Fuel 88 (2009) 2130–2134

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Effect of methanol content on enzymatic production of biodiesel from waste frying oil

R. Maceiras [b,*], M. Vega [a], C. Costa [a], P. Ramos [a], M.C. Márquez [a,*]

[a] Chemical and Textile Engineering Department, Pza. de los Caídos 1-5, University of Salamanca, 37008 Salamanca, Spain
[b] Chemical Engineering Department, ETSEI, Rúa Maxwell s/n, University of Vigo, 37210 Vigo, Spain

ARTICLE INFO

Article history:
Received 29 October 2008
Received in revised form 12 May 2009
Accepted 20 May 2009
Available online 6 June 2009

Keywords:
Waste frying oil
Transesterification
Biodiesel
Candida antarctica lipase

ABSTRACT

The enzymatic production of biodiesel from waste frying oil with methanol has been studied using immobilized lipase Novozym 435 as catalyst. The effects of methanol to oil molar ratio, dosage of enzyme and reaction time were investigated. The optimum reaction conditions were methanol to oil molar ratio of 25:1, 10% of Novozym 435 based on oil weight and reaction period of 4 h at 50 °C obtaining a biodiesel yield of 89.1%. Moreover, the reusability of the lipase over repeated cycles was also investigated under standard conditions.

1. Introduction

Nowadays, vegetable used oils are a potential renewable source for the production of an alternative for petroleum based diesel fuel due to the diminishing petroleum reserves and the environmental consequences of exhaust gases from diesel engines. The biodiesel production from vegetable oils involves the transesterification of vegetable oils using a short chain alcohol in the presence of a suitable catalyst obtaining a fuel quite similar to conventional diesel fuel in its main characteristics [1]. The transesterification process can be carried out in different ways such as alkali catalyst, acid catalyst or bio-catalyst. Of all these methods, only the alkali process is used in an industrial scale but it presents serious problems of separation of catalyst and unreacted methanol from biodiesel [2]. Biodiesel production with biocatalyst eliminates these disadvantages so producing biodiesel with a very high purity [3]. Many authors [4,5] have observed that the enzymatic catalysis provides a better glycerol recovery than chemical catalysts. However, the process has not been implemented in an industrial scale due to different factors such as high cost of enzymes, low lipase activity or enzyme inhibition by methanol.

Enzymatic biodiesel production from raw vegetable oils has been extensively studied for many authors in the last years [3,6,7]. Nevertheless, the raw material costs and limited availability of raw vegetable oil are being recently critical issues for the biodiesel production. Therefore, it has been necessary to look for another raw material to produce biodiesel. Some authors have investigated the use of waste cooking oil to obtain biodiesel using a chemical catalyst and their studies have shown that biodiesel from waste cooking oil can be used in different types of diesel engines with no loss of efficiency and significant reduction in the emissions of particulate matter, carbon monoxide and total hydrocarbons with respect to conventional diesel obtained from fossil fuel [8–11].

On the other hand, different lipases, such as *Candida antarctica* [12], *Pseudomonas cepacia* [13] and *Thermomyces lanuginosus* [14] have been employed as biocatalysts in the production of biodiesel from vegetable oil but there is no enzymatic studies that involve the use of waste frying oil. These enzymes can be used free or immobilized. Several researchers [15–17] have reported that the commercially available Novozym 435 (*C. antarctica* lipase B immobilized on acrylic resin) was the most effective catalyst among tested lipases for biodiesel production.

In this study, waste frying oil was used as raw material for enzymatic biodiesel production instead of crude vegetable oil because this offers the possibility of transform a pollutant waste in a sustainable and renewable energy source. The purpose of this work is to analyze the influence of operating parameters, such as methanol to oil molar ratio, dosage of enzyme (Novozym 435), reaction time and enzyme reuse on transesterification process.

2. Methods

2.1. Materials

The waste frying oil (WFO) was procured from local restaurants. The numerous fractions were blended in order to obtain

* Corresponding authors.
E-mail addresses: rmaceiras@uvigo.es (R. Maceiras), mcm@usal.es (M.C. Márquez).

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doi:10.1016/j.fuel.2009.05.007

homogeneous oil. *C. antarctica lipase B* immobilized on acrylic resin (Novozym 435), with an activity of 10,000 PLU/g (propyl laurate units/g), was provided by Novozymes A/S (Denmark). Methanol was used as acyl acceptor and was supplied by Panreac. Standard fatty acid methyl esters were taken as reference and were purchased from Supelco. All other chemicals used were obtained commercially and were of analytical grade.

2.2. Waste frying oil characterization

The samples of waste frying oil were filtered to remove the suspended matter. Then, the water content was determined by drying the sample until constant weight in an oven at 105 °C. The acid value was determined by UNE-EN 14104 [18]. The obtained results were water content of 0.04% by weight and acid value of 1.35 mg KOH/g oil. The fatty acid composition of the samples (Table 1) was obtained by GC according to Section 2.4.

2.3. Transesterification process

The enzymatic transesterification reactions were carried out in a test tube containing 2 g of waste frying oil, 200 mg of Novozym 435 (10%) and a 1:1 methanol to oil molar ratio. The molar amount of the oil was calculated from the acid value. The reaction was carried out in an incubator at 50 °C during 8 h with constant stirring at 150 rpm. Additional experiments were carried out to optimize the reaction conditions varying methanol to oil molar ratio, dosage of enzyme and reaction time.

At the end of the reaction period, 500 μL were taken from the reaction mixture and centrifuged to obtain the upper layer. 10 mg of the upper layer, 250 μL of methanol solution of trimethylsulfonium hydroxide (TMSH) (0.2 mol/L) and 500 μL of methyl-terbutyl-ether were precisely measured and mixed vigorously for gas chromatography analysis.

2.4. Analysis of fatty acid methyl esters

The methyl ester contents were quantified using a gas chromatograph Agilent 6890 N connected to a forte BP-20 capillary column (0.25 mm × 30 m) from SGE. The temperature program was as follows: 155 °C for 1 min and programmed from 155 to 180 °C at a rate of 2 °C/min, kept for 2 min, and finally raised to 220 °C at 4 °C/min and maintained for 6 min. The injector was set up for 250 °C and the FID detector at 260 °C. Nitrogen was used as carrier gas, at constant flow of 1.6 mL/min.

Table 1
Fatty acid composition (wt%) of methyl esters prepared from the WFO.

Component	Composition (wt%)
C6:0	0.1
C8:0	0.5
C12:0	0.1
C14:0	0.5
C16:0	9.5
C16:1	0.9
C17:0	1.0
C18:0	4.6
C18:1	54.1
C18:2	26.6
C18:3	0.4
C20:0	0.3
C20:1	0.3
C20:2	0.4
C22:2	0.8

3. Results and discussion

3.1. Effect of methanol to oil molar ratio

The methanol to oil molar ratio is one of the most important parameters in biodiesel production. However, this value depends on the properties of WCO and the type of catalyst used. For example, Centikaya and Karaosmanoglu [19] found that a ratio of methanol below 5:1 is insufficient for the base-catalyzed transesterification while Zheng et al. [20] observed that the methanol ratio could be up to 250:1 in the presence of acidic catalyst. To our knowledge, no information is available on the optimal methanol/WFO molar ratio when the reaction is catalyzed with Novozym 435. Fig. 1 shows the effect of methanol to oil molar ratio on the methyl esters yield with 10% enzyme dosage at 50 °C for 8 h. An increase of the molar ratio from 1:1 to 25:1 increased by 40% the methyl esters yield. However, a further increase above 25:1 causes a decrease on the amount of methyl esters. This reduction could be due to two factors: the excess of methanol deactivates the enzyme or the depletion of substrate. To justify this result, it is necessary to analyze the other factors involved in the transesterification process. Although, we can affirm that the optimum molar ratio of methanol/WFO is 25:1 obtaining 89.1% of methyl esters produced per 100 g of WFO. The optimal ratio of methanol/WFO different from the usual ratio of methanol/vegetable oils shows the influence of the type of oil in the transesterification process.

3.2. Effect of reaction time

Time course of methanolysis reaction of WFO in a series of experiments involving different methanol to oil molar ratio have been carried out. In both cases, the yield of methyl esters is practically constant after 4 h of reaction (Fig. 2). This indicates that the optimum reaction time is 4 h since after that time there is no change in the methyl esters yield. Therefore, it could be that four hours is the time required for enzyme deactivation or depletion of substrate.

3.3. Effect of dosage of enzyme

In order to investigate the effect of the dosage of enzyme on methanolysis of WFO, the amount of Novozym 435 was varied from 5% to 15% (wt%/wt oil) while keeping the amount of oil constant and using the above optimized reaction conditions. Fig. 3

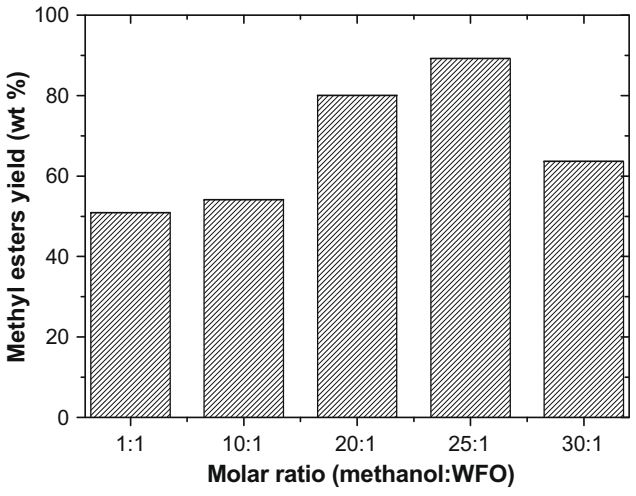

Fig. 1. Effect methanol/WFO molar ratio on biodiesel yield. Reaction conditions: WFO 2 g; enzyme dosage 10%; reaction temperature 50 °C; reaction time 8 h.

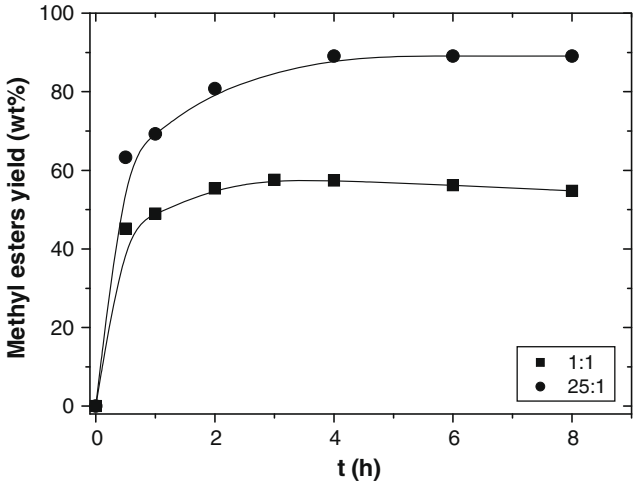

Fig. 2. Time course during transesterification process of WFO at different methanol/WFO molar ratio. Reaction conditions: WFO 2 g; enzyme dosage 10%; reaction temperature 50 °C.

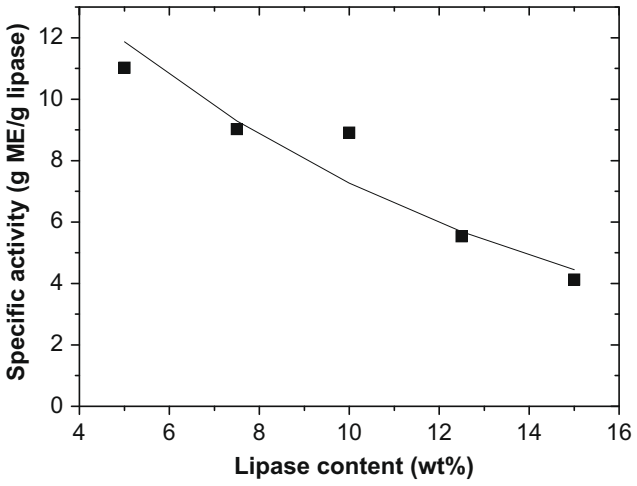

Fig. 4. Specific activity of Novozym 435. Reaction conditions: WFO 2 g; alcohol–oil molar ratio 25:1; reaction temperature 50 °C; reaction time 4 h.

shows a gradual increase in the methyl esters yield for lipase concentrations below 10% and a decrease for higher concentrations. This result suggests an excess of enzyme over required, where the liquid volume is insufficient to carry out the reaction. Moreover, visual observations during the trials with enzyme concentration higher than 10% indicated that the liquid phase was not of sufficient volume to completely suspend the solid catalyst. In these circumstances the external mass transfer resistance becomes the limiting step for the oil transesterification. This behaviour is in accordance with the obtained from other researchers [21–23].

In order to check this theory, the effect of lipase amount on specific activity of Novozym 435 (g methyl esters/g enzyme) was determined. As it can be seen in Fig. 4 the enzyme specific activity decreases with the dosage of enzyme. This effect proves that the amount of Novozyme added was much greater than the required and external mass transfer resistance had limited the rate of transesterification reaction [23].

3.4. Enzyme reuse

The main advantage of the use of an immobilized enzyme is that the enzyme can be repeatedly used. To test the reusability of Novozym 435, different experiments were carried out with the enzyme filtered and washed with 1-butanol after the reaction and reused consecutive times. Fig. 5 shows that a significant increase in methyl esters yield (93.1%) is observed after one repeated cycle but the yield remain constant in the following cycles. Similar behaviour has been reported in similar systems [21,23,24]. The slower methanolysis rate with fresh immobilized lipase was explained by Samukawa et al. [25]: when a fresh enzyme preparation was used as a catalyst for methanolysis of oil, penetration of the substrate into the preparation was restricted. Pretreatment of immobilized lipases in oil appears to be a way to improve the apparent performance of the lipases as proved by Wei et al. [26]. Therefore, Novozym 435 can be used four times without loss of any activity in these conditions. Furthermore, this behaviour shows that the enzyme is not deactivated during the transesterification process in these operational conditions.

3.5. Effect of additional enzyme

Once the optimum conditions of transesterification process were established, the effect of a new addition of Novozym on reaction was analyzed in order to verify the possible depletion of substrate on the obtained results. The first step was conducted for 4 h

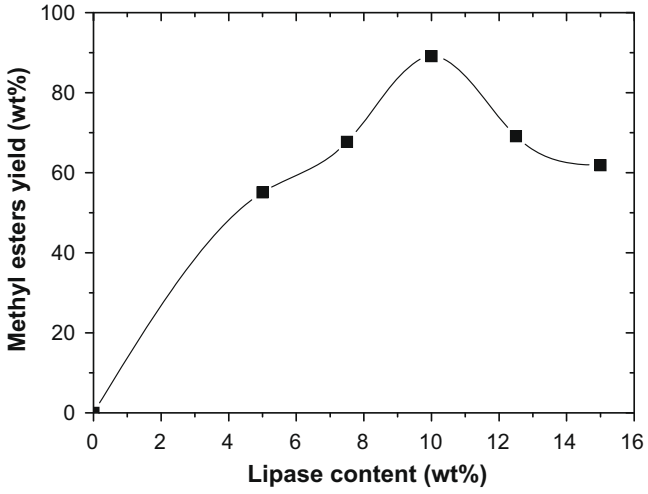

Fig. 3. Effect of dosage of enzyme on methanolysis WFO. Reaction conditions: WFO 2 g; alcohol–oil molar ratio 25:1; reaction temperature 50 °C; reaction time 4 h.

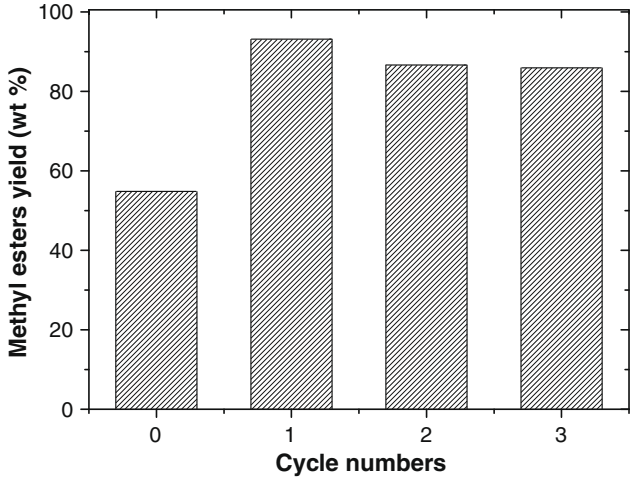

Fig. 5. Reusability of enzyme. Reaction conditions: WFO 2 g; alcohol–oil molar ratio 1:1; reaction temperature 50 °C; reaction time 8 h.

in a mixture of waste frying oil, 1:1 and 25:1 molar equivalent of methanol and 10% of Novozym 435. The methyl esters yield reached 57.4% and 89.1%, respectively. The addition of a second 10% of immobilized lipase at 4 h permitted to obtain a 64% of methyl esters in the case of methanol to oil molar ratio 1:1 (Fig. 6a), while a decrease on the FAME yield was observed for a methanol to oil molar ratio 25:1 (Fig. 6b). These results show that there is no increase in the production of methyl ester with the addition of enzyme at optimum conditions (25:1); however, there is a low increment when the amount of methanol is lower (1:1). From Fig. 6a we can deduce that (i) there is no substrate depletion as the methyl esters yield increases when a new amount of Novozym 435 is added and (ii) that external mass transfer resistance is not the only cause of the reduction in the reaction yield because the excess of enzyme over required is higher for a methanol to oil molar ratio 1:1 than for a methanol to oil molar ratio 25:1.

Therefore it is necessary to look for other causes in order to justify the results obtained. A possible cause could be interferences in the separation of methyl esters but adsorption on catalyst is also possible because Novozym 435 is immobilized on acrylic resin particles which could adsorb polar compounds such as methanol. When methanol–oil ratio is high, the immiscible methanol droplets attached to the materials reducing or blocking the entry of WFO to enzyme [27].

For determining the cause of the reduction in methyl ester yield, two other experiences were carried out under the same conditions stated above for the methanol to oil molar ratio of 25:1 but using unwashed reused enzyme, where methanol adsorption is not possible because lipase is impregnated with oil and glycerol, and reused enzyme previously washed with 1-butanol where methanol adsorption could be possible. After the experimental runs, the time course of methyl ester yield in both cases (Fig. 7a and b) was similar and similar as that obtained from the fresh enzyme (Fig. 6b). We can thus rule out the possibility of methanol adsorption on lipase because adsorption means a lower methyl ester yield in the second step of the reaction for fresh and washed reuse enzyme than for unwashed reuse enzyme. As a consequence of these results, the only reason of the observed reduction could be that the excess of methanol interferes with the separation of methyl ester and other products by increasing solubility of glycerol.

These findings are consistent with those obtained by other authors. Krisnangkura and Simamaharnnop [28] observed that when glycerol remained in solution it helped to drive the equilibrium back to the left, lowering the yield of esters. Fillieres et al. [29] found that an excess of alcohol favors a conversion of di- to mono-

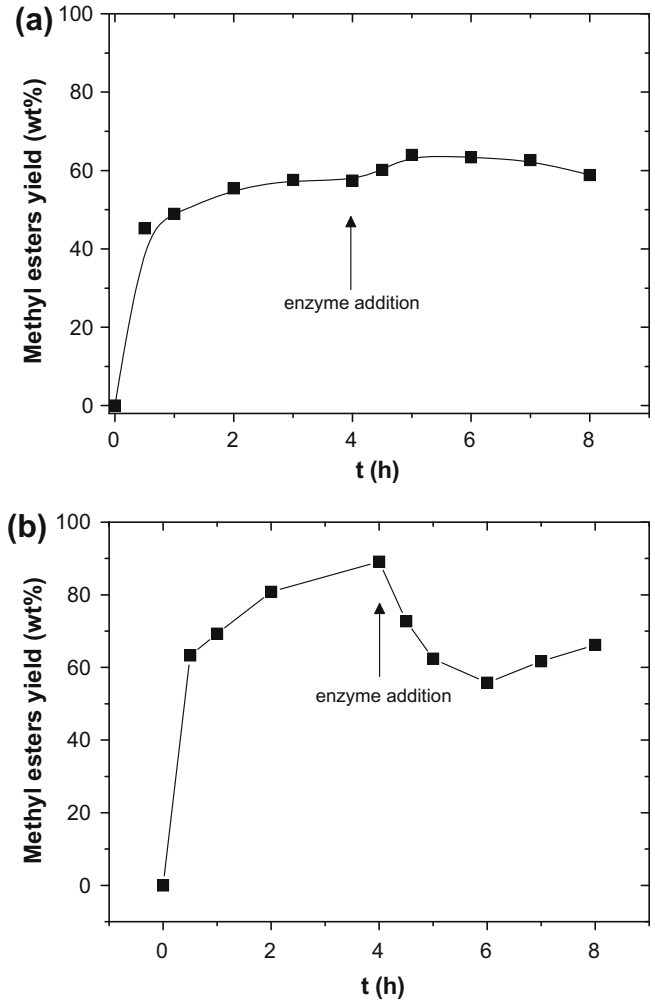

Fig. 6. Effect of enzyme addition on methanolysis WFO at different alcohol–oil molar ratio: (a) 1:1, (b) 25:1. Reaction conditions: first-step: WFO 2 g; 10 wt% immobilized *C. antarctica*; reaction temperature 50 °C; reaction time 4 h. Second-step was conducted under the same conditions after 10% of fresh enzyme was added in the reaction mixture.

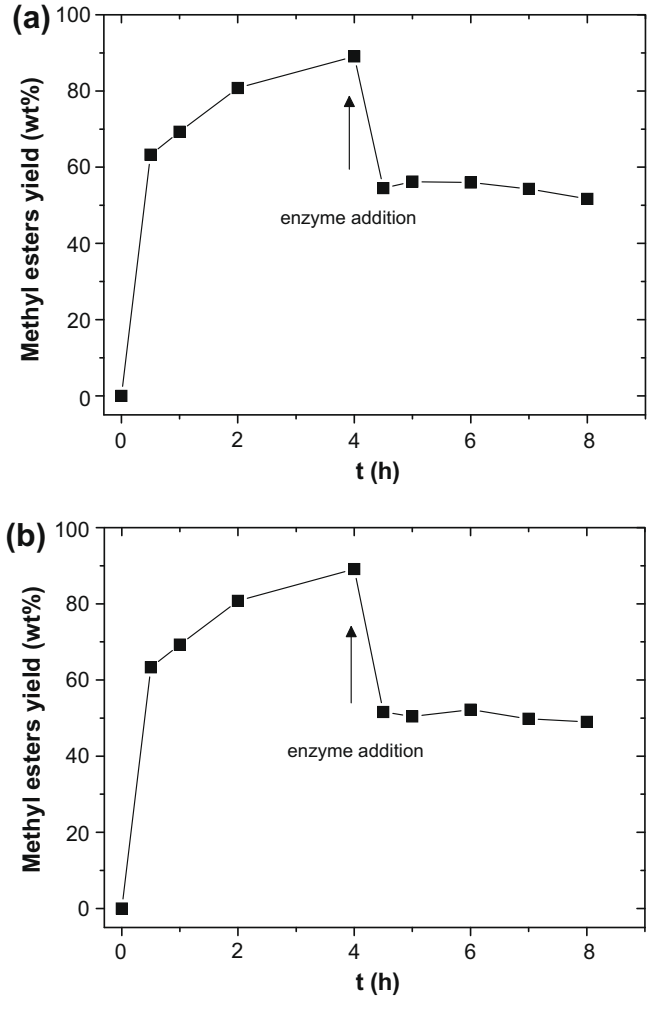

Fig. 7. Effect of enzyme addition on methanolysis WFO at different enzyme pretreating conditions: (a) reused lipase, (b) reused lipase previously washed with 1-butanol. Reaction conditions: first-step: WFO 2 g; 10 wt% immobilized *C. antarctica*; alcohol–oil molar ratio 25:1; reaction temperature 50 °C; reaction time 4 h. Second-step was conducted under the same conditions after 10% of pretreated enzyme was added in the reaction mixture.

glycerides, and a slight recombination of esters and glycerol to monoglycerides because their concentration keeps increasing during the course of the reaction, in contrast to reactions conducted with low molar ratios. Encinar et al. [30] used the same explanations to justify the decrease in the ester yield obtained from the transesterification reaction of used frying oil by means of large amounts of methanol.

Phan et al. [11] also reported a reduction in methyl ester yield for high methanol/WFO ratios and they also suggest the interference of the excess of methanol with the separation of ester product and by-products by increasing solubility of glycerol as cause of the yield decreases with two consequences: (a) part of the diluted glycerol remains in the ester phase, leading to foam formation and therefore apparent lost ester product, (b) the excess of methanol drives the combination of ester product and glycerol into monoglycerides.

From the results of our research and the cited supporting documents, it can be concluded that, because of substrate depletion, enzymatic deactivation and adsorption are not noticed for the operating conditions, decrease on the methyl esters yield observed for high methanol concentrations only can be attributed to interferences in the separation of methyl esters.

4. Conclusions

Biodiesel production from waste frying oils is feasible by enzymatic transesterification with an appropriated molar ratio of methanol/WFO in the reaction mixture. For fresh enzyme, the findings of this study show that the highest methyl esters yield of 89.1% was obtained to methanol/oil molar ratio of 25:1 and with 10% of enzyme after 4 h of reaction. However, best results were obtained with the reused enzyme and low oil molar ratio (1:1).

From the results of this work, it can be concluded that (i) Novozym 435 is not deactivated by methanol in the operating conditions used, (ii) external mass transfer can be the limiting step in the oil transesterification working with high concentration on immobilized enzyme and (iii) the excess of methanol interferes with the separation of methyl ester causing an apparent lost of product by increasing solubility of glycerol.

Acknowledgements

This work was supported by MCYT (Project No. CTM2007-64369/TECNO). The grant of Xunta de Galicia is gratefully acknowledged.

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