



Transesterification of babassu oil catalyzed by *Burkholderia cepacia* encapsulated in sol-gel matrix employing protic ionic liquid as an additive

Maria Vanessa Souza Oliveira¹, Patricia Caroline Molgero Da Rós², Silvana Mattedi³, Heizir Ferreira de Castro², Cleide Mara Faria Soares¹ and Álvaro Silva Lima^{1*}

¹Instituto de Tecnologia e Pesquisa, Universidade Tiradentes, Av. Murilo Dantas, 300, 49032-490, Aracaju, Sergipe, Brazil. ²Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, São Paulo, Brazil. ³Departamento de Engenharia Química, Universidade Federal da Bahia, Salvador, Bahia, Brazil. *Author for correspondence. E-mail: aslima2001@yahoo.com.br

ABSTRACT. Enzymatic transesterification from non-edible vegetable oil (babassu oil) and ethanol is provided. A set of seven experiments featuring a full 2² factorial design with three central points and different combinations of molar ratio and temperature as independent variables was employed. Transesterification reactions were catalyzed by *Burkholderia cepacia* lipase encapsulated in a hydrophobic matrix obtained by the sol-gel technique using protic ionic liquid (N-methylmonoethanolamine pentanoate) as additive and with conventional heating (40 – 56°C). Ethyl esters highest yield (51.90%) was obtained by experimental design with 1:7 molar ratio (oil:alcohol) and temperature at 40°C during 48h reaction. The process with a 5-fold increase of enzymatic load provided 98.69% ethyl esters yield with 4.29 mm²s⁻¹ viscosity.

Keywords: protic ionic liquid, enzymatic loading, factorial design, molar ratio, temperature, ethyl esters.

Transesterificação de óleo de babaçu catalisada por *Burkholderia cepacia* encapsulada em matriz sol-gel empregando líquido iônico prótico como aditivo

RESUMO. O presente trabalho aborda o estudo da transesterificação enzimática de um óleo vegetal não comestível (óleo de babaçu) e etanol utilizando planejamento fatorial completo 2² com três pontos centrais. As variáveis independentes foram a razão molar e a temperatura. As reações de transesterificação foram catalisadas por lipase de *Burkholderia cepacia* encapsulada em matriz hidrofóbica obtida pela técnica sol-gel, usando líquido iônico prótico (pentanoato de N-metilmonoetanolamina) como aditivo e em aquecimento convencional (40 - 56°C). O maior rendimento de ésteres etílicos (51,90%) foi obtido por meio do planejamento experimental com razão molar (óleo:álcool) de 1:7 e temperatura de 40°C em 48h de reação. Entretanto, o processo com aumento do carregamento enzimático em cinco vezes permitiu um rendimento em ésteres etílicos de 98,69% com viscosidade de 4,29 mm²s⁻¹.

Palavras-chave: líquido iônico prótico, carregamento enzimático, planejamento fatorial, razão molar, temperatura, ésteres etílicos.

Introduction

Enzymatic transesterification has certain advantages over chemical transesterification, comprising easy recovery of glycerol, transesterification of glycerides with high free fatty acid contents, less energy intensive, which transforms the process for ethyl esters production into a cleaner process (BAJAJ et al., 2010). Moreover, the biocatalysts are eco-friendly (ANTCZAK et al., 2009). The steadily growing interest in lipases over the last two decades stems from their biotechnological versatility and the enzymes' ability to catalyze a broad spectrum of bioconversion reactions such as hydrolysis, esterifications, transesterifications, aminolysis and others (MINOVSKA et al., 2005).

Immobilization is an advantageous method that improves the stability of biocatalysts and provides for their repeated use and easy separation from the reaction medium (MINOVSKA et al., 2005; KATO et al., 2011). Lipases have been entrapped by sol-gel matrix employing different additives (KANDIMALLA et al., 2006; REETZ et al., 1996; SOUZA et al., 2012).

The use of additives in the immobilization process yields a significant improvement in the activity and stability of immobilized enzymes (REETZ et al., 1996; SOARES et al., 2003). Although specific literature has often reported the use of additives, such as albumin and polyethylene glycol (SOARES et al., 2003), sporopollenin (YILMAZ et al., 2010), zeolite

(VIDINHA et al., 2006), calixarene derivatives (SAHIN et al., 2009), ionic liquid (SOUZA et al., 2013), the use of ionic liquids (IL) in all areas of chemical industries has an excellent prospective due to their unique properties (KESKIN et al., 2007). Ionic liquids are used as an additive in the immobilization process as agents that protect the enzyme from deactivation and modify the support's morphology surface (SOUZA et al., 2013; FAUZI; AMIN, 2012).

Ionic liquids (ILs) are organic salts with melting points around or below the ambient temperature, negligible vapor pressure, high chemical and thermal stabilities, and constituted by organic cations and organic or inorganic anions (LIU et al., 2012). Protic ionic liquids (PILs) comprise a subset of the ionic liquids formed by the stoichiometric (equimolar) combination of a Brønsted acid with a Brønsted base and are characterized by their great ability to form H-bond and consequently show a strong interaction with polar solvents (ANOUTI et al., 2010).

The use of PILs in the process of enzyme immobilization is not usually reported in the literature. However, a study by Souza et al. (2013) using lipase *Burkholderia cepacia* encapsulated on hydrophobic matrixes obtained by sol-gel technique using protic ionic liquid as additive in the immobilization process showed promising results. The authors demonstrated an outstanding increase in the immobilization yield (over 1000%) compared to the activity offered by the immobilized enzymes without PIL.

Current research analyzes the effects of lipase from *Burkholderia cepacia* encapsulated in sol-gel matrix using protic ionic liquid in the transesterification of non-edible vegetable oil under conventional heating by employing a full factorial design 2^2 with three replicates of the center point.

Material and methods

Materials and reagents

Commercial lipase from *Burkholderia cepacia* (1,317.16 U mg⁻¹) was purchased from Sigma Aldrich. Tetraethoxysilane (TEOS) was supplied by Across Organic (New Jersey, United States). Ethanol (> 99% pure), ammonia (28% pure), hydrochloric acid (minimum 36% pure) and Arabic gum (85% pure) were obtained from Synth (São Paulo State, Brazil). Ultrapure water, double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus, was used throughout the experiments. The protic ionic liquid N-methylmonoethanolamine pentanoate, encoded as C₅ (Figure 1), was supplied by the Federal University of

Bahia (UFBA). Babassu oil, kindly donated by Cognis (Jacareí, São Paulo State, Brazil), contained the following fatty acids (% w v⁻¹): 3.5 caprylic, 4.5 capric, 44.7 lauric, 17.5 myristic, 9.7 palmitic, 3.1 steric, 15.2 oleic and 1.8 linoleic. The average molecular weight of babassu oil is 709.90 g mol⁻¹. All chemicals were of analytical grade.

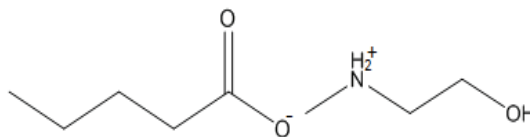


Figure 1. Protic ionic liquid structure N-methylmonoethanolamine pentanoate.

Lipase immobilization

The sol-gel matrixes encapsulation methodology applied by Souza et al. (2013) was used with some modifications. It may be briefly described as follows: 30 mL of TEOS were dissolved in 36 mL of absolute ethanol under an inert nitrogen atmosphere. Further, 0.22 mL of hydrochloric acid was dissolved in 5 mL of slowly added ultra-pure water. The mixture was stirred (200 rpm) for 90 min. at 35°C. The commercial lipolytic enzyme from *B. cepacia* (2.7 g) was dissolved in a solution of 10 mL of ultra-pure water to which 1% (w v⁻¹) of N-methylmonoethanolamine pentanoate (protic ionic liquid) was simultaneously added. Further, 1.0 mL of ammonium hydroxide dissolved in 6.0 mL of ethanol (hydrolysis solution) was added to the sol-gel reaction and the mixture was kept under static conditions for 24h until complete polycondensation. The bulk gel was washed with heptane and acetone and dried in a vacuum at room temperature for 72h.

The reloading of the previously immobilized system was carried by adsorption, following Freitas et al. (2009). The immobilized system was soaked in hexane and stirred (100 rpm) at 25°C. After 1h the excess of hexane was removed and lipase was added at a ratio of 1:4 gram of enzyme per gram of support. PEG-1500 and the enzyme solution were added together at a fixed amount (100 μL g⁻¹ of support). Lipase-support system was maintained in contact for 16h at 4°C under static conditions, filtered and thoroughly rinsed with hexane.

Lipase activity

Enzymatic activities of free and immobilized lipase samples were assayed by olive oil emulsion method according to modifications by Soares et al. (1999). One unit (U) of enzyme activity was defined as the amount of enzyme that released 1 mol of free fatty acid per min

under assay conditions (37°C; pH 7.0; 5 min. incubation).

Ethyl esters synthesis

The reactions were performed according to the methodology described previously by Freitas et al. (2009) in closed reactors with a capacity of 25 mL containing 12 g of substrate with babassu oil and anhydrous ethanol and without the addition of solvents. The mixtures were incubated with the immobilized lipase in the proportion of 20% (w w⁻¹) in relation to the total weight of reactants involved in the reaction media. Reactions were performed for a maximum period of 48h under constant magnetic agitation of 150 rpm, stressing that two reactions entitled 1 and 2 were performed for 96h. An aliquot of reaction medium was taken at various time intervals for GC-analysis.

The ethyl esters formed 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., São Paulo, São Paulo State, Brazil). Nitrogen constituted the gas carrier, with a flow rate of 25 mL min.⁻¹. Temperature programming was used. 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 minutes. Further, temperature was programmed from 25°C min.⁻¹ to 170°C and kept constant for 15 minutes. The temperatures of the injector and detector were set at 250°C. Data collection and analyses were performed with Galaxie Chromatography Data System 1.9. Calibration curves were built from standard ethyl esters using hexanol as internal standard. The transesterification yield was calculated by taking into account the mass of ester content detected by GC analysis and the total theoretical ester mass based on the molar ratio of the reaction.

Experimental design

Experiments were carried out according to the full 2² factorial design, with three replicates at the central point to study temperature effect, ethanol to babassu molar ratio (independents variables) on yield of ethyl esters.

Ethanol to babassu molar ratio (X₁) and temperature (X₂) were chosen as independent variables. Level selection was carried out from results obtained in a preliminary study (Table 1).

Table 1. Factor levels used according to the 2² full factorial design.

Variables	Symbols	Levels
Molar ratio (babassu oil to ethanol)	X ₁	1:7 1:10 1:13
Temperature (°C)	X ₂	40 48 56

The transesterification yield corresponded to response variable. Results were analyzed with Statistica 8.0 to verify the effect of the independent variable, assuming levels at p < 0.1 as statistical significance criterion. Student's test assayed the statistical significance of the regression coefficient.

Downstream procedure

At the end of the reaction with the highest yield on ethyl esters, the lipase was separated from the medium and washed three times with water to remove the free glycerol formed as a by-product. Residual ethanol was removed by pervaporation and the remaining water was removed by adding sodium sulfate salt to attain the final fatty acid ethyl ester product (ANDRADE et al., 2012).

Determination of viscosity

The absolute viscosity of biodiesel was measured with LVDV-II cone and plate spindle Brookfield viscosimeter (Brookfield Viscometers Ltd. UK) utilizing a CP 42 cone. A circulating water bath maintained temperature at 40°C for each analysis. Samples of 0.5 mL were used and measurements were replicated three times. Shear stress measurements were taken as a function of shear rate and the dynamic viscosity was determined as a slope constant.

Results and discussion

The lipase catalyzed reaction rate depends on the concentrations of enzyme, substrate, temperature and other factors (DA RÓS et al., 2012). The study of the molar ratio (X₁) and temperature (X₂) effect in the transesterification reaction was carried out in a solvent free system catalyzed by *Burkholderia cepacia* encapsulated in sol-gel matrix using protic ionic liquid (N-methylmonoethanolamine pentanoate) as additive (hydrolytic activity: 428.58 ± 25.79 U g⁻¹). The enzymatic transesterification reaction is generally performed at lower temperature than that of the chemical reaction to prevent the loss of lipase activity (GOG et al., 2012). The temperature studied ranged between 40 and 56°C and the molar ratio oil: alcohol was 1:7 - 1:13.

Results shown in Table 2 clearly indicated that the highest conversion babassu oil in ethyl esters, or rather, 51.90%, was obtained with the smallest excess alcohol (1:7) at 40°C for 48h reaction.

Table 2. Experimental design and values for yield conversion (%) according to the 2² full factorial design (variables in coded values with true values in parenthesis).

Runs	Oil-to-Ethanol Molar Ratio	Temperature (°C)	Yield (%)
1	-1 (1:7)	-1 (40)	51.90
2	+1 (1:13)	-1 (40)	44.07
3	-1 (1:7)	+1 (56)	35.41
4	+1 (1:13)	+1 (56)	35.01
5	0 (1:10)	0 (48)	46.66
6	0 (1:10)	0 (48)	40.15
7	0 (1:10)	0 (48)	41.79

Figure 2 shows that when molar ratio and temperature rates passed from +1 (1:13, 56°C) to -1 (1:7, 40°C), the highest conversions of babassu oil in ethyl esters were obtained. Table 3 shows that factors such as molar ratio, curvature and interaction between molar ratio and temperature were not significant at $p > 0.1$.

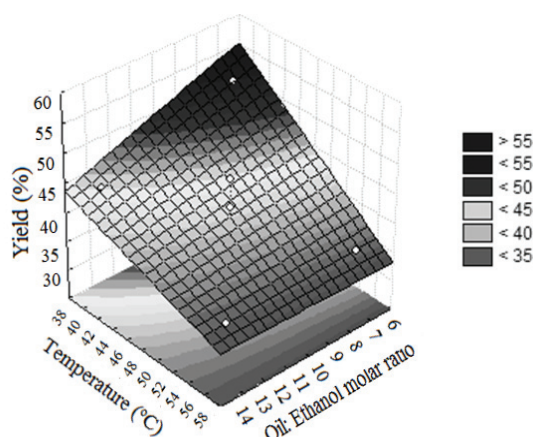


Figure 2. Response surface representing the transesterification yield of babassu oil catalyzed by lipase from *Burkholderia cepacia* encapsulated in sol-gel matrix using protic ionic liquid as additive.

Table 3. Analysis of variance (ANOVA) for transesterification yield (%) of babassu oil using the 2² full factorial design.

Factor	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Curvature	2.76	1	2.76	0.24	0.672
(X ₁) Molar Ratio	16.93	1	16.93	1.48	0.348
(X ₂) Temperature	163.20*	1*	163.20*	14.23*	0.064*
X ₁ X ₂	13.80	1	13.80	1.20	0.387
Pure Error	22.93	2	11.46		
Total	219.62	6			

*Significant at 90% confidence level.

Regression coefficient and p-values were calculated from the experimental results. Tables 3 and 4 show that temperature was the most significant independent variable. Thus, transesterification yields may be fitted to a three-dimensional surface described by a first-order model as a function of X₁ and X₂ (Equation 1; Figure 2). Rates of R² (0.89) and R² adj (0.69) of the model suggest a close agreement between the experimental data and the theoretical values predicted

by the model. No optimal conditions were observed in the response surface for the transesterification of babassu oil. Therefore, only the region corresponding to the best response may be identified.

The model equation for the response may therefore be written as ($p < 0.1$) in the following first-order equation that describes the response surface (1):

$$\text{Yield (\%)} = 41.60 - 2.06 \cdot X_1 - 6.39 \cdot X_2 + 1.86 \cdot X_1 \cdot X_2 \quad (1)$$

Table 4. Regression coefficient, standard errors, and Student's t test for transesterification yield of babassu oil (%) using the 2² full factorial design.

Factor	Regression coefficient	Standard Error Pure Error	t value	P value
Mean/Interc.	41.60	1.69	24.57	0.002
Curvature	1.27	2.59	0.49	0.672
(X ₁) Molar Ratio	-2.06	1.69	-1.21	0.348
(X ₂) Temperature	-6.39*	1.69*	-3.77*	0.064*
X ₁ X ₂	1.86	1.69	1.10	0.387

* $p < 0.1$

In current assay, the amount of alcohol added in the babassu oil transesterification, equivalent to a molar ratio of 1:10 and 1:13, yielded ethyl esters lower than 50%. According to Tongboriboon et al. (2010), a greater amount of ethanol would cause an inhibitory effect on the enzymatic activity of the mixed lipase (*Pseudomonas fluorescens* and *Candida rugosa*). Alcohols are able to bind with proteins and induce their dehydration, resulting in a drastic loss of enzyme activity (CAMBON et al., 2009). Although theoretically transesterification reaction of one mole of triglyceride required three moles of ethanol, the yield of ethylic esters of fatty acids depended on the preferred equilibrium under various conditions (CHEIRSILP et al., 2008). Further, another hypothesis (CAMBON et al., 2009) demonstrated that when alcohols were dissolved in the oil phase, the effect of denaturation of the enzyme was greatly reduced due to the hydrogen bonds with the lipid species. It may therefore be suggested that the amount of alcohol in the reaction medium prepared at molar ratio oil to ethanol of 1:7 produced the highest transesterification yield possible by decreasing the substrate mass transfer limitations.

Moreover, temperature also influenced enzymatic activities. According to the Arrhenius equation, the reaction rate increased as temperatures rose. However, enzyme inactivation occurred and catalytic activity decreased above a certain temperature degree (SU et al., 2007). Cambon et al. (2009) reported that decreasing temperature from 55°C to 30°C increased the production of esters, with a growth in conversion rate from 55 to 73%.

Freitas et al. (2009) achieved the highest transesterification yield while operating under the lowest excess ethanol (1:7) and temperature (39°C) when they used babassu oil as a triglycerides source. The authors used as catalyst the lipase from *Burkholderia cepacia* immobilized on SiO₂-PVA and thus confirmed the lipase's profile in this condition. The above is very similar to current study using *Burkholderia cepacia* lipase encapsulated in sol-gel matrix using protic ionic liquid (N-methylmonoethanolamine pentanoate) as an additive.

The performance of *Burkholderia cepacia* lipase encapsulated in current assay may be considered satisfactory when compared with performances reported by other lipase preparations, such as *Burkholderia cepacia* immobilized on niobium oxide hydrate (Nb₂O₅) (DA RÓS et al., 2010), in which the immobilized biocatalyst provided 40.21% of the ethyl esters yield for a system composed of beef tallow and ethanol, for 48h. Lipase from immobilized *Pseudomonas fluorescens*, studied by Iso et al. (2001), showed a conversion of safflower oil between 0 and 50% for 50h when reaction consisting of the absence or 90 wt% of 1,4-dioxane.

Current assay showed promising results when compared to that by Souza et al. (2013). These researchers evaluated the production of ethyl esters from soybean oil and ethanol using lipase from *Burkholderia cepacia* encapsulated in matrix obtained by sol-gel technique and the same protic ionic liquid as additive. The authors reported 46.5% yield in 72h of reaction at 1:15.2 molar ratio and 0.075 g water.

Catalytic efficiency was the ratio between catalytic activity and enzyme loading. It provided the activity per milligram of immobilized enzyme. Therefore, this parameter was useful for comparative purposes among biocatalysts with different enzyme loading (SERRA et al., 2010). Rates obtained for hydrolytic activity (2176.5 U g⁻¹ ± 99.48 U g⁻¹) showed that catalytic efficiency of immobilized lipase by adsorption with enzyme loading increased more than five times than other enzyme loading studied (*Burkholderia cepacia* lipase encapsulated in sol-gel matrix using protic ionic liquid as additive).

In an attempt to evaluate the transesterification reaction by a biocatalyst with high lipase loading, a further experiment was performed and the results were compared with those attained by the biocatalyst with low lipase loading. Both reactions were carried out for 96h with babassu oil and ethanol as raw materials. Results may be analyzed under two different perspectives: the transesterification yield (%) and formation of ethyl monoesters as a function of time (Figures 3 and 4). The above results indicated that

maximal transesterification yield (98.69%) was achieved by immobilized enzyme with high lipase loading (Reaction 2, Figure 4). Compared with reaction 1, the biocatalyst used in the experimental design exhibited a much lower transesterification yield (78.26%) (Figure 3).

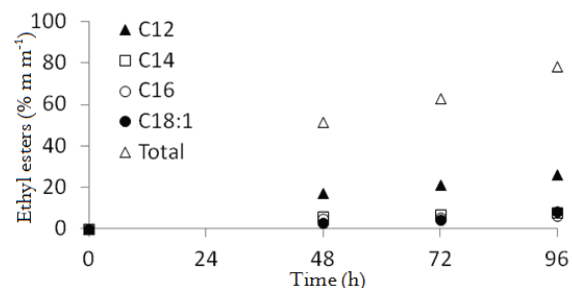


Figure 3. Formation profile of ethyl monoesters as a function of time for reaction 1 (babassu oil transesterification with molar ratio 1:7 at 40°C) catalyzed by *Burkholderia cepacia* lipase encapsulated in sol-gel matrix using protic ionic liquid as additive (20 %, w w⁻¹).

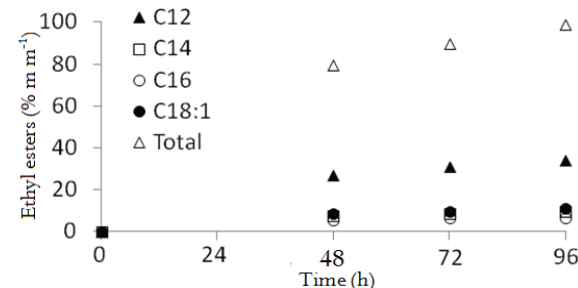


Figure 4. Formation profile of ethyl monoesters as a function of time for reaction 2 (babassu oil transesterification with molar ratio 1:7 at 40°C) catalyzed by *Burkholderia cepacia* lipase encapsulated in sol-gel matrix using protic ionic liquid as additive after enzyme loading increase (20 %, w w⁻¹).

The second reaction revealed that total conversion of the fatty acids in the babassu oil reached their corresponding esters in the 96h reaction. The highest ethyl ester concentrations referred to ethyl laurate. In the two experiments approximately 30% of the C12 was formed consonant to the babassu oil fatty acids composition.

Comparatively, transesterification yield (%) in other studies with lipase immobilized on SiO₂-PVA indicated similar results to those obtained in current research after enzyme loading increase (DA RÓS et al., 2010). Nouredini et al. (2005) also reported that when enzyme loading (*Pseudomonas cepacia* lipase) increased, there was a sudden surge in the formation of alkyl esters from the transesterification of soybean oil.

The difference between the percentages of conversion of babassu oil in the corresponding esters during the two reactions showed the influence of catalytic activity of the biocatalysts used. The viscosity values determined at 40°C were 6.67 and 4.29 mm² s⁻¹

for reactions 1 and 2, respectively. According to the effective specified standards by the European Union EN-14214: (3.5 - 5.0 mm² s⁻¹), American Society for Testing and Materials ASTM D6751 (1.9 - 6.0 mm² s⁻¹) (BSG, 2012) and ANP 255 Brazil (2.5 - 5.5 cSt) (DA RÓS et al., 2012), the viscosity rate attained in reaction 2 was within the recommended range.

Conclusion

Experimental design was employed to study ethyl esters production catalyzed by lipase from *Burkholderia cepacia* encapsulated in sol-gel matrix. A mathematical model was composed by the above technique which explained the variation of transesterification yields as a function of independent variables (molar ratio and temperature). The maximal transesterification yield was 51.9%, obtained in the molar ratio 1:7 and 40°C. When the reaction was performed with biocatalyst with high lipase loading, the yield on ethyl esters increased to 98.69%, which in turn, originated a sample with appropriate property to be used as a fuel (viscosity value of 4.29 mm² s⁻¹).

Acknowledgements

The authors would like to thank the Brazilian Council for Scientific and Technological Development (CNPq) for its financial support and for the scholarship awarded to Maria Vanessa Souza Oliveira.

References

ANDRADE, G. S. S.; FREITAS, L.; OLIVEIRA, P. C.; CASTRO, H. F. Screening, immobilization and utilization of whole cell biocatalysts to mediate the ethanolysis of babassu oil. **Journal of Molecular Catalysis B: Enzymatic**, v. 84, p. 183-188, 2012.

ANOUTI, M.; JACQUEMIN, J.; LEMORDANT, D. Transport properties of protic ionic liquids, pure and in aqueous solutions: Effects of the anion and cation structure. **Fluid Phase Equilibria**, v. 297, n. 1, p. 13-22, 2010.

ANTCZAK, M. S.; KUBIAK, A.; ANTCZAK, T.; BIELECKI, S. Enzymatic biodiesel synthesis – key factors affecting efficiency of the process. **Renewable Energy**, v. 34, n. 5, p. 1185-1194, 2009.

BAJAJ, A.; LOHAN, P.; JHA, P. N.; MEHROTRA, R. Biodiesel production through lipase catalyzed transesterification: an overview. **Journal of Molecular Catalysis B: Enzymatic**, v. 62, n. 1, p. 9-14, 2010.

BSG-Biofuel Systems Group. **Biodiesel standards**. Available from: <<http://www.biofuelsystems.com/specification.htm>>. Access on: Aug. 13, 2012.

CAMBON, E.; BOURLIEU, C.; SALUM, T. F. C.; PIOMBO, G.; DUBREUCQ, E.; VILLENEUVE, P. Ability of *Vasconcellea heilbornii* lipase to catalyze the synthesis of alkyl esters from vegetable oils. **Process Biochemistry**, v. 44, n. 11, p. 1265-1269, 2009.

CHEIRSILP, B.; H-KITTIKUN, A.; LIMKATANYU, S. Impact of transesterification mechanisms on the kinetic modeling of biodiesel production by immobilized lipase. **Biochemical Engineering Journal**, v. 42, n. 3, p. 261-269, 2008.

DA RÓS, P. C. M.; SILVA, G. A. M.; MENDES, A. A.; SANTOS, J. C.; CASTRO, H. F. Evaluation of the catalytic properties of *Burkholderia cepacia* lipase immobilized on non-commercial matrices to be used in biodiesel synthesis from different feedstocks. **Bioresource Technology**, v. 101, n. 14, p. 5508-5516, 2010.

DA RÓS, P. C. M.; CASTRO, H. F.; CARVALHO, A. K. F.; SOARES, C. M. F.; MORAES, F. F.; ZANIN, G. M. Microwave-assisted enzymatic synthesis of beef tallow biodiesel. **Journal of Industrial Microbiology and Biotechnology**, v. 39, n. 4, p. 529-536, 2012.

FAUZI, A. H. M.; AMIN, N. A. S. An overview of ionic liquids as solvents in biodiesel synthesis. **Renewable and Sustainable Energy Reviews**, v. 16, n. 8, p. 5770-5786, 2012.

FREITAS, L.; DA RÓS, P. C. M.; SANTOS, J. C.; DE CASTRO, H. F. An integrated approach to produce biodiesel and monoglycerides by enzymatic interesterification of babassu oil (*Orbinya* sp.). **Process Biochemistry**, v. 44, p. 1068-1074, 2009.

GOG, A.; ROMAN, M.; TOS, M.; PAIZS, C.; IRIMIE, F. D. Biodiesel production using enzymatic transesterification - Current state and perspectives. **Renewable Energy**, v. 39, n. 1, p. 10-16, 2012.

ISO, M.; CHEN, B.; EGUCHI, M.; KUDO, T.; SHRESTHA, S. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. **Journal of Molecular Catalysis B: Enzymatic**, v. 16, n. 1, p. 53-58, 2001.

KANDIMALLA, V. B.; TRIPATHI, V. S.; JU, H. Immobilization of biomolecules in sol-gels: biological and analytical applications. **Critical Reviews in Analytical Chemistry**, v. 36, n. 2, p. 73-106, 2006.

KATO, K.; NAKAGAKI, S.; NISHIDA, M.; HIRAO, K. Enzyme encapsulation in silica particles prepared using enzyme-assisted sol-gel reactions in ionic liquids. **Journal of the Ceramic Society of Japan**, v. 119, n. 2, p. 140-143, 2011.

KESKIN, S.; KAYRAK-TALAY, D.; AKMAN, U.; HORTAÇSU, O. A review of ionic liquids towards supercritical fluid applications. **Journal of Supercritical Fluids**, v. 43, n. 1, p. 150-180, 2007.

LIU, C.; WANG, F.; STILES, A. R.; GUO, C. Ionic liquids for biofuel production: Opportunities and challenges. **Applied Energy**, v. 92, p. 406-414, 2012.

MINOVSKA, V.; WINKELHAUSEN, E.; KUZMANOVA, S. Lipase immobilized by different techniques on various support materials applied in oil hydrolysis. **Journal of the Serbian Chemical Society**, v. 70, n. 4, p. 609-624, 2005.

NOUREDDINI, H.; GAO, X.; PHILKANA, R. S. Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. **Bioresource Technology**, v. 96, n. 7, p. 769-777, 2005.

- REETZ, M. T.; ZONTA, A.; SIMPELKAMP, J. Efficient immobilization of lipases by entrapment in hydrophobic sol-gel materials. **Biotechnology and Bioengineering**, v. 49, n. 5, p. 527-534, 1996.
- SAHIN, O.; ERDEMIR, S.; UYANIK, A.; YILMAZ, M. Enantioselective hydrolysis of (R/S)-Naproxen methyl ester with sol-gel encapsulated lipase in presence of calix[n]arene derivatives. **Applied Catalysis A: General**, v. 369, n. 1-2, p. 36-41, 2009.
- SERRA, E.; DÍEZ, E.; DÍAZ, I.; BLANCO, R. M. A comparative study of periodic mesoporous organosilica and different hydrophobic mesoporous silicas for lipase immobilization. **Microporous and Mesoporous Materials**, v. 132, n. 3, p. 487-493, 2010.
- SOARES, C. M. F.; CASTRO, H. F.; MORAES, F. F.; ZANIN, G. M. Characterization and utilization of *Candida rugosa* lipase immobilized on controlled pore silica. **Applied Biochemistry and Biotechnology**, v. 77-79, n. 1-3, p. 745-757, 1999.
- SOARES, C. M. F.; SANTANA, M. H. A.; ZANIN, G. M.; CASTRO, H. F. Efeito do polietilenoglicol e da albumina na imobilização de lipase microbiana e na catálise em meio orgânico. **Química Nova**, v. 26, n. 6, p. 832-838, 2003.
- SOUZA, R. L.; FARIA, E. L.; FIGUEIREDO, R. T.; FREITAS, L. S.; IGLESIAS, M.; MATTEDI, S.; ZANIN, G. M.; SANTOS, O. A.; COUTINHO, J. A. P.; LIMA, A. S.; SOARES, C. M. F. Protic ionic liquid as additive on lipase immobilization using silica sol-gel. **Enzyme and Microbial Technology**, v. 52, n. 3, p. 141-150, 2013.
- SOUZA, R. L.; RESENDE, W. C.; BARÃO, C. E.; ZANIN, G. M.; CASTRO, H. F.; SANTOS, O. A. A.; FRICKS, A. T.; FIGUEIREDO, R. T.; LIMA, A. S.; SOARES, C. M. F. Influence of the use of Aliquat 336 in the immobilization procedure in sol-gel of lipase from *Bacillus* sp. ITP-001. **Journal of Molecular Catalysis B: Enzymatic**, v. 84, p. 152-159, 2012.
- SU, E.; ZHANG, M.; ZHANG, J.; GAO, J.; WEI, D. Lipase-catalyzed irreversible transesterification of vegetable oils for fatty acid methyl esters production with dimethyl carbonate as the acyl acceptor. **Biochemical Engineering Journal**, v. 36, n. 2, p. 167-173, 2007.
- TONGBORIBOON, K.; CHEIRSILP, B.; H-KITTIKUN, A. Mixed lipases for efficient enzymatic synthesis of biodiesel from used palm oil and ethanol in a solvent-free system. **Journal of Molecular Catalysis B: Enzymatic**, v. 67, n. 1-2, p. 52-59, 2010.
- VIDINHA, P.; AUGUSTO, V.; ALMEIDA, M.; FONSECA, I.; FIDALGO, A.; ILHARCO, L.; CABRAL, J. M. S.; BARREIROS, S. Sol-gel encapsulation: an efficient and versatile immobilization technique for cutinase in non-aqueous media. **Journal of Biotechnology**, v. 121, n. 1, p. 23-33, 2006.
- YILMAZ, E.; SEZGIN, M.; YILMAZ, M. Enantioselective hydrolysis of racemic naproxen methyl ester with sol-gel encapsulated lipase in the presence of sporopollenin. **Journal of Molecular Catalysis B: Enzymatic**, v. 62, n. 2, p. 162-168, 2010.

Received on February 20, 2013.

Accepted on June 10, 2013.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.