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# Enzymatic synthesis of soybean biodiesel using supercritical carbon dioxide as solvent in a continuous expanded-bed reactor

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ABSTRACT

The enzymatic synthesis of soybean biodiesel using supercritical carbon dioxide (SC-$CO_2$) as solvent in a continuous expanded-bed reactor was investigated. For this purpose, the commercial immobilized lipase Novozym 435 was used as catalyst and the variables investigated were the amount of enzyme (10–70 g), oil to ethanol molar ratio (1:9–1:21) and substrate to solvent mass ratio (1:1–1:3), pressure ranging from 100 to 200 bar at 70 °C. Reaction conversions as high as 90% were obtained at 200 bar, oil to ethanol molar ratio of 1:9, substrate to solvent mass ratio of 1:3 using 40 g of enzyme. The continuous process for enzymatic synthesis of biodiesel using SC-$CO_2$ showed to be a promising technology for large-scale purposes. The proposed system demonstrated to be possible to obtain high conversion using lesser enzyme than in a packed-bed reactor, which might be an important advantage, due to the exhibited enzyme high-costs.



## 1. Introduction

Biodiesel has been introduced as an alternative to conventional petroleum-based diesel due to depletion of fossil fuel resources, government actions and growing environmental concerns, particularly global warming [1]. Biodiesel is currently of great interest as it is renewable, biodegradable, and nontoxic, which can be produced by transesterification of vegetable or waste oils using chemical catalysts or lipases. Lipases can work in non-aqueous environments like organic solvents.

Recent research has focused on enhancing the reaction rate using high pressure carbon dioxide [2]. Supercritical carbon dioxide (SC-$CO_2$) has been proposed as an alternative to the organic solvents to carry out enzymatic transesterification reaction, mainly due to its low critical temperature, below the denaturation temperature of lipase and the relatively good solubility of non-polar compounds, like triglycerides. In addition, SC-$CO_2$ offers easy product separation, as it can selectively extract/recover the alkyl esters from the reaction mixture since the solubility of the esters in SC-$CO_2$ is several orders of magnitude higher than the solubility of triacylglycerols and glycerol [3]. Promising results have been reported for the use of lipase with SC-$CO_2$ in the production of biodiesel from vegetable oils. For example, Oliveira and Oliveira [4] studied batch ethanolysis of palm kernel oil in SC-$CO_2$ using Lipozyme IM and Novozym 435. Madras et al. [5], Rathore and Madras [6], Lee et al. [7], Lee et al. [2], Ciftci and Temelli [1] investigated batch enzymatic methanolysis of various edible and non-edible oils in SC-$CO_2$.

Continuous reactors are more advantageous over batch ones because of the ease of operation, increased enzyme stability, facilitated enzyme reuse, and higher enzyme to substrate ratio, which decreases reaction time, and subsequent separation and cost effectiveness [8]. Therefore, it is of great interest to investigate the synthesis of biodiesel in a continuous SC-$CO_2$ bioreactor. Even though packed bed immobilized enzyme reactors have received much attention, continuous enzymatic biodiesel production in SC-$CO_2$ is still scarce.

Dalla Rosa et al. [9] conducted continuous enzymatic production of fatty acid ethyl esters from soybean oil in compressed fluids, namely $CO_2$, propane and *n*-butane. Recently, Rodrigues et al. [3] studied the continuous enzymatic production of biodiesel from virgin and waste sunflower oil in SC-$CO_2$, whereas Lubary et al. [10] reported integrated synthesis and extraction of short-chain fatty acid ethyl esters using SC-$CO_2$. Ciftci and Temelli [11] reported continuous production of fatty acid methyl ester from corn oil in SC-$CO_2$ bioreactor using immobilized lipase Novozym 435 as

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catalyst. On the other hand, Kondo et al. [12] performed simultaneous extraction-reaction of canola oil to produce fatty acid ethyl esters and Al-Zuhair et al. [13] combined continuous process of extracting fat from lamb meat using SC-$CO_2$ and the use of the extracted fat for biodiesel production in SC-$CO_2$ in one integrated system.

It is well known that industrial-scale synthesis of biodiesel nowadays is usually performed by transesterification of vegetable oils with a short chain alcohol, mainly methanol, using chemical catalysts. As ethanol is readily available from fermentative processes using biomass from a varied source, ethanol biodiesel appears as a 100%-renewable alternative, additionally enabling the replacement of traditionally used methanol by an innocuous reagent. Besides, in the regional context, ethanol has been the natural choice since Brazil is one of the world's biggest ethanol producers, with a well-established technology of production, large industrial plant capacity installed throughout the country.

The above reported works concerning the synthesis of biodiesel using SC-$CO_2$ in continuous mode employed packed-bed bioreactors containing immobilized lipases. However, the amount of enzyme used to load the reactor is an important aspect in the technical and economical feasibility of the process if it is taken into account the high cost of enzymes. As an alternative to this drawback, the main objective of this work was to evaluate the enzymatic synthesis of soybean biodiesel using supercritical carbon dioxide (SC-$CO_2$) as solvent in a continuous expanded-bed bioreactor. For this purpose, the commercial lipase Novozym 435 was employed as catalyst and the variables investigated were the amount of enzyme (10–70 g), oil to ethanol molar ratio (1:9–1:21), substrate to solvent mass ratio (1:1–1:3), pressure ranging from 100 to 200 bar at a fixed temperature of 70 °C.

## 2. Experimental

### 2.1. Materials

Commercially refined soybean oil (Soya, Florianópolis, SC, Brazil), ethanol (Merck, 99.9%, Florianópolis, SC, Brazil), lauric acid (Vetec, 98%, São Paulo, SP, Brazil), *n*-propanol (Synth, 99.5% of purity, São Paulo, SP, Brazil), sodium hydroxide (Quimex, 97%, Florianópolis, SC, Brazil) and acetone (Quimex, analytical grade, Florianópolis, SC, Brazil) were used without any pre-treatment. A commercial immobilized lipase (Novozym 435, Araucária, PR, Brazil) from *Candida antarctica* (immobilized on a macroporous anionic resin, 1.4 wt% water) was purchased from Novozymes (Araucária, PR, Brazil) and presented an enzyme activity of around 60 U/g, determined as the initial rates in esterification reaction between lauric acid and propanol at a molar ratio of 3:1, temperature of 60 °C and enzyme content of 5 wt% in relation to the substrates. The solvent used was carbon dioxide (mass fraction purity of 99.9% in the liquid phase) purchased from White Martins S.A. with ethanol as substrate for the transesterifications reaction (Vetec, 99.8% purity, Florianópolis, SC, Brazil).

### 2.2. Apparatus and experimental procedure

The experimental setup used for ethyl esters production from soybean oil with immobilized lipase in supercritical $CO_2$ (SC-$CO_2$) is schematically presented in Fig. 1, and consists basically of a 291 mL jacketed reaction vessel made of 316L stainless (62.6 cm length, 2.4 cm inner diameter) without mechanical agitation, a $CO_2$ cylinder and a syringe pump (Isco, model 500D, Teledyne ISCO, Lincoln, NE, USA). Reactor temperature was monitored by a K-type thermocouple with an accuracy of ±0.5 °C, while pressure was measured by a zero-volume absolute pressure transducer (Ashcroft, K2, Ashcroft GmbH, Germany), with a precision of ± 0.6 bar. The substrates, ethanol and soybean oil, were kept at continuous agitation in a mechanically stirred (IKA-RW 20 digital stirrer, São Paulo, SP, Brazil) closed flask over a hot platform (with slight warming), and fed into the enzyme bed by a high-pressure liquid pump (Acuflow, Thermofisher Science Education, Hanover Park, IL, USA). In all experimental conditions studied, the reactor was packed with varying amount of enzyme, with a small amount of cotton wool placed at the reactor ends. Two independent supply lines, equipped with check valves (HIP, 14-41AF1-T, São Paulo, SP, Brazil), were used to feed the substrates mixture and the compressed fluid, with one discharge line connected to a glass trap to allow collecting samples at periodic intervals. Before entering the reactor bed, the streams pass trough a micro-mixer (Valco—MX1C96, São Paulo, SP, Brazil) and then trough a pre-heating section consisted of a 100 cm long, 1/16″ OD 316L stainless steel tube. The output of the compressed fluid was computed based on the volume decay of the syringe pump reservoir (Isco, model 500D), with a resulting accuracy of ±0.01 g in pressurized solvent delivery. With known values of pressure and temperature in the syringe pump reservoir, solvent density was estimated using the HBT (P-V-T) correlation for compressed liquids [14] or taken from experimental literature values [15,16]. Before each reaction run, the reactor was flushed with low-pressure solvent so as to remove any residual air and also to condition and rinse the enzyme bed.

Typically, around 2 min were sufficient to feed the solvent into the reactor up to the pre-established pressure, and once the system pressure had been stabilized (zero solvent flow rate in the syringe pump), the volume in the syringe pump reservoir was recorded. Then, the syringe pump was changed to volume flow operation mode, setting the appropriate flow rate value according to the experimental design (with the proper conversion to mass flow rate), the substrates mixture was fed with the adequate flow rate, and the micrometering valve (HIP 15-15AF1, São Paulo, SP, Brazil), positioned on the discharge line, was opened, thus allowing regulating the system pressure by releasing the reaction products and pressurized solvent. After start-up of the reactor, usually a reactor space-time based on the liquid substrates mixture feeding was elapsed before taking samples for analysis. At the end of the experiment, the system was depressurized, the reactor was disconnected and washed with *n*-hexane until the enzyme was completely removed from the bed, and a sample was taken for measuring the enzyme activity [17].

The independent variables studied in this work were pressure (100, 148 and 200 bar), amount of enzyme (10, 30, 40 and 70 g), soybean oil to ethanol molar ratio (1:9, 1:15 and 1:21) and substrate to solvent mass ratio (ethanol + oil to carbon dioxide, 1:1 and 1:3). The reaction kinetics was followed in terms of triglycerides conversion to fatty acid ethyl esters as a function of residence time, being collected samples at 30, 60, 90, 120, 150 and 180 min. Reaction conversion was calculated straightforwardly based on the content of esters in the analyzed samples and on the reaction stoichiometry. All reactions were conducted at 70 °C, which has been attributed to be the most appropriate for enzyme-catalyzed transesterifications with Novozym 435.

### 2.3. Analytical methods

Samples were first submitted to ethanol evaporation to constant weight in a vacuum oven (338 K, 0.5 bar) and then diluted with 2 mL of ethanol and 8 mL of *n*-heptane. Afterwards, an amount was transferred to a 1 mL flask in order to obtain a concentration of 1000 ppm and then it was added the internal standard at a concentration of 250 ppm using *n*-heptane as solvent. After that, 1 μL of solution was injected in triplicate in the gas chromatograph (Shimadzu GC-2010), equipped with FID, auto-injector AOC-20i and a capillary