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BIOCATALYSIS

Microwave-assisted enzymatic synthesis of beef tallow biodiesel

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Abstract Optimal conditions for the microwave-assisted enzymatic synthesis of biodiesel have been developed by a full 2^2 factorial design leading to a set of seven runs with different combinations of molar ratio and temperature. The main goal was to reduce the reaction time preliminarily established by a process of conventional heating. Reactions yielding biodiesel, in which beef tallow and ethanol used as raw materials were catalyzed by lipase from *Burkholderia cepacia* immobilized on silica-PVA and microwave irradiations within the range of 8–15 W were performed to reach the reaction temperature. Under optimized conditions (1:6 molar ratio of beef tallow to ethanol molar ratio at 50°C) almost total conversion of the fatty acid presented in the original beef tallow was converted into ethyl esters in a reaction that required 8 h, i.e., a productivity of about 92 mg ethyl esters g^{-1} h^{-1}. This represents an increase of sixfold for the process carried out under conventional heating. In general, the process promises low energy demand and higher biodiesel productivity. The microwave assistance speeds up the enzyme catalyzed reactions, decreases the destructive effects on the enzyme of the operational conditions such as, higher temperature, stability, and specificity to its substrate, and allows the entire reaction medium to be heated uniformly.

Keywords Biodiesel · Microwave · Beef tallow · Transesterification · Immobilized lipase

Introduction

Biodiesel (mono-alkyl esters of primary alcohol and long-chain fatty acids derived from vegetable oils or animal fats) is an important biodegradable renewable fuel for alternative diesel engines [12, 14]. Although its use with internal combustion engines is technologically well assessed, the industrial production has not yet been completely optimized.

Most processes for making biodiesel are based on transesterification reactions and require the use of a catalyst to initiate the reaction, in order to promote the solubility of the reactants and to allow the process to proceed at a satisfactory rate [22]. Several aspects are still under investigation aiming to improve the process efficiency, reduce the amount of by-products, and mitigate the ecological impact [14, 23].

The primary raw materials used in biodiesel production are from direct agricultural sources, such as sunflowers and rapeseed crops, or indirect, such as waste oils from the food industry. As biodiesel becomes an increasingly important energy source in the world, the search has increased for economically feasible feedstock that can be used to produce this renewable fuel have increased [2–4]. In many countries, several non-edible raw materials have been studied for their capacity to support growth in biodiesel

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production, reduce the competition between the use of crops for food or fuels, lower fuel costs, reduce or eliminate subsidies, bring all available lipid streams into the renewable fuels pool and minimize increases in edible oil prices resulting from their diversion to fuel usage [2, 4, 23]. One of these raw materials is beef tallow, which is non-edible and inexpensive, for it is a by-product of the abundant beef production with limited industrial uses [2, 3]. Therefore, combining the advantages of sustainability, the beef tallow becomes the second most important raw material after soybeans to produce biodiesel in many countries [2–4]. Brazil alone produces around 600,000 tons/year, being, therefore, a sustainable source for the production of biodiesel and further resulting in a reduction of the pollution impact of this by-product [4].

Although methanol has been used so far, ethanol could be a valid alternative for transesterification owing to the lower toxicity and the higher yield on a weight basis [26]. A totally green production of biodiesel should be performed in a bioprocess using immobilized lipases (EC 3.1.1.3) as catalysts [1, 8, 24]. In addition, the use of immobilized lipases tolerates the water content of oil and increases biodiesel yield by avoiding soap formation [24].

However, from an economic point of view, a major problem with lipase catalytic biodiesel production is the low reaction rate of the enzymes compared with chemical catalysts; therefore it is necessary to find a suitable method to increase the reaction rate in order to promote the application of enzyme catalytic biodiesel production. Microwave irradiation, which has been proven to be a clean, fast, and convenient energy source, has been widely used in many reactions mediated by both chemical and biochemical catalysts [1, 13, 18]. Microwave irradiation also offers a fast, easy route to biodiesel production using acid or base catalysts; a satisfactory transesterification conversion can be achieved in a few seconds under microwave irradiation, compared to a few hours under conventional heating [11, 20]. The microwave-assisted, lipase-catalyzed reaction method is therefore a significant development and several works have already been published showing the advantages of using this kind of heating system [11, 13, 17, 18, 20]. However, most of the works covered in the literature are related with commercial available immobilized lipase preparations, in particularly Novozym® 435 and Lipozyme [7, 27].

In this work, attention was paid to study the enzymatic ethanolysis of beef tallow under microwave irradiations, using lipase immobilized on a non-commercial matrix as catalysts. Besides, in the regional context, ethanol has been the natural choice since Brazil is one of the world's largest ethanol producers, with a well-established technology of production, and large industrial plant capacity installed throughout the country [26]. The lipase chosen was *Burkholderia cepacia* immobilized on epoxy SiO_2-PVA composite based on their suitability for many biotransformation applications mainly in the biodiesel enzymatic synthesis using different feedstocks [6, 9]. Moreover, this specific lipase preparation has been selected among seven lipases as the best catalyst to mediate the transesterification reaction of beef tallow with ethanol taking into consideration the product properties to be use as fuel [21].

Materials and methods

Materials

Commercial lipase *B. cepacia* (Lipase PS) from Amano Pharmaceuticals (Nagoya, Japan) in a crude form was used in this study without further purification. Tetraethoxysilane (TEOS) was acquired from Aldrich Chemical Co. (Milwaukee, WI, USA). Epichlorohydrin, hydrochloric acid (minimum 36%), ethanol (minimum 99%), polyvinyl alcohol (PVA molecular weight 72,000) and polyethylene glycol (PEG molecular weight 1,500) were supplied by Reagen (RJ, Brazil). Beef tallow was supplied by Fertibom (Catanduva, SP, Brazil) having the following composition in fatty acids: (w/v): 3% myristic, 25% palmitic, 32% stearic, and 40% oleic acid with average molecular weight 856.76 g mol^{-1}. Other characteristics of the sample of beef tallow include: acid value: 1.96 mgKOH/g; peroxide value: 2.54 mEq/kg; iodine value: 34 g I_2/g; saponification value: 197 mgKOH/g; free fatty acid: 0.95% and density = 0.85 g/cm^3. All the other reagents were of analytical grade.

Support synthesis and lipase immobilization

SiO_2-PVA composite was prepared by the hydrolysis and polycondensation of tetraethoxysilane according to the methodology previously described [6, 9] attaining particles, having the following properties: average pore diameter (22.91 Å); surface area BET (461.00 m^2 g^{-1}) and porous volume (0.275 cm^3 g^{-1}). Activation of SiO_2-PVA particles was carried out with epichlorohydrin at 2.5% (w/v) pH 7.0 for 1 h at room temperature, followed by exhaustive washings with distilled water. Epoxy SiO_2-PVA particles were soaked into hexane under stirring (100 rpm) for 1 h at 25°C. Then, excess of hexane was removed and lipase was added at a ratio of 1:4 g of enzyme per gram of support. PEG-1500 was added together with the enzyme solution at a fixed amount (5 mg g^{-1} support). Lipase-support system was maintained in contact for 16 h at 4°C under static conditions. To perform this work, three batches of immobilized derivatives were prepared and average measured hydrolytic activity was 1,800 ± 262 U g^{-1} biocatalyst. Additional properties of this immobilized

derivative were as follows [6]: optimum pH = 8.5; optimum temperature (40–60°C), and thermal stability (half-life at 60°C = 6.24 h).

Biodiesel synthesis under conventional heating

The reactions were performed in a jacket cylindrical glass reactor with a capacity of 100 ml containing 12 g of substrate consisting of beef tallow-to-ethanol molar ratio of 1:9, without the addition of solvents. The mixtures were incubated with lipase PS immobilized on epoxy SiO_2-PVA at fixed proportion of 20% in relation the total reaction volume at 45°C. Reactions were performed for 48 h under constant magnetic agitation of 150 rpm.

Biodiesel synthesis under microwave irradiations

Reactions were performed in a microwave reactor (Model Discover/University-Wave, Cem Corporation) consisting of a cylindrical internal chamber of 75 mm in diameter and 100 mm in height. The cylindrical design assures homogeneous irradiation of the working volume that, for instance, is missing in the conventional microwave ovens available on the market and frequently used in experiments without modifications. A magnetic stirrer was used to attain at three levels of the liquid mixing qualitatively classified as low, medium, or high. The maximum power of operation was about 300 W. The temperature was controlled with compressed air blown into the chamber. This chilling air was designed to operate at different levels of pressure and either on continuous or intermittent flow mode. The temperature of the reaction was monitored by an infrared sensor located in the lower part of the chamber. ChemDriver software was used to record data for each run, including variation in the microwave flux and temperature evolution.

The working volume was designed as a 100-ml spherical glass reactor containing 12 g of substrate consisting of beef tallow and anhydrous ethanol at different molar ratios in the absence of solvents. The reactor was coupled to a reflux condenser to avoid ethanol losses as vapor. The reaction medium was incubated with the immobilized derivative at a fixed proportion of 20% (w/w) in relation to the total weight of reactants involved in the reaction. For the time-course studies, an aliquot of reaction medium was taken at various time intervals and diluted in *n*-heptane for GC analysis.

Experimental design and data treatment

Experiments were performed according to full 2^2 factorial design with three replicates at central levels. The ethanol-to-beef tallow molar ratio (X_1) and reaction temperature (X_2) were chosen as independent variables to cover data established earlier [25]. The transesterification yield (Y), defined as ratio of the concentrations of the transformed beef tallow to the initial fat multiplied by 100, as previously reported [6, 25] was considered as response variable.

Results were analyzed with help of *Statistica* software version 7.0 (StatSoft Co.) to verify the independent variables effect on the response assuming levels of $p < 0.05$ as statistical significance criterion [16]. Design-Expert software version 8.0.0 (Stat-Ease, Inc.) was used for regression, variance analysis (ANOVA) and optimization of biodiesel production. The statistical significance of the individual regression coefficients and the mathematical model was determined by Fisher test and the proportion of variance explained by the model obtained, was given by the multiple coefficient of determination, R^2.

Downstream procedure

When the reaction was completed, the lipase was separated from the medium and the organic phase was washed twice with one volume of water to remove both the remaining ethanol and the free glycerol as a by-product. The residual water was removed by evaporation to attain the final fatty acid ethyl ester product. Coulometric Karl Fischer Titrometry (Mettler DL 18 model, Mettler Scientific Co. Ltd) was used to measure the concentration of the water remaining in the purified product. The specific gravity of the product was determined in accordance with the recommendations of ASTM1298.

Batch operational stability tests

The operational stability of the immobilized system was assayed using immobilized lipase (1.0 g dry weight) and substrate containing beef tallow and ethanol (molar ratio = 6) in successive batches (45°C/8 h). At the end of each batch, the transesterified product was removed from the reactor vessel and the immobilized lipase washed in situ with *tert*-butanol and hexane, in order to remove any substrate or product eventually retained in the matrix. The recovered immobilized derivative was left in the dessecator overnight to attain water in the level required (lower than 10%). A fresh medium was then introduced in the reactor and a new reaction performed. Transesterification activity was estimated and expressed as μmol of ethyl ester formed per minute per gram of catalyst. The biocatalyst half-life time ($t_{1/2}$) was determined by applying the inverted linear decay model [5, 15].

Ethyl esters analysis

Ethyl esters derived from ethanolysis of beef tallow were analyzed by gas chromatograph using a Varian CG 3800

model (Varian, Inc., Palo Alto, CA, USA) equipped with flame—ionization detector and 5% DEGS CHR-WHP 80/100 mesh 6 ft, 2.0 mm ID, in a stainless-steel-packed column (Restek, Frankel Commerce of Analytic Instruments Ltd., SP, Brazil). Nitrogen was used as the carrier gas with a flow rate of 25 ml min^{-1}. Temperature programming was performed. The column temperature was kept at 90°C for 3 min, heated to 120°C at 25°C min^{-1} and kept constant for 10 min. Then, the temperature was programmed at 25°C min^{-1} to 170°C and kept constant for 15 min. The temperatures of the injector and detector were set at 250°C. Data collection and analyses were performed using the software Galaxie Chromatography Data System version 1.9. Calibration curves were built from standard ethyl esters (ethyl oleate, stearate, palmitate. and myristate) using hexanol as internal standard. The reaction yield was calculated by taking into account the mass of ester content obtained by GC analysis and the total theoretical ester mass based on the reaction molar ratio [15, 25].

Viscosity determination

The absolute viscosity of biodiesel was determined with LVDV-II cone and plate spindle Brookfield viscosimeter (Brookfield Viscometers Ltd, England) using a CP 42 cone. A circulating water bath was used to maintain the temperature in each analysis at 40°C for biodiesel samples and 50°C for beef tallow with an accuracy of 0.1°C. The shear stress measurements were taken as a function of shear rate and the dynamic viscosity was determined as a slope constant. Samples of 0.5 ml were used and the measurements were replicated three times.

Results and discussion

Comparison of microwave and conventional heating systems

Using the conditions previously established for biodiesel synthesis from beef tallow with ethanol [21], a comparison between the transesterification reaction under conventional heating and microwave irradiation was performed. As shown in Fig. 1, under conventional heating, a full conversion of beef tallow into ethyl esters was achieved in 48 h, while the reaction reached equilibrium in 8 h using microwave heating with the same yield. This indicates that to achieve the same yield of FAEE (fatty acids ethyl esters), a shorter time was needed under microwave irradiation compared to conventional heating. Under microwave irradiation, the reaction rate improved threefold in the first 2 h compared to that under conventional heating.

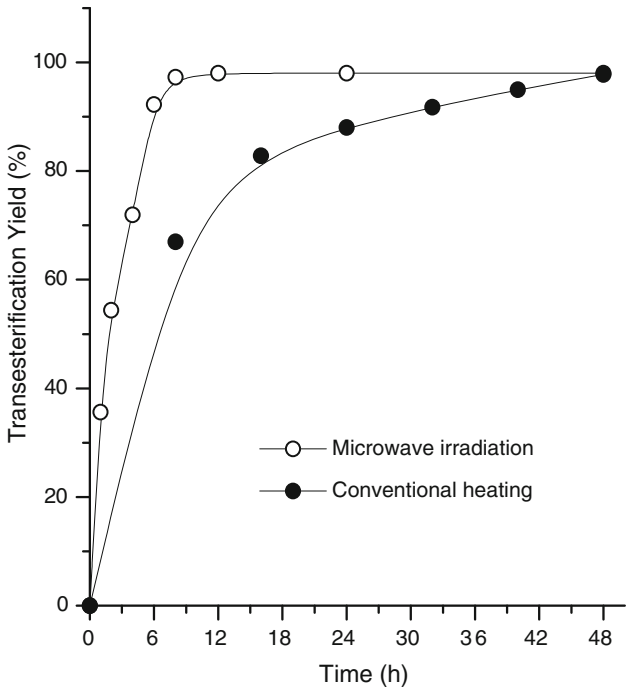

Fig. 1 Comparison of the transesterification progress under microwave irradiation (*open circle*) and conventional heating (*closed circle*). Conditions: reactions were performed at 45°C, using beef tallow-to-ethanol molar ratio of 1:9 under 150 rpm mechanical agitation

The effect of the microwave absorbing character of the substrate might contribute to the faster reaction rate. It was presumed that microwave heating involved directed energy absorption by the functional groups that bear ionic conductivity or a dipole rotational effect. In the reaction mixture, ethanol may be a good microwave radiation absorption material. Its dipole may quickly reorient under microwave radiation, which would destroy the two-tier structure of the interface between the ethanol and the oil, making the functional groups too reactive. Microwave irradiation might also increase the emulsification speed, which results in an accelerated transportation of reactants or products due to greater contact between the enzyme and the substrate, and thus an improved reaction rate. It is also possible that the enzyme behaves slightly differently and becomes more active if a conformation change in the enzyme facilitates the substrate to approach the active site of the enzyme more easily under microwave irradiation than that under conventional heating.

Factorial design

The lipase catalyzed reaction rate depends on the concentrations of the enzyme, substrate and temperature. In order to study the combined effect of reaction temperature (X_1) and molar ratio (X_2), experiments were carried out using

statistical experimental design to determine conditions at which the high conversion of beef tallow into ethyl esters can be achieved. The range of temperature and molar ratio studied was between 40 an 50°C and 6 and 12 molar ratio ethanol to beef tallow, respectively (Table 1).

Figure 2 displays the reaction progress for all runs performed. The experimental matrix and the transesterification yields (%) are shown in Table 2. Results clearly showed that the transesterification yield was strongly affected by the molar ratio for all temperatures tested. High conversions of the beef tallow into FAEE could be achieved when a lower molar ratio ethanol to beef tallow was used (runs 1 and 3). Intermediate conversions were achieved at average molar ratios (runs 5–6). However, conversion was markedly decreased when the largest excess of ethanol was present in the reaction medium (runs 2 and 4).

The statistical analysis of the results shows significant effect for the variable molar ratio (X_2) and its interaction with temperature at 95% of confidence level. Temperature effect on the activity of lipase PS immobilized on SiO_2-PVA was not significant (Table 3).

The fact that the molar ratio has influenced the transesterification yield in such a significant way may be attributed to a lack of nonthermal effects from the microwave irradiations on the molecular structure of the enzyme [20]. Because ethanol is a highly polar molecule that can easily absorb the energy created by microwaves, the lower the level of ethanol in the reaction medium, the higher will be the energy of the microwaves absorbed by the enzymatic molecule, resulting in some conformational changes in its structure, increasing in this way the enzyme selectivity for the substrate.

Regarding temperature, theoretically, an elevated temperature could help the substrate molecules obtain adequate energy to pass over the energy barrier and enhance the reaction rate. In contrast, enzymes are very sensitive to temperature and are easily deactivated at high temperature. The effect of temperature on the activity of lipase PS immobilized on SiO_2-PVA showed no significant effect, which is in agreement with thermal stability data for this immobilized lipase preparation as previously determined [6]. According to this study, lipase PS immobilized on SiO_2-PVA is a thermostable lipase preparation with a maximum activity in the range 50–60°C. However,

Table 1 Factor levels used according to the 2^2 full factorial design

Variable	Symbol	Levels		
Temperature	X_1	40	45	50
Molar ratio (ethanol to beef tallow)	X_2	6	9	12

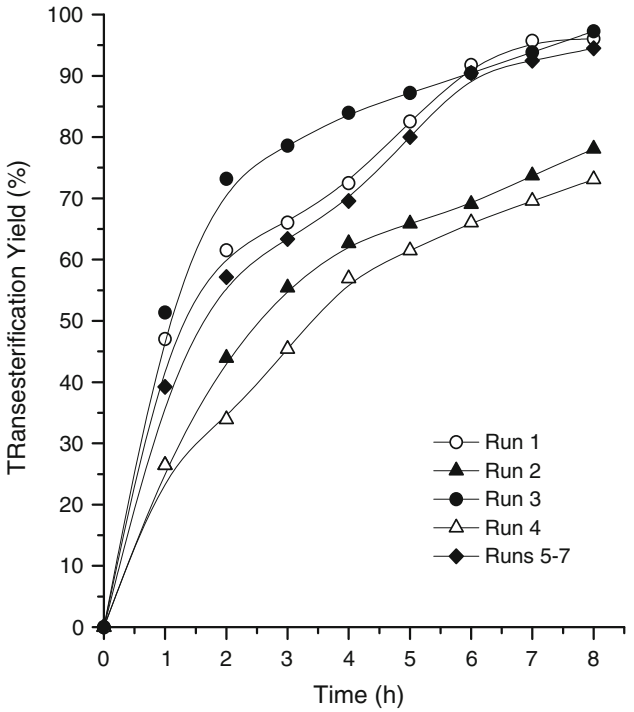

Fig. 2 Transesterification yield (%) according to results 2^2 full factorial design to evaluate the influence of the variables beef tallow-to-ethanol molar ratio and temperature using lipase from *Burkholderia cepacia* immobilized on epoxy SiO_2-PVA

Table 2 Values for transesterification yield (%) on beef tallow according to the 2^2 full factorial design

Run	Coded variables		Yield (%)
	X_1	X_2	
1	−1	−1	61.54
2	−1	+1	43.91
3	+1	−1	73.21
4	+1	+1	33.91
5	0	0	57.14
6	0	0	55.30
7	0	0	53.50

Table 3 Estimated effects, standard errors, and Student's t test for transesterification yield (%) on beef tallow using the 2^2 full factorial design

Factors	Transesterification yield (%)			P value
	Effects	Standard errors	t values	
Average	54.07	±0.68	78.60	0.00
X_1: Temperature	0.83	±1.82	0.45	0.69
X_2: Molar ratio	−28.46	±1.82	−15.63*	0.00
$X_1 \cdot X_2$	−10.83	±1.82	−5.95[a]	0.02

* $p < 0.05$

working temperatures in the range of 40–50°C are recommended to extend the operational stability.

The interaction effect of the variables was also statistically significant at the same confidence level. As a result, at lower molar ratio, the transesterification yield was improved by lowering the temperature (runs 1 and 3). However, at higher molar ratios, the transesterification yield was improved by raising the temperature (runs 2 and 4).

Based on the response evaluated (% transesterification yield), a mathematical model was developed. The criterion for acceptance of the model was based on Student's t test ($\alpha < 0.05$), and Fisher's p (also $\alpha < 0.05$) for the global validity of the resulting equation, considering the experimental error/total error and lack of fitting/experimental error ratios.

Analyzing Table 4, it can be seen that the model is highly significant ($p = 0.0106$), and presents a high determination coefficient ($R^2 = 0.9929$). Thus, the mathematical model that represents the transesterification yield, in the range studied, can be expressed by Eq. (1).

$$Y(\%) = 54.07 + 0.41 \cdot X_1 - 14.23 \cdot X_2 - 5.41 \cdot X_1 \cdot X_2 \quad (1)$$

where Y (%) is the predicted value for the transesterification yield (%); X_1 is the codified value of the variable temperature and X_2 the codified value of the variable molar ratio.

To confirm the mathematical model generated by the statistical analysis, an additional experiment was made at the predicted conditions (molar ratio 1:6 beef tallow/ethanol and temperature 50°C). At the end of the reaction, the biodiesel sample was purified and submitted to the viscosity analysis, adopting methodology previously described in the "Materials and methods" section.

The obtained results revealed that a total conversion of the fatty acids present in the beef tallow was reached in their corresponding esters in 8-h reaction (Fig. 3). The highest ethyl ester concentrations were in regard to ethyl oleate, stearate, and palmitate in accordance with the beef tallow fatty acids composition.

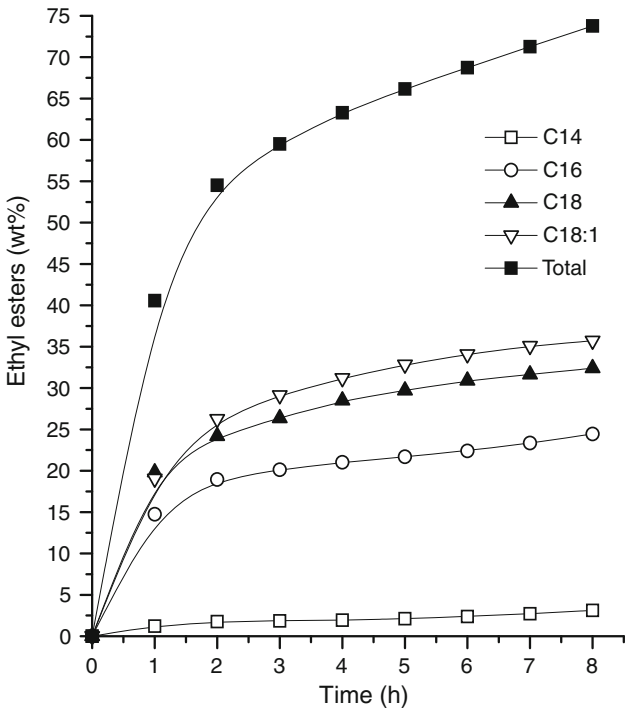

Fig. 3 Ethyl ester profile in the alcoholysis of beef tallow using lipase from *Burkholderia cepacia* immobilized on epoxy SiO_2-PVA under the predicted conditions (beef tallow-to-ethanol molar ratio of 1:6 at 50°C). *Symbols* Ethyl esters from myristic acid (*open square*); palmitic acid (*open circle*); stearic acid (*closed triangle*); oleic acid (*open inverted triangle*) and total esters (*closed square*)

Table 4 Analysis of variance for the model that represents transesterification yield (%), on beef tallow using the 2^2 full factorial design

Source	SS	DF	MS	F	P
Model	928.35	3	309.45	93.42	0.0106
X_1: Temperature	0.70	1	0.70	0.21	0.6914
X_2: Molar ratio	810.26	1	810.26	244.60	0.0041
$X_1 \cdot X_2$	117.40	1	117.40	35.44	0.0271
Curvature	8.08	1	8.08	2.44	0.2588
Pure error	6.63	2	3.31		
Cor total	943.05	6			

$R^2 = 0.9929$

SS sum of squares, *DF* degree of freedom, *MS* mean square

The purified biodiesel presented a viscosity value on the order of 5.74 cP at 40°C. This value is close to the values predicted by the effective technical standards: ASTM United D5761-States: 1.9–6.0 cSt; EN 14214-Europe: 3.0–5.0 cSt and ANP 255-Brazil: 2.5–5.5 cSt at 40°C and confirmed the occurrence of the transesterification reaction, due to the reduction of the viscosity value when compared to the value of the initial raw material: 43.82 cP, measured at 50°C. Conventional thermal heating relies on conduction currents within the sample to provide an overall rise in temperature. By comparison, microwaves transfer energy directly into the sample. This thermal energy then drives the reaction to completion. By definition, this is a relatively slow process. Microwave irradiation is a form of energy that resides fairly low in the electromagnetic spectrum. It is slightly higher in frequency than radio waves, but much lower than X-rays, ultraviolet light, and other chemical bond-breaking forms of energy. As a result, bonds are neither formed nor broken by microwave irradiation—it simply acts as an energy source, rapidly transferring energy to the molecules.

Energy transferred directly into the sample, as accomplished by microwave irradiation, is powerful enough to accomplish chemical reactions in seconds or minutes, instead of the hours or days it would take using conventional thermal heating.

Reusability of the immobilized lipase in transesterification under microwave irradiations

An important parameter in evaluating an immobilized enzyme is its lifetime or half-life for a particular reaction system, particularly under microwave irradiations. Although the microwave heating system has been shown to be a good alternative to reduce the reaction time, data concerning the ability to re-use a biocatalyst is still scarce in the literature [7, 19]. As far as lipase is concerned, the work reported by Réjasse et al. [19] is perhaps the best deep study in this matter. According to the authors, a slight higher stability was found for Novozym® 435 when assessing in batch consecutive synthesis of ethyl butyrate under microwave irradiation by comparison with conventional heating [19]. To explain these results, the workers suggested that the microwave field changes the interactions between the enzyme and its microenvironment, preventing the enzyme denaturation. However, these findings are dependent on the biocatalyst, reaction polarity, and temperature [19, 20].

In the present work, the stability of the immobilized system under microwave irradiation was also assessed by reusing the immobilized lipase seven times in the synthesis of beef tallow biodiesel. The synthesis was followed by evaluating the formation of ethyl esters per gram of biocatalyst per minute (transesterification activity). Figure 4 shows the residual activity as a function of operational time from which a slow decrease in the transesterification activity can be observed with a total reduction of 32% at the end of the seven recycles, revealing a half-life ($t_{1/2}$) of 88.46 ± 0.78 h. This result is slightly lower than the estimated for this immobilized lipase in the ethanolysis of babassu oil under conventional heating [5], which showed that after seven cycles (24 h), the enzyme retained 75% of its original activity.

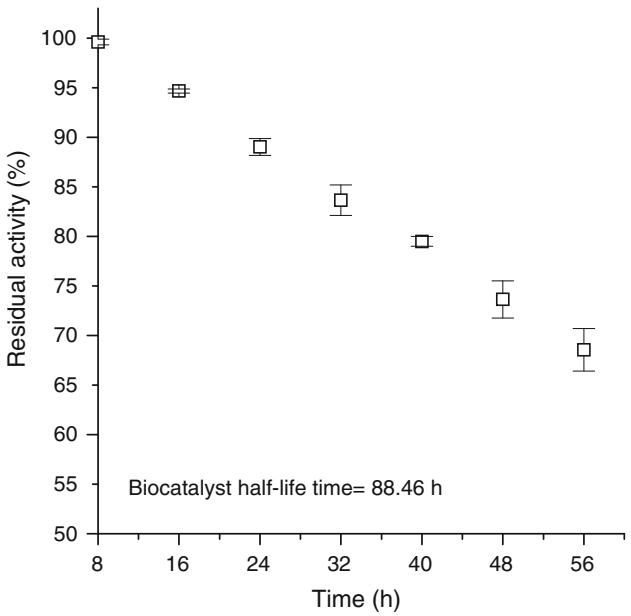

Fig. 4 Batch operational stability tests for *Burkholderia cepacia* lipase immobilized on epoxy SiO_2-PVA in the transesterification of the beef tallow with ethanol at 45°C

Although not directly comparable with results described by Réjasse et al. [19], our data suggested that microwave irradiation did not affect the stability of the lipase PS immobilized derivative. However, the enzymatic behavior under microwave radiation still need to be better exploited using various biocatalysts and feedstocks. It is also interesting to note that consecutive batch runs is subjected to many variables that can affect the enzyme performance as for example, mass loss during the repeated batch and change in the enzyme water level [15, 20]. Therefore, the better methodology to estimate the operational biocatalyst stability should be performed under continuous mode. This is also considered to be a feasible procedure to scale-up process under microwave irradiations. According to Glasnov and Kappe [10], one of the major drawbacks of microwave technology is the difficulty associated with scale-up. Because of the limited penetration depth of microwaves into absorbing media, the solution to scale-up lies in continuous-flow processing. Therefore, the combination of these two enabling technologies (microwave heating and flow processing) seems to be a logical consequence and this concept is well covered in the review published by Glasnov and Kappe [10], in which applications of microwave-assisted synthesis under flow conditions, ranging from microfluidic technology, to mesofluidic systems, stop-flow instruments, and larger-scale reactors are addressed taking into consideration the drawbacks that are still need to be overcome to enable adopting this technology at representative scale.

Conclusions

The optimization of the enzymatic synthesis of biodiesel from beef tallow performed in the microwave reactor revealed that the formation of ethyl esters was strongly influenced by the variable molar ratio and its interaction with temperature at the 95% of confidence level. The conditions that maximize the biodiesel production from beef tallow was low level of molar ratio (1:6 beef tallow: ethanol) and high level of temperature (50°C). Runs to

confirm the mathematical model revealed that a total conversion of the beef tallow was obtained in 8-h reaction, and, the final product (biodiesel), after the purification showed a viscosity of 5.74 cP at 40°C. The data presented in this work demonstrated that the reaction medium when submitted to the microwave irradiation promoted a significant increment in the reaction rate, compared to the other heating systems by conventional means. In addition, the immobilized derivative showed satisfactory stability under repeated batch runs, revealing a biocatalyst half-life of 88.46 ± 0.78 h. Thus, manufacturing biodiesel using microwave offers a fast, easy route to this biofuel with advantages of a short reaction time, a low oil/ethanol ratio, an ease of operation a drastic reduction in the quantity of by-products, and all with reduced energy consumption.

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References

1. Antczak MS, Kubiak A, Antczak T, Bielecki S (2009) Enzymatic biodiesel synthesis—key factors affecting efficiency of the process. Renew Energ 34:1185–1194
2. Araújo BQ, Nunes RCR, Moura CVR, Moura EM, Citó MGL, Santos RJ (2010) Synthesis and characterization of beef tallow biodiesel. Energ Fuel 24:4476–4480
3. Canoira L, Gamero MR, Querol E, Alcántara R, Lapuerta M, Oliva F (2008) Biodiesel from low-grade animal fat: production process assessment and biodiesel properties characterization. Ind Eng Chem Res 47:7997–8004
4. Da Cunha ME, Krause LC, Moraes MAS, Faccini CS, Jacques RA, Almeida SR, Rodrigues MRA, Camarão EB (2009) Beef tallow biodiesel produced in a pilot scale. Fuel Process Technol 90:570–575
5. Da Rós, PCM (2009) Ethanolysis of vegetal oils by enzymatic catalysis accelerated by microwave irradiation. MSc Dissertation-Engineering School of Lorena, USP. Available at: http://www.eel.usp.br, p 123
6. Da Rós PCM, Silva GAM, Mendes AA, Santos JC, De Castro HF (2010) Evaluation of the catalytic properties of *Burkholderia cepacia* lipase immobilized on non-commercial matrices to be used in biodiesel synthesis from different feedstocks. Bioresour Technol 101:5508–5516
7. Desai B, Kappe CO (2004) Microwave-assisted synthesis involving immobilized catalysts. Top Curr Chem 242:177–208. doi:10.1007/b96876
8. Fjerbaek L, Christensen KV, Norddahl B (2009) A review of the current state of biodiesel production using enzymatic transesterification. Biotechnol Bioeng 102:1298–1315
9. Freitas L, Da Rós PCM, Santos JC, De Castro HF (2009) An integrated approach to produce biodiesel and monoglycerides by enzymatic interestification of babassu oil (*Orbinya* sp.). Process Biochem 44:1068–1074
10. Glasnov TN, Kappe CO (2007) Microwave-assisted synthesis under continuous-flow conditions. Macromol Rapid Commun 28:395–410
11. Gupta M, Paul S, Gupta R (2009) General characteristics and applications of microwaves in organic synthesis. Acta Chim Sloven 56:749–764
12. Helwani Z, Othman MR, Aziz N, Fernando WJN, Kim J (2009) Technologies for production of biodiesel focusing on green catalytic techniques: a review. Fuel Process Technol 90:1502–1514
13. Leadbeater NE, Stencel LM, Wood EC (2007) Probing the effects of microwave irradiation on enzyme-catalysed organic transformations: the case of lipase-catalysed transesterification reactions. Org Biomol Chem 5:1052–1055
14. Leung DYC, Wu X, Leung MKH (2010) A review on biodiesel production using catalyzed transesterification. Appl Energ 87:1083–1095
15. Moreira ABR, Perez VH, Zanin GM, De Castro HF (2007) Biodiesel synthesis by enzymatic transesterification of palm oil with ethanol using lipases from several sources immobilized on silica-PVA composite. Energ Fuel 21:3689–3694
16. Myers RH, Montgomery DC (1995) Response surface methodology: process and product optimization using designed experiment, 1st edn. Wiley, New York
17. Nogueira BM, Carretoni C, Cruz R, Freitas S, Melo PA Jr, Costa-Félix R, Pinto JC, Nele M (2010) Microwave activation of enzymatic catalysts for biodiesel production. J Mol Catal B Enzym 67:117–121
18. Oghbaei M, Mirzaee O (2010) Microwave versus conventional sintering: a review of fundamentals, advantages and applications. J Alloy Compd 494:175–189
19. Réjasse B, Lamare S, Legoy MD, Besson T (2004) Stability improvement of immobilized *Candida antarctica* lipase B in an organic medium under microwave radiation. Org Biomol Chem 2:1086–1089
20. Réjasse B, Lamare S, Legoy MD, Besson T (2007) Influence of microwave irradiation on enzymatic properties: applications in enzyme chemistry. J Enzym Inhib Med Chem 22:518–526
21. Silva GAM (2009) Enzyme synthesis, physicochemical and thermal characterization of biodiesel from beef-tallow by ethyl route. MSc Dissertation-Engineering School of Lorena, USP. Available at: http://www.eel.usp.br, p 104
22. Solomon BD (2010) Biofuels and sustainability. Ann NY Acad Sci 185:119–134
23. Suarez PAZ, Santos ALF, Rodrigues JP, Alves MB (2009) Oils and fats based biofuels: technological challenges. Quim Nova 32:768–775
24. Tan T, Lu J, Nie K, Deng L, Wang F (2010) Biodiesel production with immobilized lipase: a review. Biotechnol Adv 28:628–634
25. Urioste D, Castro MBA, Biaggio FC, De Castro HF (2008) Synthesis of chromatographic standards and establishment of a method for the quantification of the fatty ester composition of biodiesel from babassu oil. Quim Nova 31:407–412
26. Vieitez I, Silva C, Alckmin I, Borges GR, Corazza FC, Oliveira JV, Grompone MA, Jachmanián I (2010) Continuous catalyst-free methanolysis and ethanolysis of soybean oil under supercritical alcohol/water mixtures. Renew Energ 35:1976–1981
27. Yu D, Tian L, Ma D, Wu H, Wang Z, Wang L, Fang X (2010) Microwave-assisted fatty acid methyl ester production from soybean oil by Novozym 435. Green Chem 12:844–850