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Novozym 435-catalyzed synthesis of fatty acid ethyl esters from soybean oil for biodiesel production

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ABSTRACT

This paper deals with transesterification of soybean oil with ethanol in the presence of Novozym 435 (lipase from *Candida antarctica*) catalyst. ^{1}H NMR was employed to monitor the conversion of soybean oil to fatty acid ethyl esters in a solvent-free medium. Reaction conditions such as enzyme/substrate ratio, temperature and alcohol to oil molar ratio were studied and the highest yields were found at 5 g of enzyme per 100 g of oil, 37 °C, and 3:1 respectively. Stepwise addition of ethanol was also performed, and compared with that of methanol, in order to assess the reduction of inhibition that this method causes on the enzymatic activity.

1. Introduction

Production of biodiesel from vegetable oils and fats has been the focus of several recent research studies in the recent years [1–3]. Besides its similar-to-diesel chemical structure and, consequently, the possibility of being used in the current automotive engines [4], biofuels synthesized from these raw materials have the benefit of being biodegradable and generate fewer emissions [5,6], although the total greenhouse gas contribution is still an issue going on discussion and strongly depends on the biodiesel raw material [7–9]. Current production processes employ chemical catalysts [10,11] but they face some difficulties, i.e., when working with raw materials having high contents of free fatty acids and/or water, an especially important issue using non-edible sources [11–13]. Lipases, as enzymatic catalysts, can perform this reaction overcoming these aforementioned problems, due to their ability to perform transesterification and esterification reactions in a wide range of reaction conditions [14–16]. Furthermore, milder conditions are required by enzymes, which results in lower energy consumption [17]. For example, while industrial basic catalysis often operates in the range of 100–200 °C, enzymatic catalysis can be performed at 40 °C. However, at present, lipases are not extensively used in industry because of their high cost, although several immobilization methods have been employed to reuse them repeatedly and improve their stability [18,19].

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Enzymatic catalysis has been widely studied for the production of biodiesel, with a large variety of lipases and reaction conditions using several oils and organic solvents [15,20,21]. Most of studies focus on the transesterification with methanol due to its availability and low cost. In fact, the commercial development of biodiesel is based on fatty acid methyl esters and there is no current demand of other alkyl esters, like those obtained through ethanolysis [22]. Nevertheless, use of ethanol would result in a 100% renewable fuel, as ethanol can be also produced from biomass [23,24]. Fatty acid ethyl esters have higher cetane numbers and energy contents, lower pour and cloud points, and cause less emissions of mono-nitrogen oxides [25]. Moreover, ethanol has shown a weaker inhibitory effect over enzymatic activity, thus, it could be more suitable for the enzymatic reaction [15,26]. The favourable economic potential of the production of this type of biodiesel by immobilized lipases has also been pointed out in a precedent paper [21].

This work focuses on the immobilized lipase Novozym 435 for transesterification of soybean oil with ethanol in a solvent-free medium. Organic solvents can be employed to avoid the two-phase formation resulting from the immiscibility of alcohol and triglyceride and to prevent the stripping of residue water from the enzyme's active sites, but they also have severe drawbacks: are hazardous, their selection is not easy [27,28] and additional costs for extraction are required. When the reaction mixture is only composed of reactants, solvent elimination is unnecessary, the process is safer and, in addition, higher volumetric productivity is achieved [29].

The evolution of the reaction was followed by proton nuclear magnetic resonance (^{1}H NMR). The use of this technique in quality assessment [30] and determination of fatty acid profiles [31,32] of vegetable oils has been carried out in the last decades, proving to be a good tool for organic structure determination. NMR can also be used in monitoring the formation of fatty acid alkyl esters during transesterifications with high reliability accuracy [33–36]. Furthermore, by NMR it is also possible to determine the insaturation degree of the oil, which aids in finding which enzymes can be more suitable to each raw material [37]. Some studies have found NMR advantageous with respect to other analytical methods commonly used in biodiesel production monitoring, such as GC or NIR [38–40].

2. Materials and methods

2.1. Materials

Soybean oil was used as a raw material. Its fatty acid composition (mass fraction) was: palmitic (0.139), stearic (0.073), oleic (0.21), linoleic (0.492) and linolenic (0.086). From this composition, the average molar mass was calculated at 871 g mol^{-1}. Novozym 435 (the macroporous resin immobilized form of lipase *Candida Antarctica*) was donated by Novozymes A/S (Bagsvaerd, Denmark). Standards for fatty acid ethyl esters (FAEE), monoacylglycerols (MAG), diacylglycerols (DAG) and triacylglycerol (TAG) were purchased from Sigma Aldrich (Madrid, Spain), and all other reagents used were of the highest purity and purchased from Panreac Química (Castellar del Vallès, Spain).

2.2. Alcoholysis reaction

Following previously reported studies, lipase preparation was soaked in the raw material during 6 h before reaction [15]. This treatment increases the reaction rate (probably by reducing mass transfer limitations). 50 mL of vegetable oil were mixed with the corresponding amount of alcohol (as detailed later) in 100 mL screw-capped glass flasks which were placed in a thermostated orbital shaker at 3.3 Hz. Then, the Novozym 435 was added (5 g per 100 g of oil, unless otherwise specified). Reaction variables studied were: Novozym 435 dose (from 2 to 10 g per 100 g of oil), temperature (from 37 to 47 °C) and ethanol to oil molar concentration ratio (from 1:1 to 8:1). The possible advantages of stepwise addition of alcohol were also tested, and the results compared with those of methanol. In the case of the two-step reaction, it was conducted adding a third of the corresponding amount of alcohol to the mixture at the beginning of the reaction, and the remaining two thirds after 7 h of reaction. Three-step reaction involves the addition of a third of such amount at the beginning of the reaction, the second third after 7 h of time, and the final addition at 14 h.

All the experiments were carried out without water addition. Shimada et al. found that the methanolysis of vegetable oil with Novozym 435 in a solvent-free medium does not require pretreatment with water [41] and Deng et al. indicated that 96% ethanol comprising 4% of water worsened the transesterification of sunflower oil due to the high activity of *C. antarctica* at low water levels [42].

To follow the reaction, 0.5 mL of the reaction mixture were withdrawn at selected times using a sterilized syringe, equipped with a 0.2 μm nylon filter to retain the enzyme. 100 μL of this sample were placed into the corresponding NMR tube for analysis, together with 0.5 mL of deuterated chloroform containing 0.5% of trimethylsilane (TMS) as internal standard.

2.3. Analysis

^{1}HNMR spectra were obtained on a Bruker Ultrashield AV-300 high resolution spectrometer, coupled to an autosampler robot, with a frequency of 300.13 MHz, using $CDCl_3$ in 5 mm NMR tubes. The following conditions were used: spectral width: 5175.983 Hz, acquisition time: 1.583 s, number of scans: 8. In order to analyze spectra data, a method previously published was employed [34].

3. Results and discussion

3.1. Analytical method

Prior to develop a proper analytical method, performing a suitable identification of the peaks in the ^{1}H NMR spectra is needed, as well as their meaning related to the molecules to which the protons which generate each peak belong. Firstly, to identify each species present in the reaction media, pure standards were used. However, the resonance peaks obtained in pure samples show a slight displacement when compared with those found when the compounds were part of a mixture. Thus, it is important to take this shift into account.