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Analysis of multiphasic behavior during the ethyl esterification of fatty acids catalyzed by a fermented solid with lipolytic activity in a packed-bed bioreactor in a closed-loop batch system

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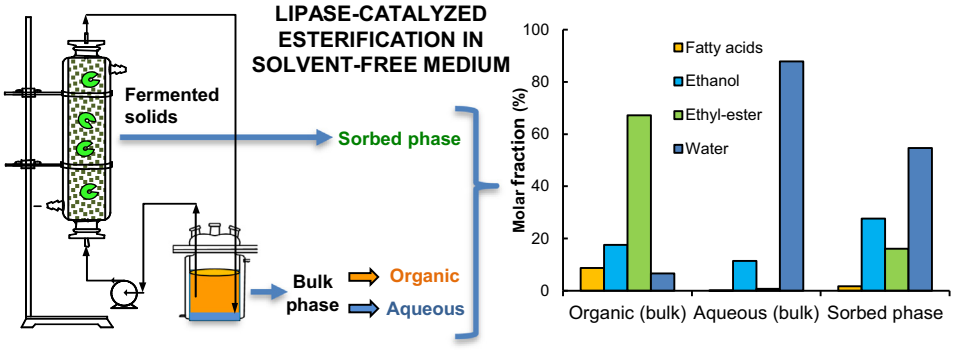
HIGHLIGHTS

- Solvent-free ethyl esterification of fatty acids from soybean soapstock acid oil.
- Catalysis by a fermented solid containing lipases from *Burkholderia cepacia*.
- Triphasic: phase sorbed on fermented solid different from organic and aqueous bulk phases.
- Sorbed phase predominantly polar, with either water or ethanol as major component.
- Improved conversion due to water sorption by sugarcane bagasse.

GRAPHICAL ABSTRACT



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ABSTRACT

A fermented solid containing lipases was produced by growing *Burkholderia cepacia* LTEB11 on a 1:1 mixture, by mass, of sugarcane bagasse and sunflower seed meal. This fermented solid was dried and then used in a packed-bed bioreactor to catalyze the esterification of fatty acids from soybean soapstock acid oil with ethanol in a solvent-free system. The bioreactor was operated in batch mode, with the medium being recirculated from the reservoir through the bed in a closed-loop system. During the reaction, the bulk reaction medium separated into organic and aqueous phases. Additionally, up to 30% of the reaction medium was held as a sorbed phase on the fermented solids. The composition of this sorbed phase, which represents the microenvironment of the lipases, was significantly different from the compositions of the bulk organic and aqueous phases. It was predominantly polar, with water or ethanol as the major component, but also contained significant amounts of ester. Importantly, the molar ratios of ethanol to fatty acid in the sorbed phase were significantly higher than those in the bulk reaction medium; this has implications for the reaction, since ethanol is known to cause inhibition and denaturation of lipases.

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1. Introduction

Hydroesterification is a promising technology for the production of biodiesel from low-value feedstocks. This process involves two steps. In the first step, triacylglycerols are hydrolyzed completely to fatty acids and glycerol. In the second step, the fatty acids recovered from the first step are esterified with an alcohol to give the corresponding ester and water [1–7]. Hydroesterification has two main advantages over the traditional alkali-catalyzed transesterification process. First, the glycerin it produces is not contaminated by alkali. Secondly, it can be used with feedstocks that contain high contents of free fatty acids without the formation of soaps.

Both the hydrolysis and esterification steps in a hydroesterification process can be carried out either chemically or enzymatically. Several authors have used lipases for the hydrolysis step, followed by esterification using a chemical catalyst [3–7]. This strategy will be problematic with low-value feedstocks since they often contain contaminants that inhibit or inactivate lipases [2].

Recently, we developed a hydroesterification process for biodiesel synthesis in which the feedstock is initially hydrolyzed in subcritical water [2]. In this process, the fatty acids are separated by distillation and then combined with ethanol to produce a solvent-free reaction medium. This medium is circulated through a packed-bed reactor that contains a fermented solid with lipase activity, with these fermented solids being produced by cultivating *Burkholderia cepacia* LTEB11 for 72 h on a 1:1 mixture (by mass) of sugarcane bagasse and sunflower seed meal. The best result that we obtained in this system was a 92% conversion in 31 h for a process undertaken at 50 °C [2]. During these studies, we observed that part of the reaction medium was sorbed by the fermented solid and that the water produced during the esterification reaction formed a second liquid phase in the reactor reservoir.

Several workers have previously observed the formation of aqueous and organic phases within the bulk reaction medium [8–11]. Also, the formation of a sorbed phase has been observed in systems involving lipases immobilized on commercial supports: Krause and Fieg [12] showed that the nonpolar organic phase sorbed onto poly-(methyl methacrylate) beads used in Novozym 435, while Sandoval et al. [10] showed that water sorbed onto the macroporous anionic resin (Duolite A568) used in Lipozyme®. However, the sorption of medium components has not previously been studied in systems involving fermented solids with lipolytic activity, neither in our own work [2,13–15] nor that of Liu et al. [16,17] and Aguieiras et al. [1]. In the present work, we investigate the sorption of the reaction medium onto our fermented solid, and also the phase behavior of the bulk reaction medium, in order to understand the effects of these phenomena on the esterification reaction.

2. Materials and methods

2.1. Raw materials

Fatty acids from soybean soapstock acid oil (FA-SSAO) were obtained by hydrolysis of SSAO in a pilot plant of the company Ubaldino Rodrigues Soares e Cia. Ltda (Ponta Grossa, Brazil), as described previously [2]. The FA-SSAO contained 0.5 wt% moisture and had an acid value of 195 mg KOH g^{-1}. Its major components (wt%) were palmitic acid (16.5%), stearic acid (4.2%), oleic acid (33.4%) and linoleic acid (44.2%), corresponding to an average molar mass of 277 g. Ethanol ($\geqslant$99.5% purity) was purchased from Vetec (Rio de Janeiro, Brazil). The standards used in the gas chromatography analyses (methyl heptadecanoate, ethyl palmitate, ethyl stearate, ethyl oleate, ethyl linoleate, ethanol and *n*-propanol) were purchased from Sigma Aldrich (St Louis, USA) and Merck (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Microorganism

B. cepacia LTEB11 was maintained in Luria Bertani medium with 50% (w/v) glycerol at −18 °C. A stock culture was transferred to an LB agar plate and incubated for 48 h at 29 °C. Isolated colonies were transferred to 30 mL of LB medium in a 250-mL Erlenmeyer flask and then incubated on a rotary shaker at 29 °C and 200 rpm for 8–10 h. This gave an optical density at 600 nm of 0.6–0.8, which represents mid-exponential phase. This culture broth was used as inoculum for the solid-state fermentation.

2.3. Solid-state fermentation

The fermented solid was obtained by solid-state fermentation (SSF) of a mixture of sugarcane bagasse and sunflower seed meal (1:1, w/w on a dry basis). Sugarcane bagasse was donated by Usina de Álcool Melhoramentos (Jussara, Paraná, Brazil) and sunflower seeds were purchased in the local market. The sugarcane bagasse and the sunflower seeds (with hulls) were milled, separately, in a knife mill, followed by sieving to obtain particles ranging between 0.85 and 2.36 mm. The SSF was done in 1000-mL Erlenmeyer flasks, each containing 40 g of milled dry substrate. Phosphate buffer solution (0.1 mol L^{-1}, pH 7.0) was added to obtain 75% moisture (w/w, wet basis). Flasks were plugged with cotton wool and autoclaved at 121 °C for 20 min. After cooling, 4 mL of inoculum was added to the substrate in each flask and the contents were immediately mixed thoroughly in order to ensure homogeneity. Flasks were then incubated at 29 °C for 72 h. After incubation, the fermented solids were dried in a column made with two polyvinyl chloride tubes (each 50 cm in height and 4.3 cm in diameter) in series. The lower tube was filled with activated silica to dry the air and the top tube was loaded with 200 g of fresh fermented solids. Air at approximately 25 °C was blown at 20 L min^{-1} into the bottom of the lower tube [2] until the fermented solids reached the desired moisture content (either 10% or 5% w/w, wet basis). We produced sufficient dry fermented solids for all the experiments in several batches. Before starting the esterification experiments, we mixed these batches together thoroughly, in order to ensure a uniform level of activity. These solids fully retained their activity during 12 months storage at 4 °C [18].

The hydrolytic and esterification activities of the fermented solid were measured, as described previously [2]. One unit (U) of hydrolytic activity corresponds to the release of 1 μmol of fatty acid per minute in the hydrolysis assay, while one unit (U) of esterification activity corresponds to the production of 1 μmol of ethyl ester per minute in the esterification assay. The hydrolytic and esterification activities of the fermented solid used in this work, per gram of dry fermented solid, were 91.6 ± 3.3 U g^{-1} and 5.8 ± 0.3 U g^{-1}, respectively.

2.4. Characterization of phase behavior during esterification in the packed-bed reactor

The reaction system was a closed-loop system operated in batch mode, in which the reaction medium was recirculated from a reservoir through a packed-bed reactor containing fermented solids. The packed-bed reactor (Fig. 1) was made of a glass column (internal diameter of 2.7 cm and height of 21 cm) with an external jacket that received water from a water bath at 45 °C [2]. The column was packed with 13.3 g of fermented solids with a moisture content of 10% w/w (equivalent to 12.0 g of completely dry