction rates (Gole and Gogate, 2012b).

Many interesting works using ultrasound to enhance biodiesel conversion with reaction catalyzed by enzymes are described in the literature (Waghmare *et al*., 2015; Lerin *et al*., 2014; Awadallak *et al*., 2013; Batistella *et al*., 2012; Fiametti *et al*., 2012; Kumar *et al*., 2011). However, we have not found reports that describe the ethanolysis of *Jatropha curcas* oil under ultrasound irradiation. Given this scenario, the present work attempts to contribute to build a platform for fatty acid ethyl ester (FAEE) production exploring non-edible raw materials. Based on these aspects, the aim of this work was to report the experimental data on lipase-catalyzed transesterification of *Jatropha curcas* oil with ethanol to produce FAEE in an ultrasound bath using commercial immobilized lipase (Novozym 435). The following process conditions were evaluated: effects of temperature, enzyme concentration, water concentration, oil to ethanol molar ratio and ultrasound irradiation power on the reaction yield in a solvent-free system.

MATERIALS AND METHODS

Materials

The *Jatropha curcas* L. seed oil used in this work, kindly donated by Biotins Energia S.A. (Brazil), extracted by cold mechanical pressing, used as received. Ethanol, n-heptane (Merck, 99.9% purity) and n-hexane (Nuclear, 99.5% purity) were used without further treatment. Commercial immobilized lipase Novozym 435 from *Candida antarctica* (immobilized on a macroporous anionic resin, 1.4 wt% water) was kindly provided by Novozymes Latin America Ltda. (Araucária, Brazil) and presented an enzyme activity of around 37.91 U/g.

Oil characterization

The *Jatropha curcas* seed oil was characterized in terms of composition of fatty acids, water content and acidity. The water content (0.33%) was determined by Karl Fischer titration, according to method AOCS Ca 2e-84, using a DL 50, Mettler-Toledo titrator. According to AOCS Cd 3d-63 method the acid value determined was 12.2 mg KOH/g. For the determination of fatty acid composition (Table 1), oil samples were previously derivatized to the corresponding methyl esters according to method AOCS Ce 2-66. Derivatized samples were analyzed using a GC Shimadzu 14B equipped with FID and a capillary column SGE BPX70 (25 m×0.32 mm×0.25 μm). The temperature program was set to heat from 160 °C to 230 °C at a rate of 4 °C/min and then holding for 10 min and nitrogen was used as the carrier gas at 50 kPa.

Table 1. Fatty acid composition of the *Jatropha curcas* seed oil.

Fatty acid	Content (wt%)
Palmitic acid (16:0)	13.73
Stearic acid (18:0)	5.79
Oleic acid (18:1)	42.37
Linoleic acid (18:2)	37.52
Linolenic acid (18:3)	0.59

Experimental procedure

Enzymatic ultrasound-assisted transesterification reactions were carried out using an ultrasonic water bath (Unique Inc., model USC-1800 A - temperature accuracy of ± 0.5 °C, São Paulo, Brazil) in which a round bottom flask of 50 mL capacity closed with a lid was placed. The ultrasonic bath was equipped with a transducer having longitudinal vibrations, operating frequency of 37 kHz and maximum rated electrical power output of 132 W. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath, horizontally along the length of the bath. To determine the best reaction conditions, a Plackett-Burman experimental design with 12 assays, with triplicate runs at the central point was used. To increase FAEE production in a solvent-free system the experimental design was studied employing as variables: oil-to-ethanol molar ratio (1:3 to 1:10), irradiation power (40 to 100%), temperature (40 to 70 °C), enzyme concentration (5 to 20 wt% by weight of substrates), and water concentration (0 to 10 wt% by weight of substrates); for all assays the reaction time was kept constant at 2 h. The software Statistica® 8.0 (Statsoft Inc., USA) was used to assist the design and the statistical analysis of experimental information, adopting a confidence level of 95% ($p < 0.05$).

Kinetic study of lipase-catalyzed transesterification of *Jatropha curcas* oil with ethanol

Based on the results obtained previously in the experimental designs, kinetic reactions were performed with different enzyme concentration of 10, 20, 30, and 40 wt% (by weight of substrates). For the kinetic study of lipase-catalyzed transesterification of *Jatropha curcas* oil with ethanol the variables oil to ethanol molar ratio (1:10), temperature (70 °C), irradiation power (100% - 132 W) were kept constant with no water addition. Samples were taken from the bulk reaction system at 15, 30 min, and 1, 1.5, 2, 3 and 4 h. It may be important to emphasize that, in all cases, destructive experiments, without sampling, were carried out.

In order to assess the real effect of ultrasound on the production of FAEE, tests without ultrasound and without enzyme were also carried out in the best experimental condition determined previously in this work (oil to ethanol molar ratio of 1:10, 70 °C, irradiation power of 100% (132 W), enzyme concentration of 30% and 1.5 h).

Reuse of biocatalyst

To evaluate the Novozym 435 enzyme reuse in the lipase-catalyzed transesterification of *Jatropha curcas* oil with ethanol the experimental condition was fixed as: 70 °C, irradiation power of 100% (132 W), without addition of water, oil to ethanol molar ratio of 1:10, enzyme concentration of 30 wt% (by weight of substrates), and 2 h of reaction time. At the end of each reaction time, the enzyme was recovered by filtration using filter paper, followed by hexane washings under moderate vacuum. Then, the product of the reaction was dried in an oven (JP 101, J. Prolab) at 40 °C for 2 h and then kept in a desiccator for 24 h. An aliquot of 0.2 g of enzyme was used for esterification activity determination. The remaining enzyme used in the previous batch was reused in subsequent cycles, until 6 cycles. The enzymatic activity was measured before and after each cycle and the residual activity was determined following the methodology described below. The reaction conversion in FAEE of each cycle was also evaluated.

Lipase esterification activity

The esterification activity of Novozym 435 was determined as the initial rate of the esterification reaction between lauric acid and n-propanol as described by Oliveira *et al.* (2006). All enzymatic activity determinations were performed in triplicate. The residual esterification activity was defined according to Eq. (1).

$$Residual\ activity\ (\%) = \left(\frac{Final\ activity}{Initial\ activity}\right) x100 \quad (1)$$

Product quantification

In the first step, samples were submitted to ethanol evaporation to constant weight in a vacuum oven (65 °C, 0.05 MPa - Quimis, model Q819V2) and then diluted with 2 mL of ethanol and 8 mL of heptane. After this, 50 µL of solution was transferred to a 1 mL volumetric flask, adding 50 µL of internal standard methyl heptadecanoate at a concentration of 5000 mg L^{-1} and filled with heptane.

Thereafter, 1 µL of solution was injected in triplicate on a gas chromatograph (Shimadzu GC-2010), equipped with FID, auto-injector AOC-20i and a capillary column RTX - Wax (30 m x 0.25 mm x 0.25 µm). Column temperature was programmed from 120 °C, holding 2 min, heating to 180 °C at 15 °C/min, holding 3 min, and to 250 °C at 5 °C/min, holding 2 min. Helium was used as carrier gas, and the injection and detector temperatures were 250 °C with a split ratio of 1:50. Compounds were quantified upon analysis following the standard UNE-EN 14103 (Standard UNE-EN 14103, 2003) and FAEE content was then calculated based on the content of ethyl esters in the analyzed sample (Eq. (2)).

$$FAEE\ yield\ (wt\%) = \left(\frac{\sum_A - A_{IS}}{A_{IS}} x \frac{C_{IS}}{C_{sample}}\right) x100 \quad (2)$$

where: FAEE yield (wt%) is fatty acid ethyl esters, $\sum_A$ is the sum of areas corresponding to ester peaks (C14:0-C24:0) and the internal standard (C17:0), A_{IS} is area of the internal standard (C17:0), C_{IS} is concentration of internal standard in the injected sample ($mg.L^{-1}$), C_{sample} is concentration of injected sample (mg/L).

RESULTS AND DISCUSSION

Effect of process variables on FAEE conversion in the solvent-free system

According to Aguieiras *et al.* (2014) a solvent-free reaction medium shows several advantages such as an increase of biodiesel productivity and reduction of environmental issues (toxicity of the organic solvent) and processing costs (recovery and losses). The results of Plackett-Burman experimental design (coded and real values) for FAEE production in solvent-free system are shown in Table 2. It can be observed from this table that the highest reaction conversion was verified in the assays 3 (42%) and 6 (47%), corresponding to the highest ethanol to oil molar ratio (10:1), irradiation power of 100%, enzyme concentration 20 wt%, and no water addition (0%).

Table 2. Matrix of the *Plackett-Burman* experimental design (coded and real values) for FAEE production in solvent-free system under sonochemical irradiation after 2 hours of reaction.

Assay	Temperature (°C)	MR[a]	[E] (wt%)[b]	[H_2O] (wt%)[c]	IA (%)[d]	FAEE yield (wt%)
1	1 (70)	-1 (1:3)	1 (20)	-1 (0)	-1 (40)	28
2	1 (70)	1 (1:10)	-1 (5)	1 (10)	-1 (40)	11
3	-1 (40)	1 (1:10)	1 (20)	-1 (0)	1 (100)	42
4	1 (70)	-1 (1:3)	-1 (5)	1 (10)	-1 (40)	19
5	1 (70)	1 (1:10)	1 (20)	1 (10)	1 (100)	39
6	1 (70)	1 (1:10)	1 (20)	-1 (0)	1 (100)	47
7	-1 (40)	1 (1:10)	1 (20)	1 (10)	-1 (40)	17
8	-1 (40)	-1 (1:3)	1 (20)	1 (10)	1 (100)	24
9	-1 (40)	-1 (1:3)	-1 (5)	1 (10)	1 (100)	15
10	1 (70)	-1 (1:3)	-1 (5)	-1 (0)	1 (100)	16
11	1 (40)	1 (1:10)	-1 (5)	-1 (0)	-1 (40)	13
12	1 (40)	-1 (1:3)	-1 (5)	-1 (0)	-1 (40)	16
13	0 (55)	0 (1:6.5)	0 (12.5)	0 (5)	0 (70)	21
14	0 (55)	0 (1:6.5)	0 (12.5)	0 (5)	0 (70)	23
15	0 (55)	0 (1:6.5)	0 (12.5)	0 (5)	0 (70)	22

[a] Oil to ethanol molar ratio. [b] Enzyme concentration. [c] Water concentration. [d] Irradiation power amplitude.

Experimental data were statistically treated and the variables effects on the FAEE yield are shown in the Pareto chart (Figure 1) at 95% confidence level ($p < 0.05$). Inspection of this figure shows that enzyme concentration and irradiation power amplitude had a positive significant effect on FAEE yield, where an increase in these variables leads to higher conversions. The oil to ethanol molar ratio, temperature, and water concentration did not show a significant effect on the reaction conversion. The transesterification reaction requires three moles of ethanol per mole of triglyceride to yield three moles of FAEE and one mole of glycerol. The theoretical minimum required molar ratio of ethanol to oil should therefore to be 1:3. However, transesterification is an equilibrium reaction in which a high molar ratio is used to drive the reaction to FAEE.

The use of 100% ultrasonic irradiation power led to higher conversions. This result is probably due to the physical effects of the cavitation phenomena, mainly in terms of the intense levels of turbulence and mixing generated in the reactor. Because of the generation of microemulsions between the two immiscible phases, the

available interfacial area between the reactants increases greatly, giving faster reaction rates and the requirement of less-severe conditions, in terms of the operating temperature (Deshmane *et al.*, 2009).

The effect caused by the ultrasound irradiation also explains the non-significant effect of temperature. Whereas the overall temperature is lower with the ultrasonic irradiation the reactants are exposed to higher temperatures locally, although for few microseconds, due to the continuous process of generation and collapse of cavities (Sutkar and Gogate, 2009; Gole and Gogate, 2012a). However, it is known that the reaction temperature increases the collision chance between enzyme and substrate molecules, increasing the interaction. Therefore, the increase of temperature decreased the reaction medium viscosity, increasing cavitation events and the rate of emulsion formation, thus increasing biodiesel production (Yu *et al.*, 2010). Based on this, for the next steps of the study the temperature of 70 °C was used.

It was observed that there was a considerable increase in ethyl ester formation with a decrease in addition of water. A maximum conversion of 47% of ethyl esters was obtained without adding water, however, the oil had a water content of 0.33%. With increasing level of water content, the hydrolytic activity of lipase increases and the transesterification yield tends to decrease. The water content could decrease the enzyme activity due to the observed enzyme particle aggregation that might consequently lead to limited access of the substrate to the enzyme active site (Karboune *et al.*, 2006). Hence, this optimum level of water plays an important role in minimizing hydrolysis and maximizing transesterification activity of immobilized lipase (Abdulla and Ravindra, 2013).

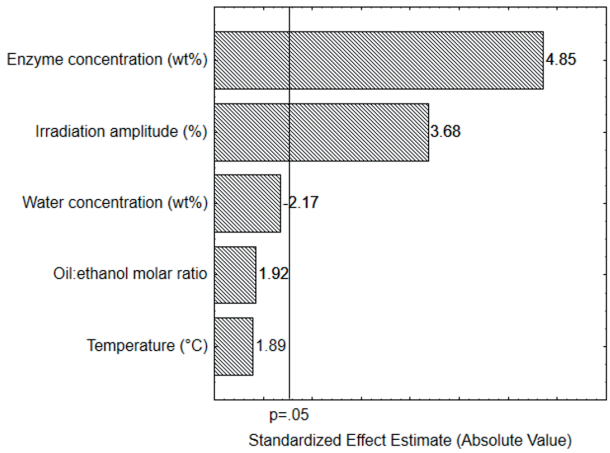

Figure 1. Pareto chart of the effects of the independent studied variables on the FAEE yield in solvent-free system ($p<0.05$). Experimental data and conditions shown in Table 1.

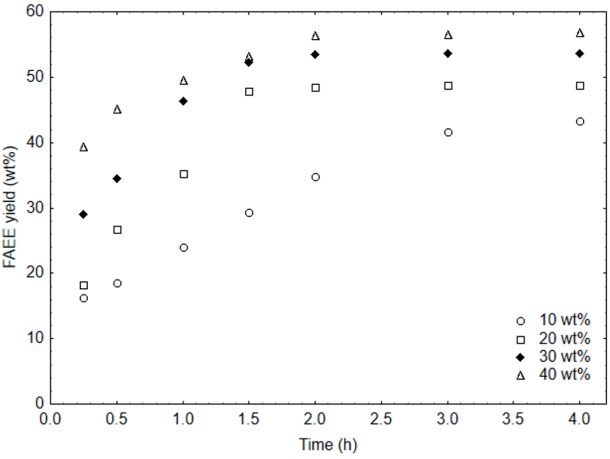

Figure 2. Kinetics of FAEE production at enzyme concentrations of 10, 20, 30, and 40 wt%. Experimental conditions: 70 °C, oil to ethanol molar ratio of 1:10, without water, and ultrasound power of 100%.

Kinetic study: effect of enzyme concentration

The effect of enzyme concentration on FAEE yield in solvent-free system under ultrasound-assisted system was evaluated at 70 °C, keeping the oil-to-ethanol molar ratio constant at 1:10, irradiation power amplitude (100%), with no water addition, and enzyme concentration in a range of 10, 20, 30 and 40 wt% (based on the substrate amount). Figure 2 shows the experimental data results obtained in this step. From the figure it is possible to observe that the initial reaction rates increased with increasing enzyme concentration, leading to reasonable yields in short reaction time.

The FAEE production in the presence of enzyme in a solvent-free system showed conversions of 48, 54, and 56% after 1.5 h of reaction, respectively for 20, 30, and 40 wt% of enzyme concentration. After 2 h of reaction time a small increase (52 to 56%) was observed in FAEE yield when 40 wt% of enzyme concentration was used. On the other hand, for 10 wt% enzyme concentration, lower conversions were achieved, reaching 34% of FAEE after 2 h. It is worth noting that the conversions obtained at higher enzyme concentrations (20, 30, and 40 wt%) after 1.5 and 2 h were similar and, considering the cost of the enzyme, it seems that 30 wt% of enzyme and 1.5 h of reaction time is the best condition for FAEE production in the present study.

The enzymatic ethanolysis reaction of *Jatropha curcas* oil using an ultrasound bath showed good conversions in a short reaction time (1.5 h), being at least 3 times shorter when compared with experiments conducted under the same experimental conditions without ultrasound irradiation (18%). For tests with ultrasound irradiation and without enzymes it was not observed the production of FAEE was not observed.

Abdulla and Ravindra (2013) using *Burkholderia cepacia* lipase immobilized in alginate and κ-carrageenan (5.25 g), 35 °C, 1:10 molar ratio of oil (10 g) to ethanol, 1 g water, 230 rpm in 20 hours reaction reached 60% ethyl esters. Soumanou *et al.* (2012) tested different immobilized enzymes for alcoholysis of *Jatropha curcas* oil and achieved the highest conversion (93%) using immobilized *Pseudomonas cepacia* lipases on Accurel 1282 after 16 h, whereas the lowest conversion was found with Novozym 435. A study of *Jatropha curcas* oil methanolysis in ultrasound showed 84.5% conversion of methyl esters using immobilized *Enterobacter aerogenes* lipase on silica, molar ratio oil to methanol 1:4, reaction time 30 min, ultrasonic amplitude 50% (100 W/m^3) and cycle 0.7 s (Kumar *et al*., 2011).

Biodiesel production using *Jatropha* seed oil reported by Shah and Gupta (2007), by *Pseudomonas cepacia* lipase immobilized on Celite and ceramic at 200 rpm, molar ratio of 1:4 (oil:ethanol) and 40 °C in 24 h reaction showed 80 and 68 wt% biodiesel yield, respectively. With *Pseudomonas cepacia* lipase immobilized on Celite with addition of 5% (w/w, enzyme) water the yield was above 85% after 12 hours of reaction (Shah and Gupta, 2007). Several studies comparing enzymatic reactions for the production of biodiesel by a conventional method and ultrasound-assisted showed that the ultrasonication process led to similar yields of esters as the conventional procedure, while significantly decreasing the reaction time, so the use of ultrasonic irradiation was a time saving and economical method of producing biodiesel fuel (Teixeira *et al*., 2009; Subhedar *et al*., 2015; Subhedar and Gogate (2016).

Biocatalyst reuse

According to Batistella *et al.* (2012), the utilization of enzymatic systems to produce biodiesel has been considered attractive due to several factors like mild reaction conditions (energy saving), the possibility of using waste oils (low price, not competing with the food chain), easy glycerol and catalyst recuperation, and low environmental impact (minimal waste water treatment). Nevertheless, the process productivity is not generally satisfactory and the cost of the catalyst remains comparatively prohibitive from an industrial point of view.

In addition, as pointed out by Wang *et al*. (2001), the cost involved in enzyme immobilization together with utilization cycles should be taken into account in the global economic analysis of an industrial biodiesel plant. It has been argued by Rahman *et al*. (2012) that lipases may lose their activity in non-aqueous medium and selectivity and stability may also be affected. Undoubtedly, the possibility of catalyst reuse is crucial for enzymatic process feasibility and accordingly reaction cycles for immobilized enzyme were performed under ultrasound irradiation.

Figure 3 presents results in terms of FAEE reaction yield and enzyme residual activity as a function of cycle number. It can be observed from this figure that the enzyme kept the initial activity only in the first cycle of reuse. In the second and third cycles, a reduction in the enzyme residual activity (84 and 64%) was accompanied by a reduction in reaction conversion (24 and 20%). From the fourth cycle, a dramatic loss of residual esterification activity was observed (from 40 to 5%) until the sixth cycle of utilization, so it seems that the enzyme activity is closely related to the reaction conversion, which can be attributed to the possible interaction enzyme-substrate. This decrease in the FAEE conversion in the solvent-free system from the second reuse cycle is due to the low solubility of glycerol (reaction mixture) and a long exposure time of the enzyme to irradiation. The glycerol has a strong negative effect expressed as a blocking effect on the enzyme activity. This effect has been attributed to the deposition of glycerol on the enzyme surface (Santin *et al.*, 2014; Michelin *et al.*, 2015). Lerin *et al.* (2014) reported that the residual enzyme activity and the product conversion are directly dependent of the energy used (potency and frequency), exposure time (to irradiation), substrates and solvents used in the reaction. The observed decrease in FAEE production and residual activity as a function of cycle number can also be related to the denaturation (alteration) of protein structure originated from the heat-induced destruction of noncovalent interactions, i.e., the breakage of the weak ionic and hydrogen bonding that stabilizes the three dimensional structure of the enzyme (Yadav and Lathi, 2006).

Different results were reported in the literature for the stability of immobilized lipases during the reuse in batch

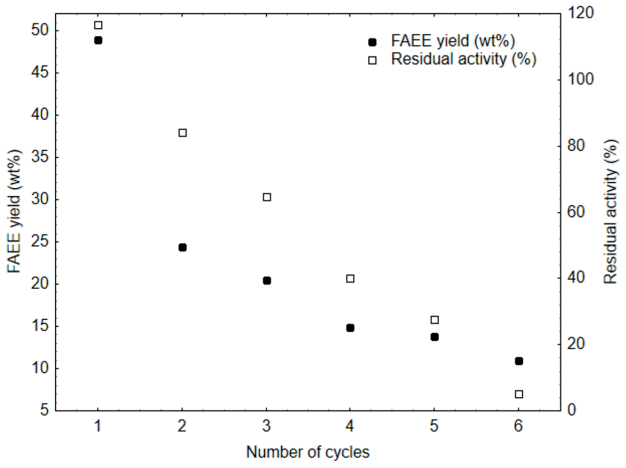

Figure 3. Cycles of Novozym 435 use for FAEE production in solvent-free system under sonochemical irradiation. Experimental conditions: oil to ethanol molar ratio of 1:10, enzyme concentration of 20 wt%, 70 °C, without water, and ultrasound power of 100% at 2 h reaction.

process. The results obtained by Subhedar and Gogate (2016) using ultrasound showed that the biodiesel yield and catalytic activity of immobilized lipase decreases after each cycle of catalyst use. Shah and Gupta (2007) studied biodiesel production from crude *Jatropha* oil using *Pseudomonas cepacia* lipase immobilized on Celite and on ceramic; after four times, a rapid decline in activity was observed for both enzyme preparations. However, Abdulla and Ravindra (2013), found that *Burkholderia cepacia* lipase immobilized in alginate and κ-carrageenan for esterification of crude *Jatropha curcas* oil was stable and retained 73% relative transesterification activity after six cycles of reuse.

CONCLUSIONS

The use of non-edible oil for biodiesel production allows a diversification of oil crops in Brazil and provides the social seal for biodiesel producers that buy this oil from family farmers. Results from this work show a promising perspective for the use of an environmentally benign technique to produce FAEE under mild conditions and relatively short reaction time, being at least 2 times shorter when compared with the reaction performed under the same conditions without ultrasound irradiation. FAEE showed conversions of 48, 54, and 56% after 1.5 h of reaction time, respectively, for 20, 30, and 40 wt% of enzyme concentration under ultrasound-assisted system at 70 °C, oil to ethanol molar ratio constant at 1:10, irradiation power amplitude (100%), and with no water addition. Enzyme reuse brought interesting insights to the enzyme-catalysis field, showing the complex behavior of lipases in the reaction system. In this work 6 cycles of reuse were possible before complete enzyme denaturation. The enzyme transesterification process described in this study appears to be a promising alternative to the traditional process of biodiesel production and can contribute to develop an economically feasible enzymatic biodiesel process.

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