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ESTERIFICATION OF OLEIC ACID AND ETHYL ALCOHOL FOR SYNTHESIS OF ETHYL OLEATE CATALYZED BY LIPASE IMMOBILIZED IN POLYURETHANE

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ABSTRACT: Esters are one of the most important classes of organic compounds that are synthesized by various mechanisms, including the reaction between an alcohol and carboxylic acid, with the elimination of water, called esterification. Accordingly, the purpose of this study was to evaluate the production of ethyl oleate by esterification of oleic acid with ethanol in the presence of lipase from Candida antarctica B free and immobilized in polyurethane subjected to a mechanical and ultrasonic system and organic solvent free. The experiments for the synthesis of ethyl oleate were conducted by varying the temperature (°C) and mass catalyst (g). The maximization of esterification results is obtained for the free enzyme after 25 minutes (688.24 U g^{-1}). For polyurethane immobilized enzyme after 40 minutes of mechanical stirring reaction system was reached 1553.83 U g^{-1} and 19 cycles of reuse. Considering the ultrasonic agitation system after 15 minutes of reaction was reached 3087.60 U g^{-1} and 14 cycles of reuse. From the obtained results, we can consider that the established process was efficient, mainly in relation to the significant reduction of the reaction time, with low instrumental requirements employed and the improvement of the general performance of the bioprocess, through the results obtained in terms of esterification. In addition, the established process can be considered an environmentally friendly and economically viable technology, and can be used in cosmetics, pharmaceuticals, biodiesel and food industry.

Palavras-chave: *Candida antarctica*. Lipase B. Ester synthesis. Isoamyl acetate. Biocatalysis.

ESTERIFICAÇÃO DE ÁCIDO OLEICO E ÁLCOOL ETÍLICO PARA SÍNTESE DE OLEATO DE ETILA CATALIZADA POR LIPASE IMOBILIZADA EM POLIURETANO

RESUMO: Os ésteres são uma das mais importantes classes de compostos orgânicos que são sintetizados por vários mecanismos, incluindo a reação entre um álcool e ácido carboxílico, com a eliminação de água, denominada esterificação. Nesse sentido, o objetivo desse estudo foi avaliar a produção de oleato de etila via esterificação do ácido oleico com álcool etílico na presença da enzima lipase B de *Candida antarctica* livre e imobilizada em poliuretano submetido a um sistema de agitação mecânico e ultrassônico e livre de solvente orgânico. Os experimentos para a síntese de oleato de etila foram realizados variando a temperatura (°C) de massa de catalisador (g). A maximização dos resultados de esterificação é obtida para a enzima livre após 25 minutos com 688.24 U g^{-1}. Para a enzima imobilizada em poliuretano após 40 minutos de reação em sistema de agitação mecânico foi de 1553.83 U g^{-1} e 19 ciclos de reutilização. Para o sistema de agitação ultrassônico de 3087,60 U g^{-1} com 14 ciclos de reutilização após 15 minutos de reação. A partir dos resultados obtidos podemos considerar que o processo estabelecido foi eficiente, principalmente em relação a redução significativa do tempo de reação, com baixos requisitos instrumentais empregados e a melhoria do desempenho geral do bioprocesso, através dos resultados obtidos em termos de esterificação. Além disso, o processo estabelecido pode ser considerado uma tecnologia ambientalmente correta e economicamente viável, e pode ser usada em produtos cosméticos, farmacêuticos, biodiesel e indústria de alimentos.

Keywords: *Candida antarctica* lipase B, síntese de ésteres, acetato de isoamila, biocatalise.

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INTRODUCTION

Ethyl oleate is useful as biological additive, PVC plasticiser, water resisting agent and for hydraulic fluid. The enzymatic technology presents significant relevance since represents promising tools for compounds synthesis of high commercial value. Besides, enzymes show many advantages compared to inorganic catalysts, as higher specificity, lower energy consumption and higher catalytic activity (NYARI et al., 2016; CAZABAN et al., 2017; SCHREIBER et al., 2017). Even with the benefit of this technology, replacement of chemical catalysts by enzymes is not generalized in industrial processes due to the instability, final product contamination by the catalyst solution, difficulty of eliminating the catalyst in final product and impossibility of recuperation and reutilization (ADLERCREUTZ, 2013; FERNANDEZ-LOPEZ et al., 2017; TOLEDO et al., 2017; NYARI et al., 2018; MATTIASSON, 2018).

Due to several advantages of enzyme catalysis in organic solvents, the synthesis of ethyl oleate and other esters of oleic acid have been studied by exploiting the catalytic activities of enzymes (ITSEKSON et al., 2011; DESHMUKH et al., 2013; KHAN; RATHOD, 2015; MADALOZZO et al., 2015; VESCOVI et al., 2017; CANET et al., 2017; DILL et al., 2018).

One of the cheap and commercially available nonmicrobial enzymes is *Candida antarctica* lipase B which has high thermostability, such as high selectivity and specificity, mild reaction conditions, wide pH range, activity in anhydrous reaction mixtures as demonstrated for esterification and transesterification reactions, allowing to obtain products with high purity, reduction of co - products and/or toxic waste, consequently reducing the environmental impact (TOMIN et al., 2010; DHAKE et al., 2013; HIRATA et al., 2016).

To be economically viable and an efficient process, the biocatalyst immobilization is an alternative for the use of a free enzyme, mainly for its reuse and for the possibility of performs the continuous process (GARCIA - GALAN et al., 2011; BATISTELLA et al., 2012). Besides allowing the enzyme its recovery, with displacement in pH value, adjustable porosity, low toxicity, favorable mechanical properties, temperature and stability (thermal, operational and storage), allowing the biotechnology process becomes economically viable (POOJARI et al., 2013; BABAKI et al., 2015; BABAKI et al., 2016).

Preliminary results demonstrated the potential use of polyurethane as support for *Candida antarctica* B lipase (CALB lipase) immobilization, increasing significantly the reaction yield and the enzyme thermal stability (NYARI et al., 2016).

The ultrasonic system is considered a green technology, little explored yet, is an alternative technology for the conventional mechanical agitation that provides significant reductions in the processing time and can increase conversion yields. These characteristics can be explained by the better mass transfer between substrates and enzyme and protection to the exterior, permitting an increase of lipolytic activity, as a result of improvement on the microenvironment created by the polymeric lattice, protecting the enzyme of adverse effects of temperature and also simulating the effects of agglomeration and confinement of living cells (ZHANG et al., 2008; ABOU-OKEIL et al., 2010; MATTE et al., 2016).

Considering the peculiarities of each process, which, depending on the operating conditions used, can interfere or contribute in a positive or negative way in the biocatalytic esterification process of ethyl oleate. Accordingly, the purpose of this study was to evaluate the production of ethyl oleate by esterification of oleic acid with ethanol in the presence of lipase B from *Candida antarctica* free and immobilized in polyurethane subjected to a mechanical and ultrasonic system, varying the temperature (°C) and mass catalyst (g) and organic solvent free.

MATERIAL AND METHODS

Lipase B from *Candida antarctica* - CALB (Novozyme NZL - 102), oleic acid (Vetec, 99%), ethanol (Merck, P.A), and dichloromethane (Vetec, 95%). Toluene diisocyanate (TDI) and polyether polyol were kindle donated by Manes Industry (Santa Catarina, Brazil).

Immobilization of CALB lipase in polyurethane (PU)

CALB immobilization on PU was performed using 6 mL polyol and 4 mL isocyanate (60–40%, v/v), with 1 mL of enzymatic solution (0.8 g enzyme in 5 mL distilled water). The enzymatic solution was added in the polyol and homogenized. After, isocyanate was added at constant stirring. The in situ polymerization was conducted at 20 ºC, until growing the PU foam (5 minutes), remaining stationary for 3 h and then crushed, producing a homogeneous product. 1 g of this product was washed with 5 mL of buffer solution (sodium phosphate pH 7). It was evaluated the enzyme leaching from the support by the measurement of esterification activity of the wash solution by methodology Nyari et al. (2016).

Immobilization of CALB lipase in polyurethane (PU)

The immobilization yield was defined as the relationship between the total activity offered (UT0) (611.5 UT0), calculated considering the amount of free enzyme (0.2 g) used in the incorporation step, and the experimental total activity (UTExp.) (immobilized enzyme is 1002.98 U mg^{-1} and free enzyme is 1753.24 U mg^{-1}), calculated by taking account the total activity of the PU (3271.5 UTExp) support incorporated with the enzyme (10.8 g) respectively. The UT0 and values obtained for the free and immobilized enzymes. There

was an increase in the activity of the immobilized enzyme was 302.9 U mg^{-1} with yield 5535.3%. These results suggest a beneficial effect of immobilization in the enzyme activity and associated with several factors, such as the easy acces-sibility of new active sites by previous study Nyari et al. (2016).

Esterification of ethyl oleate

The esterification of oleic acid and ethyl alcohol to ethyl oleate ester was carried out in triplicate (n=3) in 50 mL glass flask keeping constant molar ratio oleic acid to ethanol in 1:1 (5 g). After each reaction, the reaction medium was filtered to separate the immobilized biocatalyst to the reactional medium. 500 µL aliquots, performed in triplicate, were taken from the reaction mixture. 15 mL of acetone - ethanol solution was added in each sample. Titration with NaOH 0,05 mol L^{-1} was the method used to determine the amount of oleic acid that have reacted until the system reach the pH 11. The blank samples were made by mixing 500 µL of standard mixture and 15 mL of acetone - ethanol solution.

Enzyme activity unit was defined as the amount of enzyme that is able to convert 1 µmol of fatty acid per minute, calculated by the equation (1).

$$AE = \frac{(V_b - V_a) \times M \times 1000 \times V_f}{t.M_{EL} \times V_c} \quad (1)$$

Where: AE: Esterification (U g^{-1}); Va: Volume of NaOH consumed during the sample titration (mL); Vb: Volume of NaOH consumed during the blank sample titration (mL) M: Molarity of NaOH solution; Vf: Final Volume of the reaction medium; t: time (minutes); m: free enzyme mass or immobilized enzyme mass (g); Vc: Aliquot Volume of the reaction medium withdrawal from the titration (mL).

Reactions: mechanical and ultrasonic systems

The kinetic study was conducted to evaluate the effect of the reaction time (0 to 90 minutes) in terms of esterification to ethyl oleate. For the system with mechanical agitation, the variables studied were catalyst mass (0.018 - 0.582 g) and reaction temperature (35.9 - 64.1 °C), keeping mechanical stirring at 160 rpm and reaction time in 40 minutes. For the ultrasonic system, the variables studied were catalyst content (0.068 - 0.532 g), reaction temperature (25 – 75 °C) and ultrasonic power (26 - 93%), relative to maximum power 1800 A, US 40 KHz, US 132 W), the reaction time was fixed in 15 minutes and 160 rpm according to the methodology used for the mechanical stirring system.

Operational stability

The study of the operating cycles number for the immobilized catalyst used in the ethyl oleate ester synthesis was evaluated using optimized condition from Central Rotational Compound Design (RCCD). After each reaction, the catalyst was filtered to remove the reaction medium and reused in a new reaction. This process was successively repeated until conversion less than 50% of initial activity esterification. The results were expressed in terms of conversion, considering the initial esterification to 100%, calculated according to equation (2).

$$RA(\%) = \frac{U_{final}}{U_{initial}} \; x \; 100 \quad (2)$$

Where: RA (%) = Residual activity; U_{final} = Enzymatic activity after recycles or storage time; $U_{initial}$ = Enzymatic activity of reference (initial).

Statistical analysis

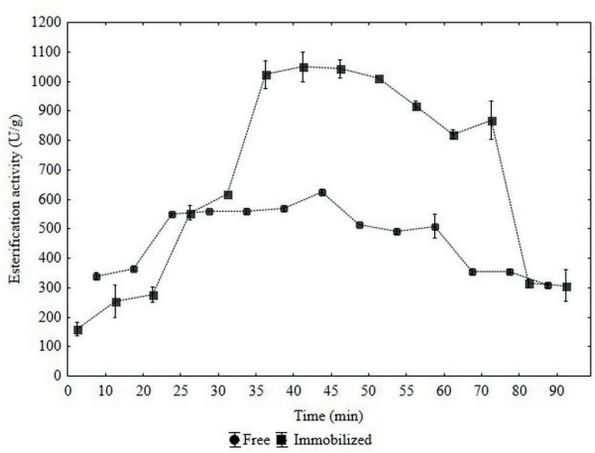
Each experiment was done in triplicate. Data were expressed as means ± standard deviation, and subjected to one-way analysis of variance (Tukey) using Statistica 8.0 (StatSoft) software. A significance level of 95% ($p < 0.05$) was used.

RESULTS AND DISCUSSION

Mechanical system

Figure 1 shows the evolution of ethyl oleate esterification of oleic acid and ethyl alcohol (90 minutes), for the catalyst free and immobilized according to the condition designed by the full RCCD 2^2 (Table 1).

Figure 1. Kinetics of ethyl oleate esterification for mechanical system immobilized and free



Source: Author

From the results, it was observed the maximal esterification of 558.78 U g^{-1} and 1071.42 U g^{-1}, for the catalyst free and immobilized after 25 and 40 minutes of reaction time, respectively.

Moreover, both assays show the same trend, increasing the acid precursor (esterification) conversion, besides the ester, water up to a certain time reaction. Followed by the reduction (hydrolysis), increasing the water concentration in the reaction system, through ester consumption, bound to lipases (LI et al., 2015).

In a general context, it is noted that in an esterification process were the reaction is reversible, it is extremely important to optimize reaction times, since it interferes significantly in the energy costs and in the economic feasibility of the process (AZUDIN et al., 2013).

Table 1 shows the full RCCD 22 for the esterification of oleic acid and ethyl alcohol, as a function of the studied variables, catalyst content (g) and temperature (°C).

Table 1. Matrix of full 22 RCCD experimental design using a free and immobilized catalyst in terms of ethyl oleate esterication by mechanical system

Run	Temperature (°C)	Catalyst content (g)	Free	Immobilized
1	-1 (40)	-1 (0.1)	140.26 ± 11.61	196.92 ± 13.77
2	1 (60)	-1 (0.1)	162.51 ± 11.51	540.00 ± 16.00
3	-1 (40)	1 (0.5)	250.00 ± 20.00	864.01 ± 13.58
4	1 (60)	1 (0.5)	553.85 ± 16.62	1223.26 ± 22.79
5	0 (50)	-1.41 (0.018)	189.66 ± 19.31	903.39 ± 11.02
6	0 (50)	1.41 (0.582)	688.24 ± 13.03	1302.98 ± 3.99
7	-1.41 (35.9)	0 (0.3)	358.33 ± 22.17	379.28 ± 11.07
8	1.41 (64.1)	0 (0.3)	448.10 ± 10.50	1372.32 ± 5.82
9	0 (50)	0 (0.3)	483.33 ± 28.68	1524.73 ± 17.57
10	0 (50)	0 (0.3)	433.33 ± 25.34	1553.83 ± 19.55
11	0 (50)	0 (0.3)	467.76 ± 26.88	1505.27 ± 14.51

***** Fixed parameters: substrate mass 5 g, oleic acid to ethanol molar ratio 1:1, reaction time 40 minutes and 160 rpm of mechanical agitation.

Source: Author

According to the results, the maximum esterification in terms of oleic acid was obtained in the assay 6 (688.24 U g^{-1}) to free after 25 minutes and assay 9, 10 and 11 (1553.83 U g^{-1}) to immobilized catalyst after 40 minutes of reaction time.

From the assays 1 and 3, 2 and 4, 5 and 7, 6 and 8, it was observed that esterification was directly proportional to the reaction temperature and catalyst content, indicating a positive effect of the temperature and catalyst (free or immobilized) in the oleic acid esterification.

This positive effect of temperature is consistent with the endothermic nature of the esterification reactions, which is characterized by the reversibility, that is, it presents a chemical equilibrium, indicates that it occurs with heat absorption, and that the increase of temperature provides an equilibrium in the reaction system, shifting the reaction for the products side, increasing reaction yield. The temperature effect can be related to the reduction in the system viscosity, reducing the mass transfer limitation.

Another variable of extreme importance, combined with temperature was the biocatalyst content, according to the Table 1, it was possible to observe an increase in the oleic acid conversion with the increase of the biocatalyst content. The increase in the biocatalyst content increases the number of active sites present in the reaction favoring the esterification.

In general, all tests using the immobilized catalyst showed higher conversions of butanoic acid in relation to the free catalyst, which is the main advantage through an efficient immobilization method, as presented in our study, besides the possibility of reuse and reduction of inactivation by distortion of its native structure by the influence of temperature, pH and solvents.

According to Nyari et al. (2016), this performance involves factors such as: any enzyme added in the immobilization process is adhered to the support, there is no leaching caused by the reaction medium and the interaction of the support material with the active center of the enzyme, leading to the opening of the hydrophobic lid and leaving the exposed site, Providing an increase in the activity / conversion of the esterification reaction. According to Orellana-Coca et al. (2005) and Colombo et al. (2015), an excess in the catalyst content is necessary to keep the enzyme activity during the reaction time.

Equation 3 and 4 (catalyst free and immobilized, respectively) presents the second - order coded model, which describes the ethyl oleate esterification as a function of the independent variables (factors) analyzed (catalyst content and temperature) within the studied range.

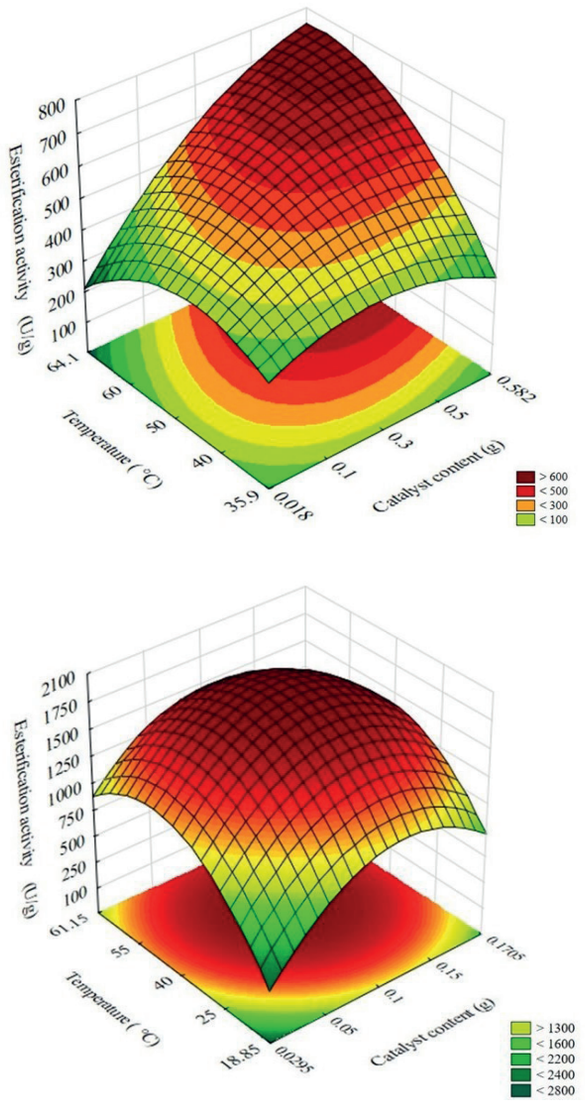
The correlation coefficient obtained for catalyst free (R^2 = 0.90) with F_{cal} (11.11) > F_{tab} (4.01) (Fcal > Ftab = 2.71) allowed the construction of the surface response presented equation 3 the construction of the contour plot in Figure 2 (a) where catalyst content (g) (M) and temperature (°C) (T).

Esterification activity (U g^{-1}) = 461.57 + 78.72T – 65.74T^2 + 128.95M – 47.88M^2 + 70.25M.T (3)

The correlation coefficient obtained for catalyst immobilized (R^2 = 0.94) with F_{cal} (2.71) > F_{tab} (4.01) (Fcal > Ftab = 0.72) allowed the construction of the surface response presented in equation 4 the construction of the contour plot Figure 2 (b) where catalyst content (g) (M) and temperature (°C) (T).

Esterification activity (U g^{-1}) = 15.28 + 307.30T – 392.36T2 - 191.76M – 378.78M2 – 71.25M.T (4)

Figure 2. Surface response using a free (a) and immobilized (b) catalyst in terms of ethyl oleate esterification by mechanical system



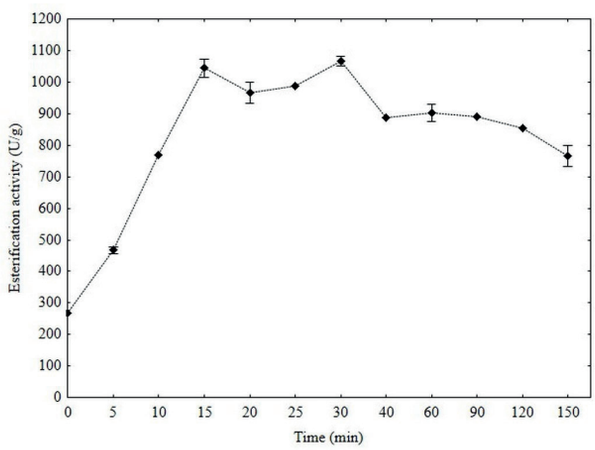
Source: Author

Figure 2 shows the contour plot for the interactions between the variables: catalyst content and temperature for the batch reaction under the mechanical stirring. It was observed that the higher esterifications for the ethyl oleate were achieved in the temperature range of 40-60 °C and biocatalyst content from 0.5 - 0.582 g (central point condition) to immobilized catalyst and temperature >40 °C and biocatalyst content > 0.5 g to free catalyst. In this sense for the ultrasonic system, only the immobilized catalyst was studied, the immobilization process can alter the characteristics of the enzymes, making a comparison not very fair. In these cases, optimizing conditions for each type of enzyme is always better due to the better results in terms of esterification of ethyl oleate.

Ultrasonic system

Figure 3 shows the evolution of ethyl oleate esterification of oleic acid and ethyl alcohol (90 minutes), for the catalyst free and immobilized according to the condition designed by the full RCCD 2^3 (Table 2).

Figure 3. Kinetics of ethyl oleate esterification for ultrasonic system



Source: Author

From the results, it was observed the maximal esterification of 1034.08 U g^{-1} after 15 minutes of reaction time. From this results, it was possible to relate the initial reaction velocity with the agitation type. It can be related to modification and opening of the three-dimensional structure of the enzyme, second Liu et al. (2008), the reduction in the reaction time can be linked to the increase in the reaction rate, which can be obtained using ultrasonic agitation system, mainly due the formation of microscopic droplets in the system, increasing the interfacial area by increasing surface contact reducing mass transference limitations between substrate and catalyst. In addition to the excellent performance, such as heat dissipation, the higher contact time between substrates and catalyst, suitable for industrial scale-up, due the catalyst is not affected by the agitation and rupture by the mechanical system (Figure 1) showing that the ultrasonic system and more efficient (Figure 3) (HO et al., 2016; MATTE et al., 2016).

Table 2 shows the full RCCD 2^3 for the synthesis of ethyl oleate ester in batch mode: ultrasonic - assisted system, as a function of the studied variables, immobilized catalyst content (g), temperature (°C), and ultrasonic power (%).

Table 2. Matrix of full 2^3 RCCD experimental design in terms of ethyl oleate esterification by ultrasonic system

Run	Temperature (°C)	Enzyme content (g)	Ultrassonic power (%)	Esterification (U g^{-1})
1	-1 (25)	-1 (0.1)	-1 (40)	416.67 ± 34.17
2	-1 (25)	-1 (0.1)	1 (80)	1641.36 ± 55.28
3	-1 (25)	1 (0.5)	-1 (40)	1785.71 ± 21.43
4	-1 (25)	1 (0.5)	1 (80)	1707.07 ± 15.03
5	1 (55)	-1 (0.1)	-1 (40)	1139.88 ± 52.99
6	1 (55)	-1 (0.1)	1 (80)	1795.70 ± 48.60
7	1 (55)	1 (0.5)	-1 (40)	1250.71 ± 36.44
8	1 (55)	1 (0.5)	1 (80)	3087.63 ± 22.14
9	- 1.68 (24.8)	0 (0.3)	0 (60)	1064.86 ± 26.13
10	1.68 (75.2)	0 (0.3)	0 (60)	867.71 ± 22.60
11	0 (40)	-1.68 (0.068)	0 (60)	939.35 ± 47.08
12	0 (40)	1.68 (0.532)	0 (60)	3054.24 ± 31,21
13	0 (40)	0 (0.3)	- 1.68 (26.4)	3005.40 ± 18.41
14	0 (40)	0 (0.3)	1.68 (93.6)	1398.50 ± 32.27
15	0 (40)	0 (0.3)	0 (60)	2122.93 ± 10.29
16	0 (40)	0 (0.3)	0 (60)	2272.47 ± 13.52
17	0 (40)	0 (0.3)	0 (60)	2128.31 ± 16.70

* Fixed parameters: substrate mass 5 g, oleic acid to ethanol molar ratio 1:1, reaction time 15 minutes.

Source: Author

The highest esterification, 3087.60 (U g^{-1}) was observed in the assay 12 (55 °C, 0.532 g of the immobilized biocatalyst, and ultrasonic power of 80%). In general, as in the study conducted with mechanical agitation, the variables evaluated, when analyzed independently, had a positive effect.

The increase in the catalyst (assays 1 and 3, 2 and 4, 5 and 7, 6 and 8, 11 and 12) content showed an increase in the esterification, independent of the temperature and the ultrasonic power. The same behavior was observed for the ultrasonic power (assays 1 and 2, 3 and 4, 5 and 6, 7 and 8), and temperature (assays 1 and 5, 2 and 6, 3 and 7, 4 and 8), independent of the other variables studied.

The temperature usually influences the chemical equilibrium of endothermic systems, due to the diffusional effect, while the enzyme content increases the active sites in the reaction medium. In both cases, increasing this ranges, tend to favor the reaction process, usually in terms to improve esterification.

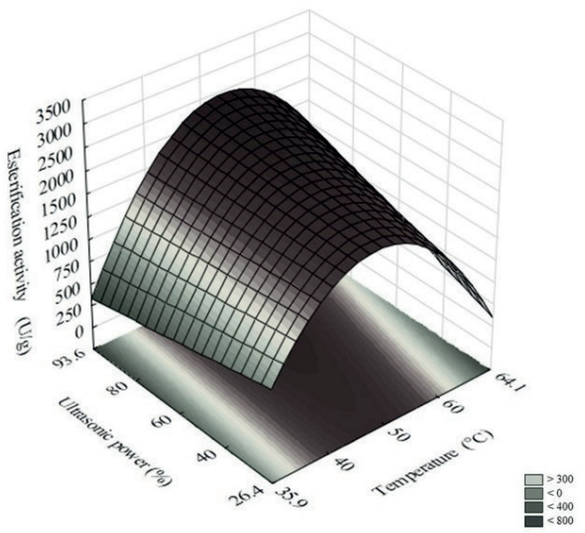
In relation to ultrasonics power, was observed from the results that increasing ultrasonics power was possible to increase ethyl oleate esterification and conversion. Choudhury et al. (2013) and Khan et al. (2015) relate the higher conversion due to the cavitation bubbles increasing the solubility of the molecule consequently increasing the reaction rate, and providing a low energy use (KWIATKOWSKA et al., 2011; MARTINS et al., 2013).

Equation 5 presents the second - order coded model, which describes the ethyl oleate esterification as a function of the independent variables (factors) analyzed (catalyst content, temperature and ultrasonic power) within the studied range.

The correlation coefficient obtained for obtained (R^2 = 0.94) with F_{cal} (2.91) > F_{tab} (3.63) (Fcal > Ftab = 0.80), equation 5 e the construction of the contour plot presented in Figure 4 where catalyst content (g) (M), temperature (°C) (T) and ultrasonic power (%) (P).

Esterification activity (U g^{-1}) = 4.18 + 120.59T – 574.65T^2 + 485.97M + 106.49P + 168.25T.P (4)

Figure 4. Surface response in terms of ethyl oleate esterification by ultrasonic system



Source: Author

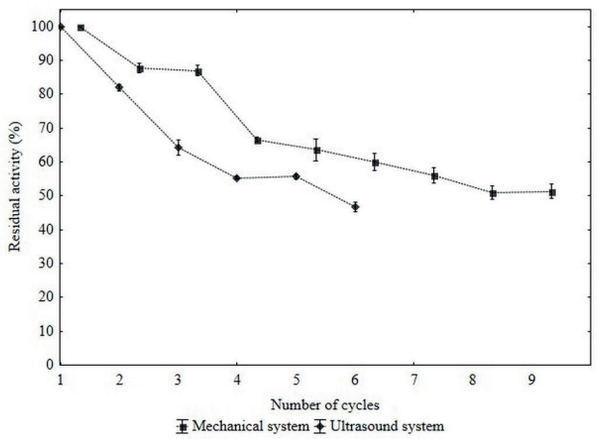
The highest esterification for the synthesis of ethyl oleate esters were achieved in the region corresponding to high temperature (40 – 60 °C). The literature reports several concerns regarding the application of the ultrasonic system as a tool in the reactions of ester synthesis.

Through the results obtained in this study, it was shown that by using ultrasonic - assisted system it was possible to obtain ethyl oleate esters in a relatively short reaction time (15 minutes), especially when compared to the conventional system, presenting as an alternative process, simple, and promising to improve reaction conditions and conversion yield.

Operational stability

Figure 5 shows the operational stability (number of reuse cycles) for the esterification of ethyl oleate (mechanical agitation and ultrasonic - assisted system). Considering 100% as residual activity with 5 cycles in mechanical agitation.

Figure 5. Operational stability for the esterification of ethyl oleate using mechanical system (40 minutes) and ultrasound system (15 minutes)



Source: Author

In a more extensive analysis, it was observed that the mechanical system showed 19 cycles with residual activity higher than 60% of residual activity and 14 cycles higher than 50% residual activity. The observed reduction in the conversion during the reuse cycles may be related to the biocatalyst loss of mass between cycles, by the leaching of the enzyme from the support and by the catalyst denaturation according to successive reuses (CARVALHO et al., 2015; BANSODE; RATHOD, 2014; WAGHMARE et al., 2015).

According to a study of Nyari et al. (2016) the immobilized enzyme to the reaction medium, favore the increased stability of immobilized CALB on PU. It is therefore purposed that this immobilized enzyme can be used in future applications employing a continuous flow rate at different temperatures. Thus, evidencing good resistance to elevated temperatures, theresults demonstrate the effectiveness of the catalyst in PU matrix. In addition, the catalyst may have been more stable, or modifications in the support structure may have favored immobilized enzyme stability. The enzyme in soluble form has some flexibility, which causes conformational changes in active site, often irreversible, and makes it inactive for direct temperature influences. When immobilized, it become more rigid due to their bonds with the support, decreasing the flexibility while maintaining the shape of the active site, which is responsible for its activity. The PU support has great mechanical stability, this contributed to present a higher thermal stability immobilized enzyme to the reaction medium, in this case, favoredthe increased stability of immobilized CALB on PU.

Poppe et al. (2015) using mechanical agitation for the synthesis of methyl esters using immobilized Novozym 435 obtained 8 cycles of reuse keeping 70% of the initial. Khan et al. (2015) in the synthesis of cetyl oleate ester by an ultrasonic - assisted system and mechanical agitation using commercial lipase *Candida antarctica* lipase B CALB™ 10000, obtained only one reuse cycle with 80% of initial enzyme activity for both systems.

Adewale et al. (2016) reported the transesterification of biodiesel by an ultrasonic - assisted system using *Candida antarctica* B lipase (CALB), the authors reported 3 and 5 cycles of reuse keeping 40% of the initial enzyme activity. Michelin (2015) in the synthesis of ethyl esters using immobilized lipase (Novozym 435) reported the possibility of 5 cycles of reuse keeping enzyme activity up to 50% of the initial activity.

CONCLUSIONS

The operating mechanical system the maximum esterification of ethyl oleate used immobilized catalyst was 1553.83 U g^{-1} with 19 cycles of reuse after 40 minutes of and ultrasound system reaction time in and 3087.60 U g^{-1} was observed and 14 cycles de reuse after 15 minutes of reaction time used *Candida antarctica* lipase B immobilized on polyurethane. Thus, the process was considered efficient with significant reduction of the reaction time, low instrumental requirements and improve the bioprocess performance. Until now, there were no studies available in the open literature in relation to the ester synthesis catalyzed by immobilized lipase in polyurethane as support in the ultrasound system. Thus, the results obtained in this work are promising in relation to the results observed in the literature for different lipases immobilized on different supports and applied in the synthesis of different esters.

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