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Ultrasound-assisted lipase-catalyzed transesterification of soybean oil in organic solvent system

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ABSTRACT

This work reports the transesterification of soybean oil with ethanol using two commercial immobilized lipases under the influence of ultrasound irradiation. The experiments were performed in an ultrasonic water bath, following a sequence of experimental designs to assess the effects of temperature, enzyme and water concentrations, oil to ethanol molar ratio and output irradiation power on the reaction yield. Results show that ultrasound-assisted lipase-catalyzed transesterification of soybean oil with ethanol might be a potential alternative route to conventional alkali-catalyzed method, as high reaction yields (~90 wt.%) were obtained at mild irradiation power supply (~100 W), and temperature (60 °C) in a relatively short reaction time, 4 h, using Lipozyme RM IM as catalyst. The repeated use of the catalyst under the optimum experimental condition resulted in a decay in both enzyme activity and product conversion after two cycles. The use of Novozym 435 led to lower conversions (about 57%) but the enzyme activity was stable after eight cycles of use, showing, however, a reduction in product conversion after the forth cycle.

1. Introduction

The application of biodiesel to diesel engines for daily activities is advantageous for its environmental friendliness over petro-diesel fuel. The main advantages of using biodiesel is that it is biodegradable, can be used without modifying existing engines, and produces less harmful gas emissions such as sulfur oxide [1]. Chemically, biodiesel is a mixture of alkyl esters with long-chain fatty acids and is typically made from nontoxic, biological resources such as vegetable oils, animal fats [2,3], or even used cooking oils [4].

Transesterification has been the most common way to produce biodiesel [5,6]. Traditionally, transesterification can be performed using alkaline, acid or enzyme catalysts [5–7] and though chemical transesterification through alkali-catalyzed processes provides high conversion levels of triglycerides to their corresponding fatty acid alkyl esters in short reaction times, it suffers from several well-known drawbacks [5,6], while transesterification using acid catalysts brings undesirable features [5,6].

Though nowadays industrial-scale synthesis of biodiesel has been generally performed by transesterification of vegetable oils with methanol, ethanol has also been considered as a potential substrate, due to the fact that ethanol biodiesel appears as a 100%-renewable alternative, especially in Brazil, one of the world's biggest ethanol producers, with a well-established technology of production, large industrial plant capacity installed throughout the country.

In an attempt to overcome the drawbacks of chemical-catalyzed processes, a free-catalyst technique for the transesterification of vegetable oils using an alcohol at supercritical conditions has been recently proposed [8–10]. However, the so-called supercritical method usually requires the use of high temperatures and pressures, which mean high operating costs associated and significant install investments.

In the case of enzymatic catalysis, although the rates of triglycerides conversion to fatty acids alkyl esters are known to be slower compared to the alkali-catalyzed route, it has attracted much attention, because it is more eco-friendly and holds the potential for industrial implementation due to the simplicity of product refining process and the allowance for the use of lower reaction temperature [11–13]. Besides, research efforts have been made nowadays towards developing cost-effective systems using enzyme catalysts for biodiesel production [5,13–18].

In enzyme-based applications, ultrasound has been shown to increase enzyme stability and catalytic activity, as well as the longevity of enzyme biocatalysts. The areas of biotechnology that have already profited from ultrasound continue to expand, and further improvements might be achieved by determining the optimum

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reaction conditions and parameters for ultrasound-assisted applications [19]. Ultrasonic irradiation causes cavitation of bubbles near the phase boundary between the alcohol and oil phases and hence may lead to a raise in temperature at the phase boundary, enhancing the transesterification reaction, which means that neither agitation nor heating are generally required to produce biodiesel by ultrasound application [20–23].

Literature is relatively vast on the production of biodiesel via ultrasound irradiation [3,20–45], but only two, very recent reports, have been found dealing with enzyme-catalyzed transesterification through ultrasonic technique [23,46]. In fact, as mentioned by Yu et al. [46], little research has been done to date on lipase-catalyzed biodiesel production through ultrasound irradiation and hence demonstration of technique utility remains a pressing concern.

In this context, this work is aimed to report experimental data on lipase-catalyzed transesterification of soybean oil with ethanol (fatty acid ethyl esters – FAEE) under the influence of ultrasound irradiation, with two commercial immobilized enzymes, assessing the effects of temperature, enzyme and water concentrations, oil to ethanol molar ratio and output irradiation power on the reaction yield. Taking into account the aforementioned lack of experimental information on enzyme-catalyzed production of biodiesel in ultrasound system, our main intention is to contribute to literature survey through a systematic study on the subject.

2. Materials and methods

2.1. Materials

Commercial refined soybean oil (Soya), ethanol (Merck, 99.9%), *n*-hexane and *n*-heptane (Nuclear, 99.5%), lauric acid (Vetec, 98%), *n*-propanol (Synth, 99.5% of purity), sodium hydroxide (Quimex, 97%) and acetone (Quimex, analytical grade) were used without further treatment. Two commercial immobilized lipases, Novozym 435 from *Candida antarctica* (immobilized on a macroporous anionic resin, 1.4 wt.% water) and Lipozyme RM IM from *Rhizomucor miehei* (immobilized on a macroporous anion exchange resin, 3 wt.% water) were purchased from Novozymes (Araucária, PR, Brazil) and presented an enzyme activity of around 37.91 U/g (Novozym 435) and 31.63 U/g (Lipozyme RM IM). The acid value (mg KOH/g) and water content (wt.%) (Karl Fischer titration method, DL 50, Mettler-Toledo) of the soybean oil were determined as 0.11 and 0.04, respectively.

2.2. Experimental procedure

Enzymatic ultrasound-assisted transesterification reactions were carried out using an ultrasonic water bath (Unique apparatus – temperature accuracy of ±0.5 °C) in which a round bottom flask of 250 mL capacity coupled with a total reflux condenser mounted on it (water circulating fluid at 7 °C) was immersed. The experimental setup consists of an ultrasonic bath (Unique Inc., model USC 1800A, Brazil, BR) with an operating frequency of 37 kHz and a maximum rated electrical power output of 132 W, which has been found as a suitable ultrasonic system [47]. Towards establishing the reaction time, an experimental condition, oil to ethanol molar ratio of 1:3, temperature of 65 °C, enzyme concentration, [*E*], of 5 wt.% (by weight of substrates, oil + ethanol), without water addition and 100% ultrasonic power was carried out for both enzymes taking samples at each 1 h up to 8 h reaction. Throughout this work reactions were accomplished using 1 g of oil and 40 mL of *n*-hexane.

2.3. Strategy of sequential experimental design

At first, a Plackett and Burman experimental design with 12 assays (PB-12), with triplicate runs at the central point, was employed for both enzymes to evaluate the effects of five variables (temperature, oil to ethanol molar ratio, enzyme and water concentrations, and power output rate), keeping the reaction time fixed at 4 h and solvent volume (*n*-hexane) of 40 mL. Then, two Central Composite Rotatable Design (CCRD – 2^2) with two axial points for each variable and triplicate at the central point, totalizing 11 experiments for each enzyme, were adopted to assess the influence of enzyme concentration and temperature, while maintaining fixed reaction time (4 h), oil to ethanol molar ratio (1:3), water concentration (0 wt.%) and irradiation power amplitude at 40% for Novozym 435 and 100% for Lipozyme RM IM.

Table 1 presents the reaction variables (design factors) and ranges investigated for the experimental designs used in this work. The software Statistica® 6.0 (Statsoft Inc., USA) was used to assist the design and statistical analysis of the results.

2.4. Reuse of immobilized catalysts

At the optimum temperature and enzyme concentration conditions found from the application of CCRD – 2^2 designs, successive cycles of utilization of lipases were carried out. A total of seven and eight cycles, for Novozym 435 and Lipozyme RM IM, respectively, were evaluated for each reaction system. Before starting the first reaction cycle, 4 h reaction, the enzyme was activated at 40 °C for 60 min. After the pre-defined reaction time, the enzyme was recuperated and kept in desiccator for at least 24 h. Then the enzyme was used in a new batch. The enzymatic activity was measured before and after each cycle and the residual activity was determined following the methodology described after. The product conversion of each cycle was also evaluated.

Another approach (called "simulated reuse") was also tested in this step, using the same experimental conditions established in the experimental designs for each catalyst. Here a batch of fresh enzyme was used in each cycle, just increasing 4 h in the transesterification reaction, for posterior measurement of lipase activity and product conversion, i.e., performing the reaction at multiple times of 4 h up eight cycles, 32 h of reaction.

2.5. Analytical methods

2.5.1. Product quantification

Samples were first submitted to ethanol evaporation to constant weight in a vacuum oven (338 K, 0.05 MPa) and then diluted

Table 1
Factors and levels investigated in the experimental designs employed in this work: PB-12 and CCRD 2^2 (reaction time fixed at 4 h).

Variable	Level				
	−1.41	−1	0	1	1.41
PB-12					
Temperature (°C), *T*	–	40	55	70	–
Oil:ethanol molar ratio, O:E	–	1:3	1:6.5	1:10	–
Enzyme concentration (wt.%), [*E*]	–	5	12,5	20	–
Water concentration (wt.%), [H_2O]	–	0	5	10	–
Irradiation amplitude (%), IA	–	40	70	100	–
CCRD 2^2 Novozym 435					
Enzyme concentration (wt.%), [*E*]	10	12.9	20	27.1	30
Temperature (°C), *T*	60	63	70	77	80
CCRD 2^2 Lipozyme RM IM					
Enzyme concentration (wt.%), [*E*]	10	12.9	20	27.1	30
Temperature (°C), *T*	40	43	50	57	60