A fluidized bed reactor as an approach to enzymatic biodiesel production in a process with simultaneous glycerol removal

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A B S T R A C T

The hydrodynamic characteristics and operational conditions to produce biodiesel by the ethanolysis of babassu oil catalyzed by immobilized lipase (Novozym® 435) were established in a fluidized bed reactor coupling with a column to simultaneous remove glycerol formed as byproduct. Hydrodynamics was determined by means of pulse tracer trials and results showed that the flow pattern can be described as an ideal continuous flow stirred tank. The best performance was obtained by running the reactor with biocatalyst loading of 12% and a space–time of 8 h, attaining an average yield of 98.1% and productivity of 9.9 mol_{ester}/g_{cat}/min.

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Introduction

The search for alternative fuels to mineral-derived diesel has intensified over the last few decades, resulting in the proposition of using fuels derived from renewable sources, such as vegetable oils and animal fats, obtained by transesterification with short chain alcohols to generate a mixture of fatty acid alkyl esters (FAAE) designated as biodiesel [1,2] "Bio" means the use of renewable biological sources in contrast with petroleum-derived diesel fuel, while "diesel" refers to its use in diesel engines [1]. As an alternative fuel, biodiesel can be used in its pure form or blended with petroleum-derived diesel [3].

Conventional biodiesel plants adopt transesterification processes using chemical catalysts such as alkalis and acids [1–4]. Although in low numbers, industrial plants that employ enzymatic processes for biodiesel production have been already implemented [5–7]. Unlike the conventional chemical routes for the synthesis of diesel fuels, the biocatalytic route allows carrying out the transesterification of a wide variety of oil feedstocks in the presence of impurities, such as free fatty acids [5,6]. In addition, the process meets the requirements of green chemistry, reducing the environmental impact by minimizing the waste generated in the process [6]. Despite these features, there is still a need for strategies to increase the efficiency and make it more suitable for large-scale applications [7–9]. The number of patents and reviews issued appears to indicate that the biochemical problems, such as finding a lipase with the desired characteristics, including the ability to utilize all monoglycerides, diglycerides and triglycerides; low product inhibition; high activity and yield in non-aqueous media; temperature and alcohol resistance and establishing optimal conditions, have all been solved at the laboratory research level [5–8]. However, the development of a high-efficiency reactor for the lipase-catalyzed production of biodiesel has proceeded slowly [9].

Commonly used reactors are the stirred tank reactor (STR), packed-bed reactor (PBR) and fluidized bed reactors (FBR) [9,10].

Abbreviations: A, cross-sectional area of the reactor (mm²); C, tracer concentration (wt%); C_{ee}, ethyl ester concentration (g_{ester}/g_{medium}); CSTR, continuous flow stirred tank reactor; C_v, coefficient of variation; DG, diacylglycerol; d_i, reactor internal diameter (mm); d_p, biocatalyst particle diameter (mm); $E(t)$, residence-time distribution function; F, volumetric flow (mL/min); FAAE, fatty acid alkyl esters; FAEE, fatty acid ethyl esters; h, bed height (mm); h/D, height/reactor diameter ratio; h_{min}, minimum FBR height (mm); m, amount of biocatalyst (g); MG, monoacylglycerol; MMester, molar mass of the esters (g_{ester}/mol); n, expansion coefficient; N, number of tanks-in-series that will give approximately the same residence time distribution as a non-ideal reactor; P, productivity ($mol_{ester}/g_{cat}/min$); PBR, packed-bed reactor; Q_{min}, minimum fluidization flow (mL/min); RTD, residence time distribution; s^3, skewness; STR, stirred tank reactor; t, time (h); t_m, mean residence time (h); u, fluid superficial velocity (cm/min); u_{mf}, minimum fluidization velocity (cm/min); u_t, particle terminal velocity (cm/min); u_{tc}, particle terminal velocity corrected due to the column wall effect (cm/min); V, void volume of the bed (cm³); V_b, volume occupied by the biocatalyst (cm³); V_t, total volume (cm³); ε, bed porosity; ε_{min}, minimum bed porosity; ρ, biocatalyst density (g/mL); ρ_m, density of the reactor output medium (g/mL); σ^2, variance; τ, space time (h).

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Compared with PBRs, FBRs exhibit a lower pressure drop, more uniform flow, and lower formation of preferential channels [9–11]. Although FBRs have some advantages over fixed-bed and stirred-tank reactors, few examples of enzyme-catalyzed biodiesel production in a continuous FBR have been reported [12,13]. Ricca et al. [12], using Lipozyme® as the biocatalyst and olive husk oil as the feedstock, reported the use of an FBR as a tool to increase the productivity and reaction rate for ethyl ester production. The best results were achieved in a configuration without recycling, with a relative conversion of 3%, corresponding to 0.02 mol ester/gcat/min. In a more recent work, Zhou et al. [13] described the use of *Rhizopus oryzae* lipase immobilized in magnetic chitosan microspheres to produce methyl esters from soybean oil in a magnetically stabilized FBR. In that work, the maximum methyl ester content reached was 91.3 (w/v), using a magnetic field intensity of 150 Oe.

In this study, the continuous enzymatic production of biodiesel from babassu oil in an FBR using Novozym® 435 as a catalyst was assessed. Ethanol, instead of methanol, was used as an acyl acceptor due to its being a cleaner and more sustainable alternative [14]. Furthermore, ethanol is a larger and heavier, which means a mass yield gain in the enzymatic synthesis of fatty acid ethyl esters (FAEE), resulting in a higher quantity of biodiesel per unit of oil [15]. Babassu oil was chosen as a lipid source because there are a great number of palm trees (*Orbinya* sp.) in some parts of tropical countries, such as Brazil [16]. The feasibility of producing biodiesel by the enzymatic transesterification of babassu oil has been demonstrated in previous works [17–19]. The large amount of lauric acid triglycerides ($C_{12}H_{24}O_2$) is a positive factor for the reaction kinetics due to the small relative size of these acids [19]. The FBR was operated in a recycle configuration with a cation exchange resin (Lewatit® GF202) column for glycerol removal [7]. Variables such as the enzyme loading and space–time were assessed to define the best operating parameters that satisfying both criteria of high biodiesel productivity and overall efficiency of the process, with minimal use of the enzyme.

Experimental

Materials

The biocatalyst used was the lipase from *Candida antarctica* (CalB), physically adsorbed on a methyl-methacrylate resin, trademarked as Novozym® 435 and purchased from Sigma-Aldrich Chemical Co (Milwaukee, WI, USA). Refined babassu oil was kindly supplied by Pulcra Chemicals (Jacarei, SP, Brazil), having the following fatty acid composition: 3.5 wt% caprylic acid, 4.5 wt% capric acid, 44.7 wt% lauric acid, 17.5 wt% myristic acid, 9.7 wt% palmitic acid, 3.1 wt% stearic acid, 15.2 wt% oleic acid and 1.8 wt% linoleic acid, with an average molecular weight of 709.90 g/mol [19]. Ethanol (minimum 99.8% purity) from Synth (São Paulo, Brazil) was used as an acyl acceptor. Lewatit® GF202, a macroporous cation-exchange acid resin, was kindly donated by Lanxess (São Paulo, Brazil) and used to reduce the negative effect of the glycerol adsorption on the biocatalyst surface. The resin beads were uniformly 0.65 mm in diameter, with a density of 1.24 g/cm³ and a bulk density of 0.74 g/cm³. The ion-exchange resin was activated by washing with methanol and then drying at room temperature. Each liter of the resin is able to absorb up to 250 g of glycerol, and its regeneration was carried out with methanol, according to the procedure provided by Lenntech on their website (http://www.lenntech.com). A deep blue liposoluble dye organic synthetic pigment (manufactured by Glitter – Ind. Com. Imp. Exp. Ltd., Carapicuiba/SP, Brazil) was acquired from a local market and used as a tracer.

Reactor system set-up

Continuous runs were carried out in an FBR system, as shown in Fig. 1. A jacketed glass tube (internal diameter, 12 mm; height, 375 mm; total volume, 42.4 cm³) was used as the main reactor vessel. The system was also composed of a substrate storage reservoir (babassu oil and ethanol) coupled with a reflux condenser and a column packed with 7.0 g of Lewatit® GF202 located in the recycle line. The temperature in the feed vessel and the reactor column was maintained constant by a circulating water bath, and the temperature in the area surrounding the system was maintained at approximately 50 °C using a heater tape. At the edges of the reactor column, foam disks (8-mm thickness) were placed to prevent the loss of the immobilized enzyme, which would cause pipe clogging. The reactants and products were continuously pumped (peristaltic pump, Perista Pump SJ-1211, Atto Bioscience and Biotechnology, Tokyo, Japan) through Marprene® tubing (Watson Marlow). In Fig. 1, pump 6 is a two-channel peristaltic pump that was used to feed the reaction medium and remove product at the same flow rate.

Bed expansion behavior

Bed expansion assays, as a function of the ascending fluid flow in the reactor, were carried out in the column with different amounts of immobilized enzyme and substrate containing an oil-to-ethanol molar ratio of 1:12. The test was performed by placing the immobilized enzyme in the reactor and measuring the bed height in relation to the flow used, which was gradually increased. At each flow change, the bed height was measured after 5 min to ensure complete stabilization. For each level of immobilized enzyme, the space time, τ, was calculated for different volumetric flow conditions, F, according to Levenspiel [20] (Eqs. (1) and (2)):

$$\tau = \frac{V}{F} \tag{1}$$

$$V = A \cdot h - \frac{m}{\rho} \tag{2}$$

where V is the void volume of the bed; A is the cross-sectional area of the reactor; h is the bed height, m is the amount of biocatalyst, and ρ is the biocatalyst density.

Eq. (3) calculated the porosity (ε) of the bed.

$$\epsilon = \frac{(V_t - V_b)}{V_t} = \frac{V}{V_t} = 1 - \frac{m}{\rho \cdot A \cdot h} \tag{3}$$

where V_t is the total volume of the bed and V_b is the volume occupied by the biocatalyst (i.e., by the solids in the bed).
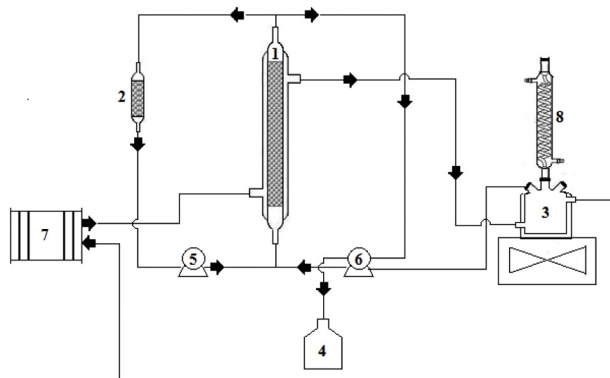

Fig. 1. FBR system used in the experiments (1 – FBR; 2 – packed column with Lewatit® GF202; 3 – substrate reservoir; 4 – product vessel; 5 and 6 – peristaltic pumps; 7 – thermostatic bath; 8 – reflux condenser).