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Study of fame production from waste cooking oil: Operation in batch and continuous regime with regeneration of enzyme catalyst

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Abstract

This work aimed to: (i) assess the performance of Novozyme® 435 for FAME production from waste cooking oil (WCO) under different oil acid values and enzyme-to-oil ratios; (ii) test different regeneration methods and assess the regenerated enzyme's performance; and (iii) determine FAME yield in continuous operation regime. Oil acid values up to 104 mg KOH.g^{-1} and enzyme-to-WCO ratios of up to 20:80 (% wt.) were studied, with maximum yield recorded for increasing acid values and enzyme-to-WCO ratio. FAME production from WCO yield ranging 50%–80% was recorded for 5 consecutive production cycles (8h each, in batch regime) with no regeneration of the enzyme. Among the enzyme regeneration methods tested, the highest FAME yield was recorded using t-butanol washing followed by enzyme incubation in the WCO before reusing it. Operation in continuous regime, including enzyme regeneration, produced the highest FAME yield (maximum of 86 %). Thus, effectiveness of the regenerating process and efficiency of continuous production of FAME from WCO (up to 576h) were observed.

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Keywords: Biodiesel; Enzyme regeneration; FAME; Novozyme® 435; Waste cooking oil

1. Introduction

Output of conventional transport biofuels grew 4% to reach 83 MToe in 2017; biodiesel from waste oil and animal fat feedstock accounted for 6%–8% of this value (IEA Website) [1]. Biodiesel is usually produced from the catalyzed reaction between triglycerides and short-chain alcohols, forming either FAME – Fatty Acid Methyl Esters – or FAEE — Fatty Acid Ethyl esters, depending on the alcohol being methanol or ethanol, respectively. Glycerol is a sub-product of the reaction. Catalysts used in biodiesel production may be homogeneous (acid or alkali), heterogeneous (e.g. metallic oxides) and enzymes (immobilized or not) [2]. The efficiency of the process

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is related with the type of catalyst, the oily mixture and the operational conditions. Some operational conditions influencing FAME yield include the oil used as raw material (its fatty acids and water contents), type of catalyst, alcohol chain length, oil/alcohol ratio, catalyst/oil ratio and reaction temperature, content [3,4].

Enzymatic catalysts are biological catalysts of protein origin, composed of long amino acid chains linked by peptide bonds. Unlike chemical catalysts, enzymes are very selective and sensitive to the conditions of operation, such as alcohol used (short-chain alcohols tend to denature/inactivate enzymes) and even stirring rate [2,5]. The immobilized enzymes have the possibility of being reused, however during their use it is necessary to regenerate/wash them to reduce mass transfer problems created by the deposition of glycerol on their surfaces. Different methods for regeneration of the enzymes have been reported in the literature, mainly washing with alcohols [6,7].

The goals of this work were: (i) to evaluate the influence of the oily mixture acid value the performance of the enzyme in the FAME production (ii) to evaluate the effect of enzyme-to-oil ratio (% wt.) on the FAME yield produced from waste cooking oil (WCO) and oleic acid blend (50:50); (ii) to select an efficient enzyme regeneration method; and (iii) to produce FAME from WCO and enzyme catalysis in continuous operation regime (fixed bed).

2. Materials and methods

2.1. Materials

Waste cooking oil (peanut) for FAME production was provided by a canteen of the University of Aveiro, Portugal. The catalyst used was an immobilized enzyme, Novozyme® 435 (9080 PLU g^{-1} in batch essays and 1075 PLU g^{-1} in continuous regime experiment), able to catalyze both transesterification and esterification reactions. Methanol (MeOH) was the alcohol used in all experiments. To protect the enzyme from inactivation by the methanol t-butanol was added to the reactional medium (0.75 v/v). All the chemicals used were analytical grade, except n-hexane (GC grade) and methyl heptadecanoate (analytical standard), from Sigma-Aldrich.

2.2. Oily mixtures characterization

WCO was characterized in terms of density, pH, acid value and viscosity. Oily mixtures with different WCO-oleic acid ratios were prepared and characterized in terms of acid value. Density was measured by picnometry; pH was measured with a Denver Instrument® model 25 pH/ion metre; acid value was determined by a Metrohm 904 automatic titration equipment; viscosity was measured using a Cannon-Fenske viscometer (tube size 200).

2.3. Influence of acid value and enzyme-to-oil ratio on catalyst performance

Aiming to assess the performance of the enzyme in the FAME production under different acid values, four oily mixtures of WCO-oleic acid were prepared according to Table 1.

Table 1. Operational conditions used for testing the influence of oily mixture acid value on performance of the enzyme (10% wt. loading; reaction time of 8 h) in the FAME production.

WCO:oleic acid (% v/v)	Acid value (mg KOH/g oily mixture)	MeOH/oily mixture (mol/mol)	T (°C)	Stirring conditions
100:0	2.302	4:1	50	Orbital stirring, 260 rpm
90:10	16.79			
75:25	68.34			
50:50	104			

The oily mixture yielding higher FAME production was adopted in the set of experiments to test the effect of enzyme loading, according to Table 2. These experiments were carried out in batch reactors (c.a.35 mL).

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Table 2. Operational conditions used for testing the effect of enzyme loading on FAME yield, from a WCO:oleic acid ratio of 50:50 (acid value of 104.0 mg KOH g^{-1} oily mixture; reaction time of 8 h).

Enzyme loading (% wt.)	MeOH /oily mixture (mol/mol)	T (°C)	Stirring conditions
5 10 20	4:1	50	Orbital stirring, 260 rpm

Table 3. Enzyme regeneration methods tested in this work.

Method reference	Description of the regeneration method
A	Wash with t-butanol
B	Wash with t-butanol and then incubate the enzyme in WCO for 8 h before (re)use
C	Wash with 1-butanol
D	Wash with 1-butanol and then incubate the enzyme in WCO for 8 h before (re)use

2.4. Enzyme regeneration methods

To test different enzyme regeneration methods and assess the regenerated enzyme's performance on FAME production from WCO, four methods were tested (see Table 3). The regeneration methods listed in Table 3 were carried after 5 consecutive cycles of enzyme use in batch regime (35 mL glass vials), for 8 h, at 50 °C, with methanol-to-WCO ratio of 4:1 (mol/mol), enzyme loading 20% (wt.) and with 260 rpm orbital stirring. A control batch was performed with no regeneration. FAME yield was measured by Gas Chromatography, in a Thermo Scientific TRACE GC ULTRA, according to EN 14103.

2.5. FAME production in continuous regime

Continuous regime FAME production was performed at 50 °C in two 20 mL fixed bed reactors set in series, with a column of resin Lewatit® GF 202 (between fixed bed reactors). The experiment lasted for 576 h. The system was fed continuously by mixture of: WCO, methanol (4:1 mol MeOH/mol WCO) and t-butanol (0.75 v/v WCO) at 0.9 mL min^{-1} from the bottom of the 1st reactor. Fig. 1 shows the experimental setup used in this essay. The continuous operation was interrupted after (c.a.) 200 h for enzyme regeneration using method B described in Table 3.

Fig. 1. Experimental setup for the continuous-regime experiment. A — stirring device; B — Feed mixture; C — Pump for reactional mixture feeding; D — 1st fixed bed bioreactor; E — Resin column; F — 2nd fixed bed bioreactor; G — Sampling point.

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Table 4. Characterization of the WCO used in this work for FAME production.

Method reference	Value
Density [g L^{-1}]	979 ± 3
pH	9.43
Acid value [mg KOH g^{-1}]	2.302
Viscosity (@25 °C) [mm^2 s^{-1}]	44.6

3. Results and discussion

3.1. Oily mixtures characterization

Main characteristics of the WCO used in this work are presented in Table 4. Waste cooking oils properties are quite dependent of the vegetable oils feedstock and their frying practiecs and conditions [8]. In comparison to the virgin peanut oil (density 916 ± 2 g L^{-1}), the WCO has a density approx. 7% higher.

3.2. Influence of oil acid value and enzyme-to-oil ratio on catalyst performance

Regarding influence of acid value on catalyst performance, maximum FAME yield was recorded for increasing acid oily mixtures. Thus, a ratio of 50:50 of WCO and oleic acid, corresponding to an acid value of 104 mg KOH g^{-1} (see Fig. 2(a)) was the best (tested) condition for FAME production. These results also show/prove that Novozyme® 435 can catalyze both transesterification and esterification reactions, i.e., the enzyme has a bifunctional character.

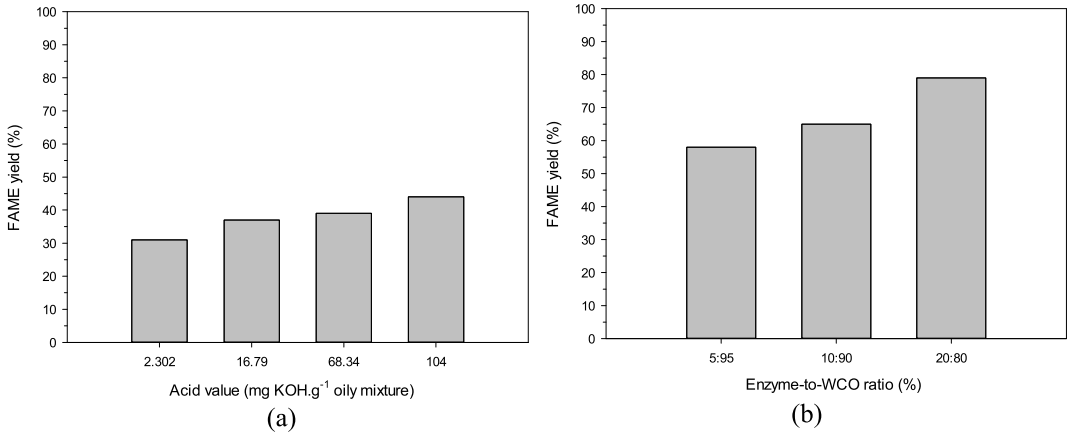

Fig. 2. FAME yield as function of (a) acidity, from different oily mixtures; and (b) enzyme-to-WCO ratio. Conditions: 50 °C, 8 h, MeOH/oily mixture = 4:1 (mol/mol), acid value of 104 mg KOH g^{-1}.

Concerning the influence of enzyme-to-oil ratio on catalyst performance, maximum FAME yield was achieved for a 20:80 ratio, i.e. 20% (wt.) loading (see Fig. 2(b)). Wang and Zhang [9] reported an increase of FAME yield, but only for enzyme loading up to 10% (wt.), so this work poses a useful contribute in this topic. Hernández-Martín and Otero [10] tested higher enzyme loads (up to 50% wt.) but with different operational conditions and reagents. These authors observed an increasing reaction yield as Novozyme® 435 loading increased. This immobilized lipase, with an active open form conformation, is absorbed on macroporous acrylic resin [11]. As its concentration increases in the reaction medium, the greater the number of active sites available for catalyzing both transesterification and esterification reactions, thus higher is the FAME yield. This tendency is expected to reach a maximum value (not tested in this work), from which there is no improvement in the reaction yield by increasing the enzyme loading.

3.3. FAME production from WCO and regeneration of enzyme Novozyme® 435

FAME yields ranging 50%–80% were recorded in batch regime experiments using only WCO, with no enzyme regeneration, for a minimum of 6 consecutive production cycles (8 h each), proving that the enzyme maintains

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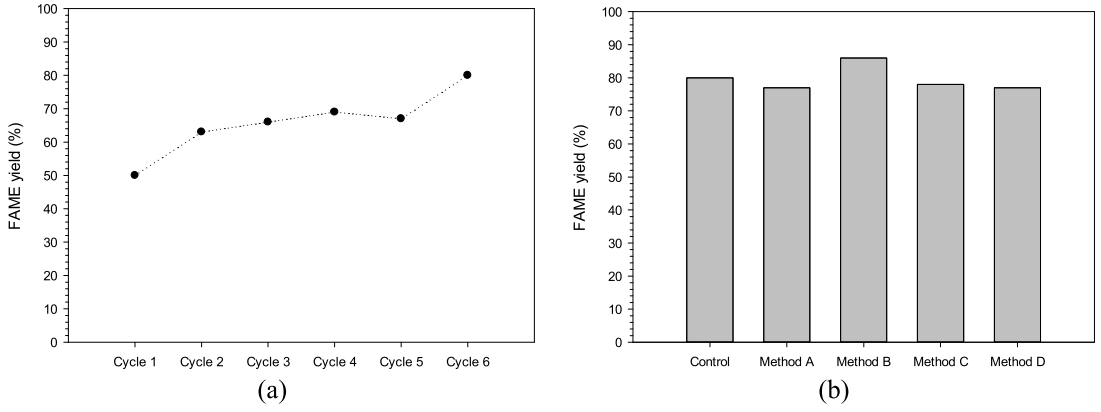

Fig. 3. (a) FAME yield of the consecutive cycles under batch regime (control). (b) FAME yield attained (6th operation cycle) for each enzyme regeneration method Conditions: 50 °C, 8 h, MeOH/oily mixture = 4:1 (mol/mol), enzyme loading 10% wt.

its activity for at least 48 h, which is very important in terms of costs upon scale-up (see Fig. 3(a)). Regarding Novozyme® 435 regeneration, the four methods tested (between 5th and 6th cycle) have similar performances, however Method B is slightly more efficient (see Fig. 3(b) and Table 3). Hydrophilic properties of t-butanol allow the removal of glycerol, which is a key feature since this sub-product tends to coat the surface of the immobilized catalyst, inhibiting its activity. Since t-butanol was used in the reaction medium any way, with the intent of protecting the enzyme from methanol, this alcohol showed a double role: protection of the enzyme from methanol and solubilization of glycerol, regenerating the enzyme.

3.4. FAME production in continuous regime

The higher FAME yield attained under continuous regime was 86% after 76 h of operation. Then, the yield started to decrease, possibly due to deposition of glycerol in the free spaces of the fixed bed enzyme column, or even on the enzyme surface. After cleaning the system and regenerating the enzyme by Method B, FAME yield increased again, reaching a maximum of 85%, very close to the maximum recorded in the first operating period. After this peak, a decrease of the yield to about 60% was recorded, probably due to glycerol deposition in the empty spaces of the fixed bed and/or on the surface of the enzyme. Yield stabilization at 60% lasted for the remaining operation time — 576 h total. These results are shown in Fig. 4 and proved the effectiveness of the regenerating process and the efficiency of continuous production of FAME from WCO.

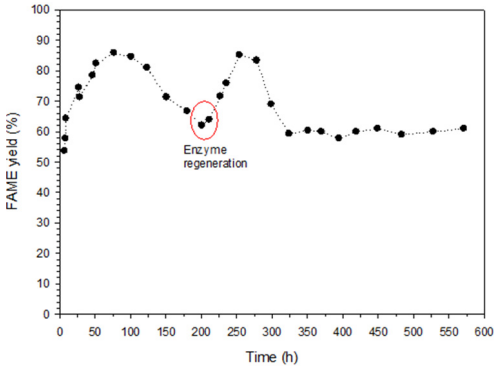

Fig. 4. FAME yield along continuous operation in a fixed bed reactor.

4. Conclusion

Maximum FAME yield was recorded for increasing oil acid value and enzyme-to-WCO ratio, so 50:50 (v/v) ratio of WCO and oleic acid and a 20% (wt.) enzyme-to-WCO ratio were selected as the best conditions for FAME

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production within the conditions tested in this work. Thus, Novozyme® 435 has a bifunctional character, being able to catalyze both transesterification and esterification reactions, so that is suitable to recover waste cooking oils with low quality (more degraded).

FAME production from waste cooking oil was successfully performed in batch and continuous operation regime. Yield ranging 50%–80% was recorded in batch operation mode for a minimum of 6 consecutive production cycles, proving that the enzyme maintains its activity for at least 48 h of use. Regarding Novozyme® 435 regeneration, it seems that is possible to achieve by all four methods tested, since in all of them the FAME yield was very similar to the control. The highest FAME yield was recorded using the enzyme regenerated by t-butanol washing and incubation in the WCO before using it, so this seemed the most promising method and was tested in FAME production from WCO in continuous regime.

The operation in continuous regime produced higher FAME yield, with maximum of 86% after 76 h. After the regeneration of the enzyme the FAME yield reached again the higher values previously observed.

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