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Enzymatic Synthesis of Biodiesel via Alcoholysis of Palm Oil

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Abstract The enzymatic alcoholysis of crude palm oil with methanol and ethanol was investigated using commercial immobilized lipases (Lipozyme RM IM, Lipozyme TL IM). The effect of alcohol (methanol or ethanol), molar ratio of alcohol to crude palm oil, and temperature on biodiesel production was determined. The best ethyl ester yield was about 25 wt.% and was obtained with ethanol/oil molar ratio of 3.0, temperature of 50 °C, enzyme concentration of 3.0 wt.%, and stepwise addition of the alcohol after 4 h of reaction. Experiments with 1 and 3 wt.% of KOH and 3 wt.% of MgO were carried out to compare their catalytic behavior with the enzymatic transesterification results. The commercial immobilized lipase, Lipozyme TL IM, showed the best catalytic performance.

Keywords Enzyme · Ethanol · Crude palm oil · Immobilized lipase · Biodiesel

Introduction

Biodiesel, an alternative to diesel fuels, is a mixture of mono-alkyl esters of higher fatty acids, industrially produced from transesterification of vegetable oils or animal fats with a short-chain alcohol, as methanol or ethanol. Currently, the vegetable oils that are used industrially to produce biodiesel are rapeseed, sunflower, palm, and soybean oil. More than 80% of worldwide biodiesel production is from rapeseed oil [1]. Even though the current

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production of biodiesel from palm oil is only 1% of total world production, palm oil has the potential as the main source of biodiesel due to its high-yield crop with the lowest price trade among the vegetable oils [1]. For instance, the average yield of palm oil is approximately 4.2 tonnes ha^{-1} $year^{-1}$, whereas rapeseed and soybean oils produce only 1.2 and 0.4 tonnes ha^{-1} $year^{-1}$, respectively [1].

Biodiesel fuel can be obtained through different reaction pathways. The transesterification process can be conducted using alkali, acid, biocatalyst, heterogeneous catalysts, or alcohols in their supercritical state [2, 3]. The transesterification of oils in the presence of an alkaline catalyst, as sodium or potassium hydroxide, is commercially used. The main disadvantage of the conventional chemical process is that the catalyst could not be recovered because it is removed from the reaction medium together with glycerol. In order to be used as raw materials for the chemical process, the oils and fats should present a low content of free fatty acids (lower than 1%). This low content of free fatty acids is achieved using refining processes that increase the oil price [4].

So, the use of oils with high content of free fatty acids such as palm oil to produce biodiesel through the conventional chemical process demands an additional step of pretreatment. Initially, the free fatty acid content should be reduced by an esterification process using acid catalysts; then, in a second step, the transesterification process could be done [5].

On the other hand, the acid-catalyzed approach is less sensitive to free fatty acids in the feedstock oil compared to the alkali-catalyzed one; however, this route is 400 times slower than that of alkali catalyst and more hazardous [6].

The lipase-catalyzed enzymatic production of biodiesel under milder conditions can overcome these problems and is a less energy-intensive and more environmental-friendly procedure. The enzymatic transesterification of palm oil with alcohol is an interesting alternative to the chemical process because the free fatty acids present in the oil could be converted into their corresponding esters using lipases that also catalyze the oil transesterification with alcohol. Moreover, that reaction employs fewer complex steps for product isolation as well as avoids the elimination of the catalyst and salt produced in the first process [7].

The productivity of palm oil per hectare is ten times higher than the productivity of other oils, such as soybean oil, and Brazil has the potential to produce this crop. This work investigated the use of commercial immobilized lipases as catalysts for the transesterification of crude palm oil to biodiesel as a model conversion process for commercial applications. The effect of reaction parameters such as temperature, oil/alcohol molar ratio, alcohol and its addition procedure, and lipase type on biodiesel yield was evaluated.

Materials and Methods

Materials

The crude palm oil was provided by Piraque S.A. (Brazil); its fatty acid composition is 0.2% of lauric acid, 0.8% of myristic acid, 41.5% of palmitic acid, 5.0% of stearic acid, 41.5 of oleic acid, and 10.4% of linoleic acid. The acid value of the crude palm oil was about 6.2 mg KOH per gram of oil. The commercial enzymes used, Lipozyme RM IM and Lipozyme TL IM, were kindly provided by Novozymes Latin America Ltda (Parana, Brazil). Other reagents employed were analytical grade ethanol (99.8%), methanol (99.9%), *n*-hexane,

MgO, and potassium hydroxide, all supplied by Vetec (Brazil). Methyl heptadecanoate (a chromatographic standard) was acquired from Sigma/Aldrich (St. Louis, USA).

Measurement of Lipase Activity

The esterification activity of Lipozyme RM IM and Lipozyme TL IM was measured by the consumption of oleic acid at 45 °C in the esterification reaction with butanol (oleic acid/butanol molar ratio of 1) with the enzyme concentration of 3 wt.%. One esterification unit of Lipozyme was defined as 1 μmol of oleic acid consumed per minute (U) under the experimental conditions described herein. The Lipozyme RM IM and Lipozyme TL IM used in this work have esterification activity of 1,510 and 454 U/g, respectively.

Reaction System

The transesterification reactions between crude palm oil and alcohol were conducted in closed 15 mL batch reactor magnetically stirred, coupled to a condenser in order to avoid alcohol loss by volatilization. The water circulating in the condenser was cooled by a thermostatic bath. The reaction temperature was kept constant by circulating ethylene glycol from a thermostatic bath (Haake DC10) into the jacket of the reactor. Reaction progress was monitored by taking duplicate samples each 30 min until 4 h of reaction. The reaction products were analyzed by gas chromatography.

Chromatography Analysis

The samples were diluted in hexane injected into a Varian gas chromatograph (GC; CP-3380 model), equipped with a flame ionization detector, a CP WAX 52 CB capillary column 30 m × 0.25 mm × 0.25 mm, and a split injection system with a 1:20 ratio. Injector and detector temperatures were kept at 280 and 300 °C, respectively. The oven was initially maintained at 200 °C for 4.5 min, then heated up to 250 °C at a 20 °C min^{-1} rate, and kept constant at the final temperature for 5 min. Hydrogen was used as the carrier gas at a 2 mL min^{-1} flow rate; column pressure was set at 20 psi. A computer loaded with the Star Workstation 6.2 software was connected to the GC by a Star 800 Module Interface to automatically integrate the peaks obtained. Methyl heptadecanoate was used as internal standard.

Transesterification Reaction

The reaction medium consisted of a mixture of the crude palm oil, alcohol, and enzyme. The experimental conditions used for the alkyl esters (biodiesel) synthesis were the following: reaction temperature=30, 40, 50, 60, 70, and 78 °C; enzyme concentration=3 wt.%; alcohol/crude palm oil molar ratio=3, 6, and 9; alcohol used, methanol or ethanol; single addition or stepwise addition of alcohol (three consecutive additions at different times).

The transesterification of crude palm oil and ethanol was also studied using KOH as catalyst under these experimental conditions: reaction temperature=78 °C; catalyst concentration=1 and 3 wt.%; ethanol/crude palm oil molar ratio=6; single addition of the alcohol.

For comparison, a single test was done using 3 wt.% of MgO as a solid basic catalyst for the aforementioned transesterification using an ethanol/crude palm oil molar ratio=3 and single addition of the alcohol at 78 °C.

Results and Discussion

Effect of Reactants Molar Ratio

The effect of the ethanol/crude palm oil molar ratio on the transesterification reaction was evaluated using a 3 wt.% commercial immobilized lipase (Lipozyme TL IM). The transesterification of vegetable oils requires at least 3 mol of alcohol to each mol of oil to accomplish a complete conversion of the oil into its esters. As this is a reversible reaction, an increase in the concentration of any reactant results in a higher yield of ester. An alcohol/oil molar ratio of 6 is commonly used in industrial processes to obtain higher yields of esters [8–10]. In such case, ethanol/crude palm oil molar ratios of 3, 6, and 9 were tested in reactions conducted at 40 °C for 4 h. The results obtained are shown in Fig. 1 and indicate that the excess of alcohol reduced the ethyl esters production. The ethyl esters yield achieved using ethanol/crude palm oil molar ratio of 3:1 was 15.3%, while higher molar ratios, 6:1 and 9:1, caused a decrease on the ethyl esters yield (10.6% and 4.7%, respectively).

An excess of alcohol could improve the solubility of glycerol, a reaction product that physically blocks the entrance of catalyst pores [11]. However, Shimada et al. [12] also showed that high ethanol/oil molar ratios cause lipases deactivation. According to the literature [7, 12–14], proteins are usually unstable in the presence of short-chain alcohols, such as ethanol and methanol. Thus, an excess of alcohol causes opposite effects on the lipase reaction: (1) a decreased biocatalyst stability and (2) increased formation of a homogeneous suspension of the reactants and biocatalyst [11]. The results obtained here suggest that the alcohol excess mainly caused the deactivation of lipase.

This confirmed the findings of Talukder et al. [15], who predicted inhibition of methanol at methanol/palm oil ratios ≥3:1. According this work, at a higher ratio, Novozym 435, a commercial immobilized lipase, is easily poisoned as a result of the contact with insoluble methanol. The insoluble methanol droplets attach to the solid support (acrylic resin) used for lipase immobilization, and the access of the substrate to the lipase active site is blocked, causing the reaction to stop.

Methanol inhibition was also verified by Al-Zuhair et al. [6]. The authors also observed that transesterification of palm oil with methanol catalyzed by lipase from *Mucor miehei* in

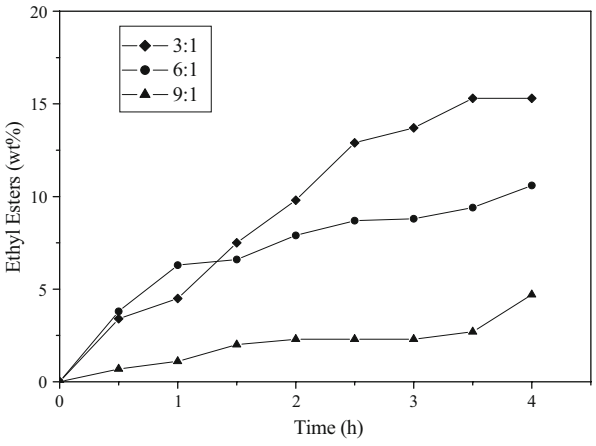

Fig. 1 Effect of ethanol/crude palm oil molar ratio on the ethyl esters yield of the transesterification reaction of crude palm oil and ethanol after 4 h using 3 wt.% Lipozyme TL IM at 40 °C

n-hexane microaqueous system has been successfully described by a Ping-Pong Bi Bi model with competitive inhibition by both reactants.

Effect of the Stepwise Addition of Ethanol

As discussed above and shown in Fig. 1, a high initial alcohol concentration causes lipase deactivation. In order to avoid this deactivation, the stepwise addition of ethanol (three consecutive steps) was studied. The reactions were conducted with 3 wt.% Lipozyme at 40 °C using reactant molar ratios of 3:1, 6:1, and 9:1. Ethanol was added in a stepwise way (i.e., 1/3 added at time 0, 1/3 after 30 min, and 1/3 after 1 h of reaction).

The results are shown in Fig. 2. For all three reactant molar ratios, the stepwise addition of ethanol produced higher biodiesel yields than those observed when ethanol was added in a single step at the beginning of the reaction. Similar results were presented by Bernardes et al. [16] in the transesterification of soybean oil and ethanol using Lipozyme RM IM and also by Hernández-Martín and Otero [11] that have verified an increase in the conversion of the transesterification of sunflower oil and ethanol using 10 wt.% of Lipozyme TL IM with a stepwise addition of the alcohol.

According to the results presented in Fig. 2, the best molar ratio is the stoichiometric one, even when ethanol is added in a stepwise manner (18.1%).

Effect of Temperature

The influence of reaction temperature was investigated in the ethanolysis of crude palm oil catalyzed by Lipozyme TL IM (3 wt.%) using an ethanol/crude palm oil molar ratio of 3:1 (stoichiometric ratio) and a stepwise addition of ethanol. The ethyl ester production was investigated at 30, 40, 50, 60, 70, and 78 °C. The results obtained are shown in Fig. 3. The highest yield was verified at 50 °C (25.2%) and the lowest at 78 °C (3.3%). The decrease in ethyl ester yields was presumably due to the deactivation of the enzyme caused by high temperatures [16]. In addition, hydrophilic solvents, such as ethanol, tend to reduce lipase thermal stability [17].

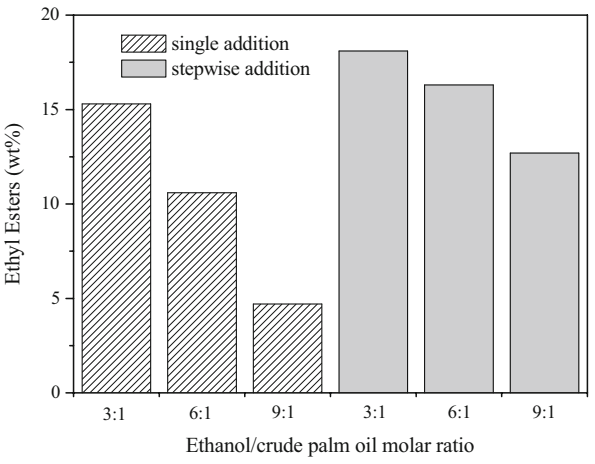

Fig. 2 Effect of stepwise ethanol addition on the transesterification reaction of crude palm oil and ethanol using ethanol/crude palm oil molar ratios of 3:1, 6:1 and 9:1, and 3 wt.% Lipozyme TL IM at 40 °C

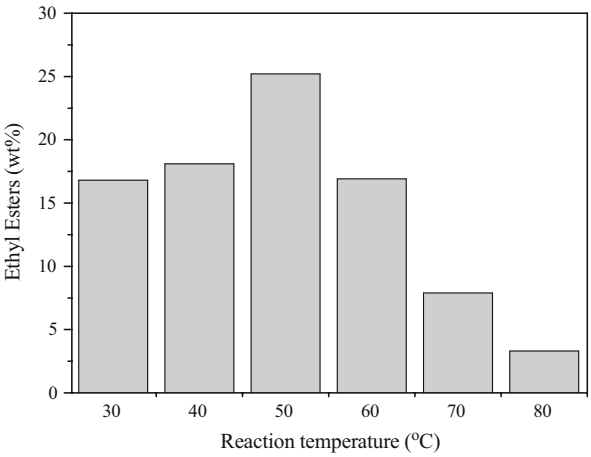

Fig. 3 Effect of temperature on the transesterification of crude palm oil and ethanol using an ethanol/crude palm oil molar ratio of 3:1, with stepwise ethanol addition (1/3 at 0 h, 1/3 after 0.5 h, and 1/3 after 1 h), and 3 wt. % Lipozyme TL IM

Talukder et al. [15] verified that the maximum Novozym 435 activity in the reaction of palm oil with methanol (methanol/palm oil ratio of 3.2:1) was observed at about 50–60 °C, after which the enzyme undergoes inactivation.

Effect of Type of Alcohol and Enzyme

It is well known that the catalytic performance of lipase is highly dependent on sources of the lipase, substrates, and reaction conditions. Thus, the effect of the type of the alcohol (methanol or ethanol) over the transesterification of crude palm oil, at 50 °C, was investigated using 3 wt.% of two different commercial enzymes, Lipozyme RM IM and Lipozyme TL IM, and an alcohol/oil molar ratio of 3:1. Figure 4a,b shows the results obtained after 4 h of reaction using ethanol and methanol, respectively.

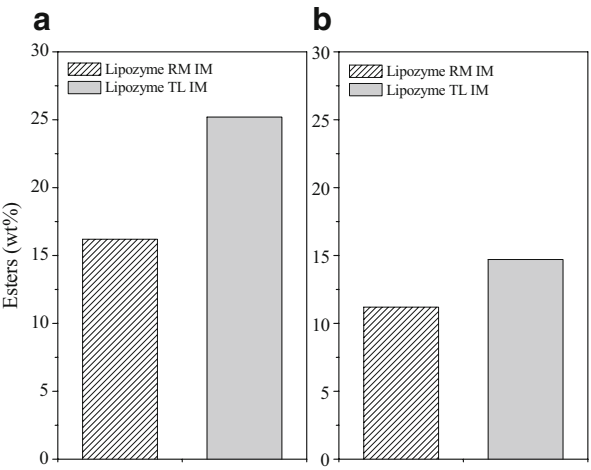

Fig. 4 Effect of different types of lipases (Lipozyme RM IM and Lipozyme TL IM) and alcohols (ethanol and methanol) on the transesterification of crude palm oil using an alcohol/crude palm oil molar ratio of 3:1, with stepwise alcohol addition (1/3 at 0 h, 1/3 after 0.5 h, and 1/3 after 1 h), 3 wt.% of enzyme at 50 °C. **a** Ethanol. **b** Methanol

The transesterification of triglycerides with methanol is a preferred enzyme-catalyzed reaction for biodiesel production due to its lower cost in many countries. However, Brazil produces ethanol on a large scale; therefore, it is cost competitive with methanol for conversion of oil into biodiesel. In this study, optimal ester yields were obtained when ethanol was used in the enzymatic transesterification of crude palm oil. According to the results presented in Fig. 4, the reaction catalyzed by Lipozyme TL produced yields 71.4% higher in the presence of ethanol (25.2% versus 14.7% for ethanol and methanol, respectively). The difference between ethanolysis and methanolysis was smaller when Lipozyme RM was tested. This can be explained by the greater enzyme deactivation by an alcohol with fewer carbon atoms [17, 18].

Hernández-Martin and Otero [11] observed that ethanolysis of sunflower oil occurs faster than methanolysis, at 25 °C, using excess of alcohol and Novozyme 435. However, when Lipozyme TL IM and Lipozyme RM IM were tested as catalysts for the methanolysis of sunflower oil under the same reaction conditions, no reactions were observed. According to the authors, the different behaviors of the two nucleophiles (methanol and ethanol) can be related to two points. First, the partial (Novozyme 435) or total (Lipozyme TL IM and Lipozyme RM IM) deactivation of the biocatalyst can occur from contact with the polar organic phase containing the alcohol and glycerol, which is a by-product of the transesterification of oils. This phase is dispersed in the reaction mixture because of its partial solubility in the oil phase. Second, as the reaction proceeds, glycerol is produced, facilitating the removal of methanol from the oil phase. A new phase composed by methanol and glycerol is formed. The concentration of methanol in the oil phase decreases, resulting in a decreased conversion rate. In agreement with this result, Watanabe et al. [19] have shown that the removal of glycerol during the reaction enhanced overall conversion.

Comparison Between Different Catalysts

The catalytic performances of commercial Lipozyme TL IM, KOH, and MgO for the transesterification of crude palm oil and ethanol were compared. Figure 5 shows the results of ethyl ester yields using the aforementioned catalysts. The traditional chemical process that uses 1 wt.% of KOH presented a low ethyl ester yield (3.5%) as well as the

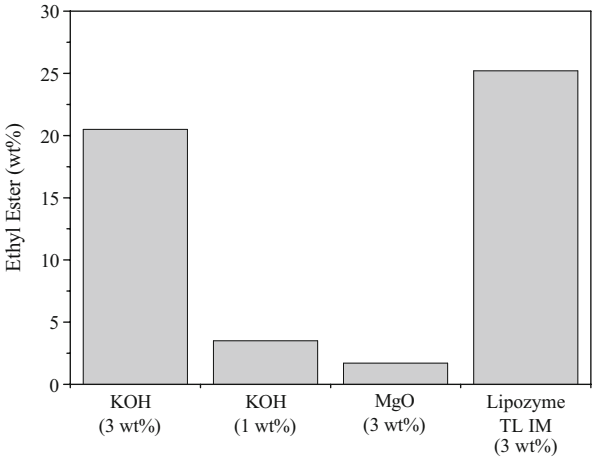

Fig. 5 Comparison of the catalytic performance of different catalysts on the transesterification of crude palm oil and ethanol

heterogeneous basic process that utilizes MgO as catalyst (1.7%). These behaviors are due to the high acidity of the crude palm oil. The free fatty acids neutralize the liquid basic catalyst (KOH) forming soap, and they adsorb on the surface basic sites of MgO. When a large amount of KOH was tested, the ethyl ester yield increased. Among the catalysts studied, Lipozyme TL IM showed the best performance under mild experimental conditions.

Conclusions

The production of biodiesel through the transesterification reaction of a crude palm oil with high acid value (6.2 mg KOH per gram of oil) using a commercial immobilized lipase, Lipozyme TL IM, was accomplished as presented by this work. Better ester yields were obtained using ethanol in presence of lipases under mild reaction conditions (temperature of 50 °C, atmospheric pressure, stepwise addition of ethanol, reactant stoichiometric ratio). These results on the use of ethanol are very promising, especially for biodiesel production in Brazil where ethanol can be obtained cheaply from biomass, for an overall low-cost, commercially viable conversion process. Biodiesel produced in this manner utilizing vegetable oils and plant-derived ethanol could also be considered a totally renewable fuel.

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