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Performance of an enzymatic packed bed reactor running on babassu oil to yield fatty ethyl esters (FAEE) in a solvent-free system

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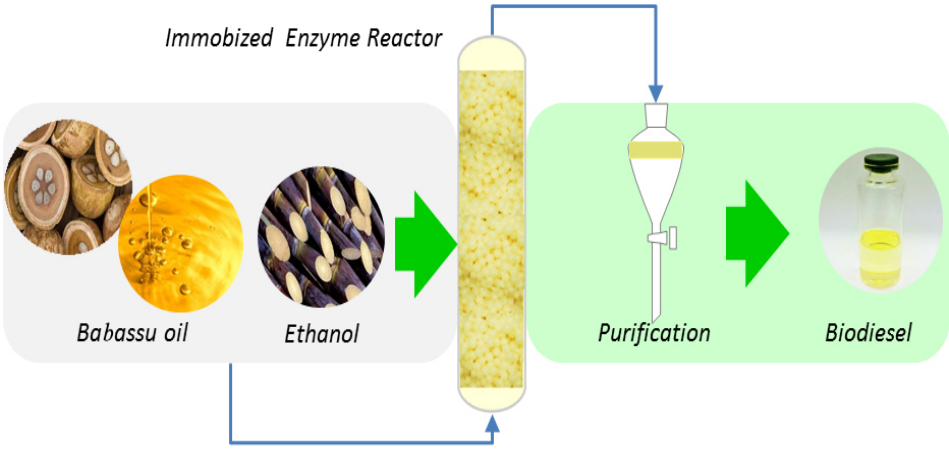
HIGHLIGHTS

- The performance of packed bed reactor running on babassu oil to yield ethyl esters was assessed.
- *Burkholderia cepacia* lipase immobilized on SiO2-PVA composite was used as a catalyst.
- The molar ratio of babassu oil and ethanol was found as a critical parameter for attaining high yields.
- High yield (96.0 ± 0.9%) and productivity (41.1 ± 1.6 $mg_{ester} \cdot g_{catalyst}^{-1} \cdot h^{-1}$) were achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

The transesterification reaction of babassu oil with ethanol mediated by *Burkholderia cepacia* lipase immobilized on SiO_2-PVA composite was assessed in a packed bed reactor running in the continuous mode. Experiments were performed in a solvent-free system at 50 °C. The performance of the reactor (14 mm ×210 mm) was evaluated using babassu oil and ethanol at two molar ratios of 1:7 and 1:12, respectively, and operational limits in terms of substrate flow rate were determined. The system's performance was quantified for different flow rates corresponding to space times between 7 and 13 h. Under each condition, the impact of the space time on the ethyl esters formation, the transesterification yield and productivity were determined. The oil to ethanol molar ratio was found as a critical parameter in the conversion of babassu oil into the correspondent ethyl esters. The highest transesterification yield of 96.0 ± 0.9% and productivity of 41.1 ± 1.6 $mg_{ester} \cdot g_{catalyst}^{-1} \cdot h^{-1}$ were achieved at the oil to ethanol molar ratio of 1:12 and for space times equal or higher than 11 h. Moreover, the immobilized lipase was found stable with respect to its catalytic characteristics, exhibiting a half-life of 32 d.

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1. Introduction

The transesterification of triglycerides (TGs) with alcohol in the presence of chemical or biochemical catalysts leads to the formation of alkyl ester, also known as biodiesel. Enzymatic transesterification using lipase (E.C. 3.1.1.3) is attractive and encouraging due to easy product separation, minimal wastewater treatment needs, easy glycerol recovery and the absence of side reactions, unlike with chemical catalysts (Jegannathan et al., 2008; Christopher et al., 2014).

Conventionally, methanol is used to produce fatty acid methyl esters (FAME), but production of fatty acid ethyl esters (FAEE) with bioethanol is considered more advantageous due to the expected improved sustainability of this type of biodiesel. More specifically, ethanol has a superior dissolving power in vegetable oils and low toxicity compared to methanol (Stamenković et al., 2011; Brunschwig et al., 2012). Additionally, unlike methanol (which is generally derived from fossil sources), ethanol is produced mainly from renewable sources *via* fermentation processes, and because its large scale production as a substitute fuel for gasoline already exists, the supply of bioethanol for the industrial production of biodiesel can be easily achieved (Brunschwig et al., 2012).

A biodiesel production process based on immobilized lipase-catalysis involves a multi-phase system throughout the reaction, including the insoluble phase of the biocatalyst, lipid phase and a polar phase when alcohol exceeds the solubility limit (or when the by-product glycerol is present). Stirred tank reactors (STRs) and packed bed reactors (PBRs) are often used as reactors for studying this type of reaction at different scales (Christopher et al., 2014).

Studies of the process conditions in reactors are necessary for the technical and economic viability of enzymatic biodiesel production. In most of the works published, transesterification is conducted in batch reactors, in which the enzyme is dispersed in the reaction mixture by agitation in STRs (Balcão et al., 2006). PBRs can be considered as a better choice than STRs for immobilized enzymes, mainly due to the lower shear stress imposed on the catalyst particles and the possibility of continuous operation (Yusuf, 2006). In addition, PBR may be operated with no need for biocatalyst separation from the reaction products and can be relatively easily scaled up (Yusuf, 2006).

The present study was set to investigate the efficiency of a PBR system through the transesterification reaction of babassu oil and ethanol mediated by lipase from *Burkholderia cepacia*. The enzyme was covalently immobilized on a hybrid matrix silica-polyvinyl alcohol (SiO_2-PVA) and the reactor was operated in a continuous mode. Variables, such as flow rate and molar ratio of the reactants, were assessed to define the operating parameters, aiming at establishing the potentials and challenges in scaling up the process based on the experimental results obtained. Babassu oil was selected as the TGs source because the tree from which it is extracted is abundant in certain countries, such as Brazil, and its primary use is for nonfood purposes (Teixeira, 2008; Carvalho et al., 2013).

2. Materials and methods

2.1. Materials

Refined, bleached and deodorized babassu oil was provided by BASF (Jacarei, SP-Brazil) with its properties summarized in **Table 1**. Commercial virgin olive oil (0.3% acidity), purchased from a local market, was used to determine the hydrolytic activity of the biocatalysts. A commercial lipase from *Burkholderia cepacia* (Batch number: 01022TD) was purchased from Amano Pharmaceuticals (Nagoya, Japan) and was used as received without further purification. Tetraethoxysilane (TEOS) and epichlorohydrin (99%) were acquired from Aldrich Chemical Co. (Milwaukee, WI, USA). Polyvinyl alcohol (MW 88000, 88%) was obtained from Acros Organic (USA). Hydrochloric acid (36%), anhydrous ethanol (99.8%), *tert*-butanol, polyethylene glycol (MM 1500) and hexane were supplied by Reagen (Rio de Janeiro, RJ, Brazil). A deep blue liposoluble dye (organic synthetic pigment) was obtained from Glitter Ind. Com. Imp. Exp. Ltd (Carapicuiba, SP, Brazil) and was used as a tracer. All solvents and reagents for analyses were of chromatographic or analytical grade.

Table 1.
The properties of the babassu oil used in this study.

Property	Value
Acid number (mg KOH·g^{-1})	0.65
Peroxide value (mEq·kg^{-1})	1.82
Iodine number (g I_2·g^{-1})	25
Saponification number (mg KOH·g^{-1})	238
Average molecular weight of TGs (g·mol^{-1})	709.90
composition of fatty acids (% wt)	
Caprylic acid (C8:0)	4.5
Capric acid (C10:0)	3.5
Lauric acid (C12:0)	44.7
Myristic acid (C14:0)	17.5
Palmitic acid (C16:0)	9.7
Stearic acid (C18:0)	3.1
Oleic acid (C18:1)	15.2
Linoleic acid (C18:2)	1.8

2.2. Support synthesis and lipase immobilization

A polysiloxane-polyvinyl alcohol composite (SiO_2-PVA) was prepared, activated with epichlorohydrin and used to immobilize the lipase according to the methodology reported by Da Rós et al. (2010). To perform this work, ten batches of immobilized derivatives were prepared, and the average measured hydrolytic activity was 1950 ± 120 IU/g biocatalyst. One international unit (IU) of enzyme activity was defined as the amount of enzyme that liberates 1 µmol of free fatty acid per min under the assay conditions (37 °C, pH 7.0, 150 rpm). The properties of the immobilized derivative were as follows: diameter (0.175 mm); average pore diameter (29.42 Å); surface area (337 $m^2 \cdot g^{-1}$); porous volume (0.25 $cm^3 \cdot g^{-1}$); and density (1.865 $g \cdot cm^{-3}$). The biochemical, kinetic properties, thermal stability and operational stability of this immobilized lipase preparation were described elsewhere (Da Ros et al., 2010).

2.3. Continuous runs of the PBR

The ethanolysis of the babassu oil was conducted in a PBR including a glass column (internal diameter: 14 mm; height: 210 mm; and total volume: 32 cm^3) with a water jacket connected to a circulating water bath to maintain the temperature at 50 ºC. The continuous operation was started by loading the reactor with the biocatalyst, and the substrates were continuously pumped using a peristaltic pump (Perista Pump SJ-1211, Atto Bioscience & Biotechnology, Tokyo, Japan) from a reservoir to the bottom end of the bioreactor at the required flow rate through Marprene tubing (Watson Marlow 913.AJ05.016). A reflux condenser system was connected to the feeding vessel to avoid ethanol loss. Heating tapes containing a thermostatic electrical resistance (25 W) were used to avoid heat loss in the inlet and outlet tubing.

For each run, 24.3 g (d= 1.865 $g \cdot cm^{-3}$) of the biocatalyst corresponding to a working volume of 19.0 cm^3 was used. The substrate was prepared at a molar ratio of oil to ethanol of 1:7 and 1:12, respectively, and flow rates ranging from 1.5 to 2.8 $mL \cdot h^{-1}$ were imposed to determine the performance limit of the reactor for both ratios. **Figure 1** shows the experimental setup of the reaction system. During the continuous runs, samples were periodically taken from the reactor vessel for analysis of the relevant variables, such as the ethyl esters concentration, viscosity and density. For each tested space time, the parameters were determined when the concentrations within the reactor remained relatively constant over a period corresponding to at least three space times. The space time was calculated according to Levenspiel (1997) as described by the following equation (**Eq. 1**).

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$$\tau = \frac{V}{v_0} \quad \text{(Eq. 1)}$$

Where τ is the space time (h), V is the working volume of the reactor (mL) and v_0 is the flow rate (mL·h^{-1}).

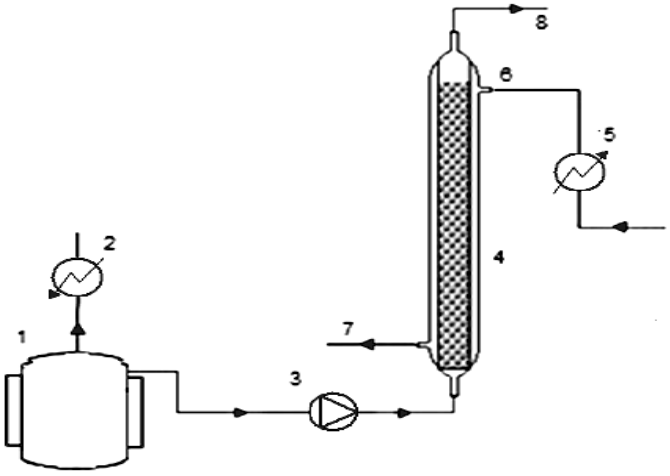

Fig.1. Schematic diagram of the packed bed experimental apparatus: 1) substrate reservoir; 2) reflux condenser; 3) peristaltic pump; 4) column; 5) thermostatic bath; 6) water in; 7) water out; 8) product output.

2.4. Operational stability of the immobilized derivative

Transesterification The biocatalyst stability was assessed by measuring the hydrolytic activity of the immobilized derivatives at the end of each continuous run, taking into account the original activity as 100%. The recovered immobilized lipase was then washed with tert-butanol to remove any substrate or product retained in the matrix. Hydrolytic activity was determined by the olive oil emulsion method according to the modification proposed by Soares et al. (1999). The inactivation constant (kd) and half-life (t1/2) for the immobilized lipase were calculated as described by Costa-Silva et al., (2014).

2.5. Purification of biodiesel

The volume of sample collected from the bioreactor was transferred into a decanting funnel, in which the same quantity of distilled water was added. Then, vigorous agitation was performed, and the mixture was allowed to stand for 6 h for phase separation. This procedure was performed three times in sequence. The upper phase, consisting of FAEE (biodiesel) was evaporated by a rotatory-evaporator. Then, the solution was dried with sodium sulfate, and the lower phase, consisting of glycerol and wastewater, was disposed of.

2.6. Hydrodynamic characterization of the PBR system

The Tracer response analysis was used to characterize and model the flow through the reactor. The PBR was filled with 24.5 g of previously denatured immobilized *B. cepacia* lipase. Then, the system was operated under a continuous flow rate of substrate (5.3 mL·h^{-1}), which corresponded to 3.9 h of spatial time.

A deep blue liposoluble dye (15 wt %) was solubilized in a substrate and was used as a tracer. A pulse input experiment was performed, and the residence time distribution (RTD) was experimentally determined by injecting the tracer into the reactor at time zero and then by spectrophotometrically measuring the tracer concentration, C, at 650 nm in the output stream as a function of time using a Varian Cary 50 UV/Vis Spectrophotometer (Varian Australia Pty Ltd, Mulgrave, VIC, Australia). The experiments were performed in duplicate.

The RTD function, $E(t)$, is defined using the following equation (**Eq. 2**) (Fogler, 1992).

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \quad \text{(Eq. 2)}$$

Where $C(t)$ is the tracer concentration at time t.
The mean residence time, t_m, for a constant volumetric flow, was calculated using the **Equation 3** (Fogler, 1992).

$$t_m = \int_0^\infty t.E(t)dt \quad \text{(Eq. 3)}$$

The integration in the Equation 3 was calculated using the ORIGIN 8.0 software (OriginLab Corporation, MA, USA).

The variance, σ, or the square of the standard deviation, is defined by the **Equation 4** (Fogler, 1992).

$$\sigma^2 = \int_0^\infty \left(t - t_m\right)^2 \cdot E\left(t\right)dt \quad \text{(Eq. 4)}$$

The skewness, s^3, is defined by the **Equation 5** (Fogler, 1992).

$$s^3 = \frac{1}{\sigma^{3/2}} \int_0^\infty \left(t - t_m\right)^3 \cdot E\left(t\right)dt \quad \text{(Eq. 5)}$$

To characterize the pipe flow through the enzyme particles in the column, the Reynolds number was calculated according to Lide (2007) (**Eq. 6**).

$$\mathrm{Re} = \frac{dp \cdot \upsilon \cdot \rho}{\mu} \quad \text{(Eq. 6)}$$

Where d_p is the enzyme particle diameter (0.175 mm), υ represents the fluid velocity calculated as the flow/cross area of the column, and, ρ denotes the fluid specific mass (848 and 863 kg·m^{-3} using substrates at oil to ethanol molar ratios of 1:7 and 1:12, respectively), μ is the fluid viscosity of the reactant mixture (8.12 and 6.95×10^{-3} kg·m^{-1}·s^{-1} using substrates at oil to ethanol molar ratios of 1:7 and 1:12, respectively) measured as 1 atm at 40°C with a Brookfield Viscometer−LVDVII (Brookfield Viscometers, England). The flow was hereafter characterized as turbulent if $R_e \geq 4000$ or laminar if $R_e \leq 2000$ (Lide, 2007).

2.7. Analytical Procedure

The FAEE formed in the transesterification reaction were analyzed by FID gas chromatography (Varian CG 3800, Inc. Corporate Headquarters, Palo Alto, CA, USA) using a 5% DEGS CHR-WHP 80/100 mesh 6 ft 2.0 mm ID and 1/8" OD column (Restek Frankel Commerce of Analytic Instruments Ltd, SP, Brazil) following previous established conditions (Urioste et al., 2008). The theoretical ester concentrations were calculated by taking into account the fatty acid composition and its initial weight mass in the reaction medium, and the transesterification yield (%) was defined as the ratio between the produced and theoretical esters concentrations (Carvalho et al., 2013).

The absolute viscosity of the purified biodiesel was determined by a Brookfield Viscometers model LVDVII (Brookfield Viscometers Ltd, England) using the cone CP 42. All assays were performed at 40 °C using a 0.5 mL aliquot of the sample. The biodiesel density was determined by a digital densimeter model DMA 35N EX (Anton Paar, Graz, Austria). In this test, all assays were performed at 20 °C using a 2.0 mL aliquot of each sample (Carvalho et al., 2013).

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3. Results and discussion

3.1. Reactor performance at oil to ethanol molar ratio of 1:7

The reactor was filled with immobilized lipase to form a well packed column, and the substrate at a oil to ethanol molar ratio of 1:7 was fed at increasing flow rates ranging from 1.5 to 2.8 mL·h^{-1}, corresponding to 13 to 7 h space times, respectively, for a total period of 30 d. Figure 2 shows the concentration of ethyl esters for different space times.

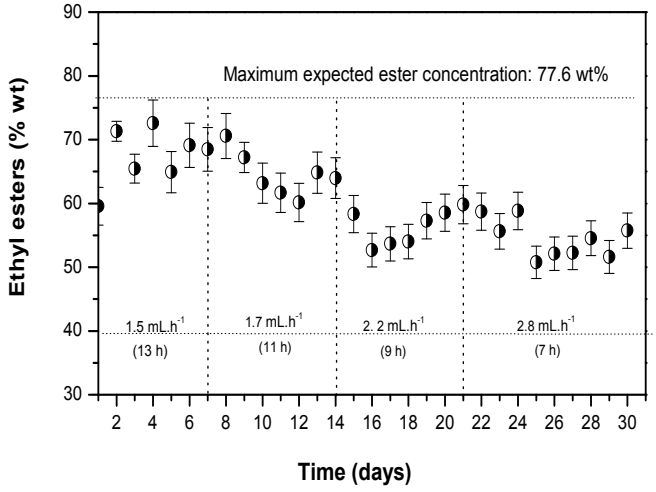

Fig.2. Ethyl esters concentrations from the transesterification of babassu oil performed in a packed bed reactor using immobilized *B. cepacia*. The reactions were carried out at a babassu oil to ethanol molar ratio of 1:7, at 50 ºC, at feed flow rates ranging from 1.5 to 2.8 mL·h^{-1} (corresponding to space times of 7 and 13 h).

Changes in the space time along the continuous run interfered with the formation of ethyl esters, resulting in a variation in their concentration from 52.8 to 69.5 wt%, corresponding to transesterification yields between 68.1 and 89.5%. The average values of the ethyl esters concentration and productivity achieved for each space time are shown in Table 2. Among the range of the flow rates studied, the best reactor performance was found at a space time of 13 h (flow rate = 1.5 mL.h^{-1}). Under such conditions, 89.5% of the fatty acids present in the babassu oil were converted into ethyl esters, attaining an average productivity of 37.0 ± 1.3 $mg_{ester}.g_{catalyst}^{-1}.h^{-1}$.

Table 2.
The average values of the ethyl esters concentration, transesterification yield and productivity obtained in the continuous ethanolysis of babassu oil in a PBR using lipase *B. cepacia* immobilized on SiO_2-PVA under different space times (oil to ethanol molar ratio of 1:7).

Parameter	Space time (h)			
	7	9	11	13
Ethyl ester concentration (% wt.)	52.8 ± 1.9	56.6 ± 2.6	62.7 ± 3.0	69.5 ± 3.6
FAEE yield (%)	68.1 ± 2.4	72.9 ± 3.8	80.8 ± 3.5	89.5 ± 3.5
Productivity ($mg_{ester}.g_{catalyst}^{-1}.h^{-1}$)	53.5 ± 1.9	44.9 ± 2.1	40.1 ± 1.9	38.3 ± 2.0

These results were favorable when compared to those previously reported by several researchers (Royon et al., 2007; Wang et al., 2011; Dors et al., 2012) using PBRs running on different feedstocks in the presence or absence of solvents. Nevertheless, the studied PBR exhibited lowered biodiesel yields compared to the batchwise reactors reported previously (Freitas et al., 2009; Carvalho et al., 2013). For instance, the performance of a STR used by Carvalho et al. (2013) to conduct lipase-catalyzed transesterification of babassu oil was superior in terms of the conversion achieved (at the same molar ratio) compared to that of the PBR studied herein. The decrease in yield in the present reactor system may be ascribed to the high viscosity of the substrate medium, which prevented a uniform forced flow through the inter-particle spaces within the support matrix. Another reason may be the steady state adsorption of glycerol on the surface, whose amount might have been larger compared to what absorbed in a non-steady state batch run. The FAEE yield at the steady state under the conditions of the lowest volumetric flow rate (1.5 mL.h^{-1}) reached 89.5%, and the maximum expected ethyl ester concentration of 77.6 wt% was not achieved.

To reduce the feedstock lost in the process, substrate containing a higher excess of ethanol was used, i.e. the molar ratio of babassu oil to ethanol was increased from 1:7 to 1:12. In addition to the investigation of the possible limitations of the process pertaining to the concentration of ethanol, the tolerance of the *B. cepacia* lipase to substrates containing high ethanol concentration was also checked. In fact, the substrate containing higher ethanol concentrations had a lower viscosity value obviously and facilitated the flow through the bed, avoiding reactor operational limitations caused by occasional obstructions. The average viscosity values of the substrate at oil to ethanol molar ratios of 1:7 and 1:12 were 7.0 and 8.5 x 10^{-5} kg m^{-1}. s^{-1}, respectively.

3.2. Reactor performance at oil to ethanol molar ratio of 1:12

Experiments were conducted using the babassu oil to ethanol molar ratio of 1:12 while keeping the other operating conditions constant. The substrate was fed at increasing flow rates and the reactor was operated for 28 d. Oscillations were observed in the ethyl esters formed as seen in Figure 3.

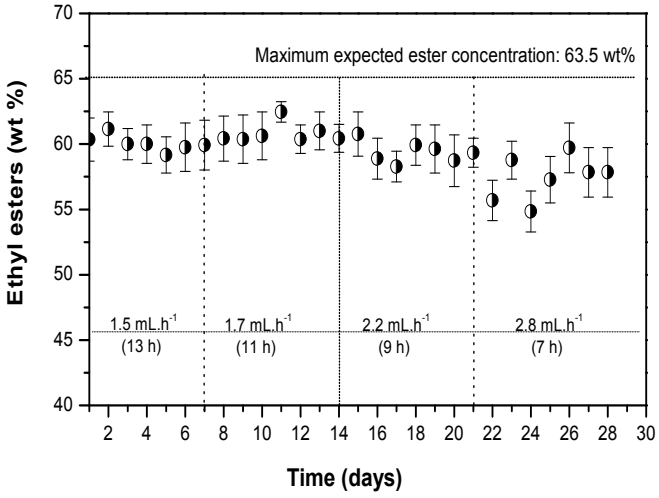

Fig.3. Ethyl esters concentrations through the transesterification of babassu oil performed in a packed bed reactor using immobilized *B. cepacia*. The reactions were carried out at a babassu oil to ethanol molar ratio of 1:12, at 50 ºC, at feed flow rates ranging from 1.5 to 2.8 mL·h^{-1} (corresponding to space times of 7 and 13 h).

In spite of this, the average concentration of ethyl esters attained throughout the process revealed lower loss of feedstock (minimum yield of 90.4%), independent of the flow rate of the input stream. In this way, the substrate composed of babassu oil and ethanol at a molar ratio of 1:12 promoted greater conversion of the fatty acids present in the babassu oil into ethyl esters.

Figure 4 presents the average productivity and transesterification yield values for the tested spatial times. Based on these results, for the substrate containing a higher amount of excess ethanol (babassu oil to ethanol molar ratio of 1:12), space times greater than or equal to 9 h provided transesterification yields higher than 92.2 ± 3.3%. The highest yield of 96.0 ±0.9% was obtained for the reactor operating at a flow rate of 1.8 mL·h^{-1} (space time = 11 h), corresponding to a productivity of 41.1 ± 1.6 $mg_{ester}.g_{catalyst}^{-1}.h^{-1}$.

3.3. Evaluation of the PBR parameters

The RTD is an essential tool to assess fluid velocity patterns, which can be used to describe the flow regime in a reactor. Because the flow regime affects the reactor performance, its description may enable better process control

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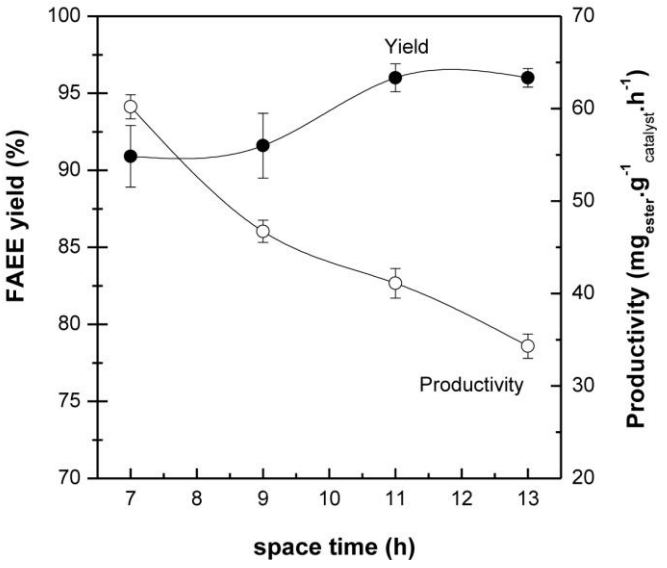

Fig.4. The average values of the transesterification yield and productivity attained in the continuous ethanolysis of babassu oil in a PBR using immobilized *B. cepacia* under different space times (oil to ethanol molar ratio of 1:12).

(Fogler, 1992; Levenspiel, 1997). In this work, for the experimental determination of the mean residence time of the reactants in the reactor, as well as the characterization of the reaction mixture in the bed, a pulse input experiment was performed.

The results obtained allowed the RTD curve E (t) to be traced as a function of the time, according to the **Equation 2** (data not shown). Using the **Equation 3**, the mean residence time was calculated at 4.1 ± 0.8 h. This value was approximately 5.9% higher than the value calculated by taking into account the experimental conditions based on the **Equation 1** and can be considered acceptable in this type of test. In better words, this finding indicated that no preferential paths or backmixing took place in the bed demonstrating the good quality of the packing.

The variance, or square of the standard deviation, indicating the spread of the distribution, was calculated using the **Equation 4**. The greater that value, the greater the distribution spread. **Equation 5** is related to the skewness and measures the extent that a distribution is skewed in one direction in reference to the mean. The main parameters of the RTD are shown in **Table 3**.

Table 3.
Mean residence time, variance, and asymmetry coefficient obtained through the pulse input experiment for the PBR system.

Parameter	Value
Mean residence time, t_m (h)	4.1
Variance, σ^2 (h^2)	0.6
Asymmetry coefficient, s^3 ($h^{3/2}$)	-3.2 ×10^2

Parameters such as the column length-to-diameter ratio, substrate flow rate and the characterization of the pipe flow through the enzyme particle in the column were also evaluated. According to Damstrup et al. (2007), a column length-to-diameter ratio of less than 25 does not interfere with the transfer of the fluid mixture through the column. In this work, the column length-to-diameter ratio obtained was 15. Damstrup et al. (2007) reported that the highest yield was achieved with a column length-to-diameter ratio in the range of 13-14.

To characterize the pipe flow through the enzyme particles in the column, the Reynolds number was calculated at 4.3×10^{-5} and 5.0×10^{-5} for the experiments performed using the oil to ethanol molar ratio of 1:7 and 1:12, respectively, indicating a laminar flow behavior (Re ≤ 2000) for both experimental conditions (Fox et al., 2013). Therefore, the flow was dominated by viscous force with uniform non-turbulent flow in parallel layers and little mixing between layers (Lide, 2007).

3.4. Overall performance of the reactor running on babassu oil and ethanol

Many processes can only reach high productivity values at the expense of high residual substrate levels (low substrate conversion rate) and/or low concentration of product formed (Rica et al., 2009). At the industrial scale, the ideal goal to be achieved is the adoption of a system that has a good productivity combined with low loss of the feedstock. For biodiesel production, an additional parameter should be attained according to the technical standards which is a minimum level of alkyl esters at 96.5% wt. In this context, the productivity data analysis took into account both economic and technical criteria. A summary of the obtained results is tabulated in **Table 4**, in which the average values achieved for both sets of experiments are compared.

Table 4.
Comparative performance of the ethanolysis of babassu oil in a PBR using lipase *B. cepacia* immobilized on SiO_2-PVA under different oil to ethanol molar ratios (biocatalyst initial activity of 1950 IU·g^{-1}).

Parameter	Substrate molar ratio (oil to ethanol)	
	1:7	1:12
Biocatalyst (residual activity, IU·g^{-1})	1229 ± 42	1092 ± 51
Total operating time (d)	30	28
Space time (h)	13	11
Deactivation coefficient (k_d, d^{-1}) ×10^{-2}	1.6 ± 0.4	2.2 ± 0.5
Biocatalyst half-life (days)	44.7 ± 1.1	32.1 ± 3.4
Ethyl ester concentration (% wt.)	69.5 ± 3.6	60.9 ± 0.6
Productivity ($mg_{ester}.g_{catalyst}^{-1}.h^{-1}$)	38.3 ± 2.0	41.1 ± 1.6
Transesterification yield (%)	89.5± 3.5	96.0 ± 0.9
Kinematic viscosity ($mm^2.s^{-1}$)	8.2±0.8	4.3 ± 0.7

Regarding the influence of the molar ratio on the continuous synthesis of biodiesel, the use of equimolar amounts of ethanol to the number of fatty acids (FA) residues is enough to obtain complete conversion of the FA residues to their corresponding FAEE. However, ethanol in excess could result in an increase in yield. Moreover, an excess of alcohol can also be advantageous because it can contribute to medium homogeneity, minimizing the diffusion limitation that could result in low yields, mainly when immobilized systems are used (Antczak et al., 2009; Christopher et al., 2014). Based on the results obtained in the present study, the best reactor performance was attained for runs in which the oil to alcohol molar ratio of 1:12 was used. Under such condition, and at space time greater than or equal to 11 h, an average transesterification yield of 96.0 ± 0.9% and a productivity of 41.1 ± 1.6 $mg_{ester}.g_{catalyst}^{-1}.h^{-1}$ were achieved. This also resulted in biodiesel samples with viscosity values (average 4.3± 0.7 $mm^2.s^{-1}$) complying with the international standard for biodiesel viscosity i.e. ASTM 6751-02 (1.0<kinematic viscosity of B100< 6.0 $mm^2.s^{-1}$.).

The feasibility of enzymatic processes can also be determined by investigating the biocatalyst half-life, which depends on a series of factors, such as linkage of the enzyme with the support, obstruction of the pores by both sludge and by-products, as well as support loss by friction and obstruction of the fixed-bed causing bypass (Zanin and Moraes, 2004). In both sets of experiments, the biocatalyst half-life was measured by determining the biocatalyst activity at the end of each run. The results obtained revealed a loss of 37.2% of the initial enzyme activity for the reactor running on babassu oil and ethanol at a molar ratio of 1 to 7. The deactivation coefficient (*kd*) and half-life ($t_{½}$) were measured at 0.016 d^{-1} and 44.7 d, respectively. Using the higher level excess ethanol (i.e. oil to ethanol molar ratio of 1:12), a lower operational stability of the biocatalyst was observed (32.1 ±6.4 d).

This is an expected behavior because the support used for immobilizing the lipase from *B. cepacia* (i.e. SiO_2-PVA particles) has a strong affinity to adsorb glycerol molecules (formed as a byproduct) due to the presence of hydroxyl groups on both the organic (PVA) and inorganic (silanol groups)

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parts of the hybrid matrix. Using the dyeing method (adsorption of Amaranth, a food grade pigment), Xu et al. (2011) verified the strong affinity of glycerol molecules produced by the ethanolysis of rapeseed oil on a silica particle support used to immobilize *Thermomyces lanuginosus* lipase. In that study, the authors reported a good correlation between the transesterification yield and the amount of glycerol adsorbed on the silica particles. According to the literature, glycerol tends to adsorb on the microenvironment of the support forming a hydrophilic layer, which in turn makes the lipases inaccessible to hydrophobic substrates, such as oil droplets (Dossat et al., 1999; Watanabe et al., 2000). Based on the results of the present study, the transesterification reaction performed using a higher oil to ethanol ratio led to FAEE yields higher than 95%, and this must have resulted in the formation of a more hydrophilic layer on the microenvironment of the biocatalyst and consequently lower operational stability. It is worth quoting that such a limitation could be overcome implementing a strategy to remove the soluble glycerol. For instance, Hama et al., (2011) proposed continuous biodiesel synthesis integrated with a glycerol-separating system through the incorporation of a column packed with resin such as Lewatit GF 202 to absorb glycerol.

4. Conclusions

The findings of the present study confirmed the feasibility of continuous enzymatic production of biodiesel from babassu oil and ethanol in the absence of any solvents using a PBR. The babassu oil and ethanol molar ratio was found as a critical parameter for attaining high FAEE yields. More specifically, the highest performance was achieved when the babassu oil to ethanol molar ratio of 1:12 was used. At this molar ratio and at space times greater than or equal to 11 h, high substrate conversion (transesterification yield of 96.0 ± 0.9%) and productivity value (average productivity 41.1 ± 1.6 $mg_{ester}.g_{catalyst}^{-1}.h^{-1}$) were obtained. Higher yields were also found to have negatively affected the operational stability of the biocatalyst caused by higher rate of glycerol production and its consequent destabilizing effects on the biocatalyst particles by forming a hydrophilic layer. Therefore, further investigation would be required to overcome the limitations imposed by glycerol to improve the overall performance of the proposed system.

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