Enzymatic Alholysis For Biodiesel Production From Waste Cooking Oil

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Huge efforts have been carried out in recent years in order to develop an alternative fuel from renewable resources, such as biodiesel fuel. Therefore, the use of cooking oil as raw material is a very interesting alternative for the production of biodiesel. In this work, waste frying oil was used as raw material for enzymatic biodiesel production. Moreover, this study analyzes the influence of the type of enzymatic catalyst on the conversion of methyl esters, as well as the influence of other variables such as reaction time.

1. Introduction

The lack of conventional fossil fuels and the increase of the polluting emissions generated by combustion have increased the necessity for alternative fuels, such as biodiesel. Biodiesel is a renewable fuel and presents advantages of producing approximately 80% less carbon dioxide emissions, and almost 100% less sulfur dioxide. The cost of biodiesel, however, is higher than that of petroleum-based diesel due to it is produced mostly from expensive high-quality virgin oil. Use of low-cost feedstock such as waste cooking oils should help make biodiesel competitive in price with petroleum diesel and provides the possibility to convert a pollutant waste into a sustainable and renewable energy source.

Biodiesel is produced from oils by a catalyzed chemical reaction of transesterification. The transesterification reaction consists of the tryglycerides transformation into fatty acid alkyl esters (biodiesel) and glycerol in presence of a short-chain alcohol, such as methanol, ethanol, or buthanol and a catalyst (Vasudevan and Briggs, 2008). Transesterification reactions can be alkali-catalyzed, acid-catalyzed or enzyme-catalyzed. Of all this methods, only the alkali process is carried out in an industrial scale but it presents problems of separation of catalyst and unreacted methanol from biodiesel (Ranganathan et al., 2008). Biodiesel production with biocatalyst eliminates the disadvantages of the other process so producing biodiesel with a very high purity (Fukuda et al., 2001). Different lipases, such as *Candida Antarctica* (Hernández-Martín and Otero, 2008), *Pseudomonas cepacia* (Kumari et al., 2007) and *Thermomyces lanuginosus* (Dizge et al., 2007) have been employed as biocatalysts in the production

of biodiesel from vegetable oil but there are only a few enzymatic studies that involve the use of waste cooking oil (Wu et al., 2003).

Therefore, the use of cooking oil as raw material and enzymes as catalyst is a very interesting alternative for the production of biodiesel. Then, in this work, waste frying oil was used as raw material for enzymatic biodiesel production instead of crude vegetable oil. The B lipase from *Candida antarctica* (CALB) was used as catalyst. The purpose of this research is to analyze the influence of operating parameters in order to optimize the process.

2. Materials and Methods

2.1 Materials

The samples of waste frying oil (WFO) obtained from local restaurants were mixed to obtain homogenous oil mixture. After that, the oil was filtered to remove the suspended matter. The fatty acid composition of the samples was obtained by gas chromatography (Table 1). Moreover, some physical and chemical properties (density, acid value and saponification value) were determined (Table 2).

Table 1. Fatty acid composition of 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

Table 2. Physical and chemical properties of WFO.

Properties	Values
Density (g/cm^3)	0.96
Acid value (mg KOH/g oil)	1.35
Saponification value (mg KOH/g oil)	180.8

Commercial enzyme used in this work was Candida Antarctica lipase B free (Calb L), with an activity of 5000 LU/g (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 purchased from Supelco. All other chemicals were obtained commercially and were of analytical grade.

2.2 Transesterification Process

The enzymatic transesterification reactions were carried out in a test tube that contained 2 g of waste frying oil, 0.2 g of enzyme (Lipozyme Calb L) (10%) and 1:2 alcohol-to-oil molar ratio.

The reaction was carried out in an incubator at 50 ºC with constant stirring at 150 rpm. Experiments were carried out to optimize the reaction conditions varying reaction time. At the end of the reaction period, 500 μL were taken from the reaction mixture and centrifuged in order to obtain the upper layer that was analysed by gas chromatography.

2.3 Analytical Procedure

The methyl ester contents were quantified using a gas chromatograph Agilent 6890N connected to a forte BP-20 capillary column (0.25 mm x 30 m) from SGE. The temperature program was as follows: 155 ºC for 1 min and programmed from 155 to 180 ºC at a rate of 2 ºC/min, kept for 2 min, and finally raised to 220 ºC at 4 ºC/min and maintained for 6 min. The injector was set up for 250 ºC and the FID detector at 260 ºC. Nitrogen was used as carrier gas, at constant flow of 1.6 mL/min. Methyl heptadecanoate was used as an internal standard (EN 14104:2003).

3. Results and Discussion

One of the most important parameters in biodiesel production is the methanol to oil molar ratio. This value depends on the properties of WFO and the type of catalyst used. Although theoretically the oil-alcohol stoichiometric ratio is 1:3, when lipases are used as catalyst, an excess of methanol can deactivate the enzyme (Kaieda, 2001). In order to minimize the enzyme inactivation, in this work a methanol to oil molar ratio below the stoichiometric ratio has been employed to carry out the transesterification process.

For a better comparison between the obtained mehtyl esters yield, the experience with the highest methyl esters percentage has been defined like reference and relativy activiy has been calculated. Then, relative activity represent the percentage of the methyl ester yield obtained in each experience with respect to the reference experience.

The first experience carried out consisted at determining the optimum reaction time. In Figure 1 can be observed that the higher methyl esters yield is obtained at 3 h and after that it is practically constant. Therefore, it could be that three hours is the time required for enzyme inactivation. This time is less than the value obtained from *Candida Antarctica* lipase B inmobilized (Maceiras et al., 2009). This faster inactivation can be due to the fact that, in contrast to the same enzymes free in solution, the enzymes immobilized show much enhanced stability (Ackerman, 2003).
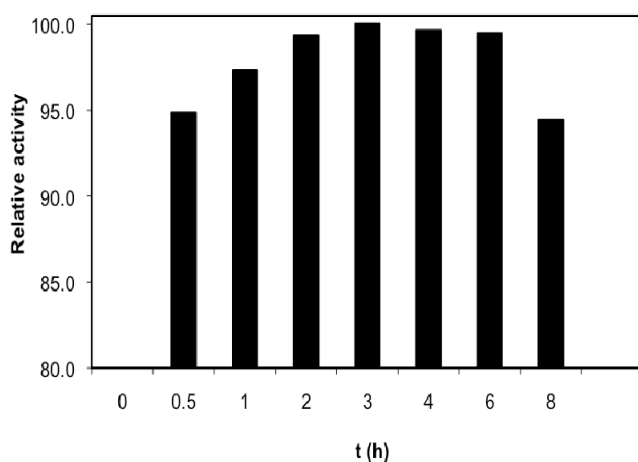
Figure 1. Effect of reaction time on relative activity.

Once the optimum reaction time was established, the effect of a new addition of 0.2 g of fresh enzyme after 4 hours of reaction time was analyzed in order to verify the possible enzyme inactivation on the obtained results. Against the foreseen increase of FAME yield, it can be observed (Figure 2) that the relative activity decreased after the enzyme addition (correction for variation of volume in test tube was made before comparison). This result indicates that there is not increase in the production of methyl ester with the addition of fresh enzyme. The reason of that reduction could be that the excess of enzyme volume could interfere with the separation of methyl ester and other products by increasing solubility of glycerol. This promotes that diluted glycerol remained in ester phase and therefore apparent lost FAME product. These findings are in accordance with the obtained from other authors (Maceiras et al., 2009; Phan and Phan, 2008; Xu et al., 2006).
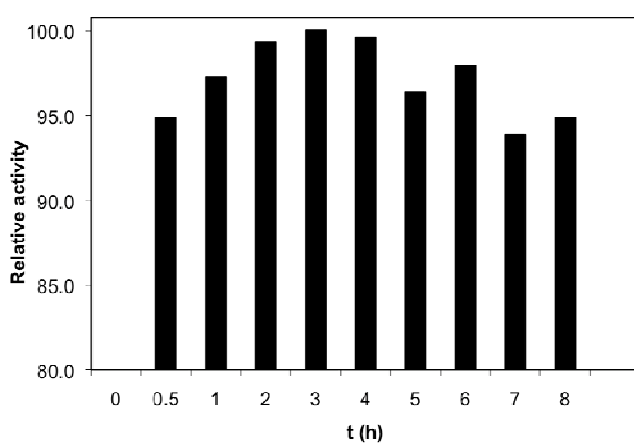

Figure 2. Effect of enzyme addition on transesterification process.

4. Conclusions

The results obtained in this research allow to conclude that biodiesel production from waste frying oils is feasible by enzymatic transesterification with an appropriated conditions. It was observed that an excess of methanol interferes with the separation of methyl ester causing an apparent lost of product by increasing solubility of glycerol.

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