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Solvent-free ethyl oleate synthesis mediated by lipase from *Candida antarctica B* adsorbed on polypropylene powder

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Abstract

The enzymatic synthesis of ethyl oleate by direct esterification of oleic acid and ethanol in solvent-free media has been studied. Native lipase from *Candida antarctica B* and *Candida antarctica B* lipase adsorbed on powdered polypropylene were used with promising results. The influence of different parameters such us temperature, mass of lipase and aqueous content of reaction medium, on conversion profiles has been carefully studied. High water contents that ensured the co-existence of two liquid phases gave the best results, with conversions of up to 78.6% in 7 h of reaction. Pre-treatment with octane/buffer mixtures significantly reduced agglomeration of the immobilized catalyst, leading to important increments in specific enzymatic activity, when compared with non-pre-treated biocatalyst. Lipase desorption from PP has also been studied.

Keywords: Ethyl oleate; Lipase; Reaction parameters; Agglomeration; Desorption

1. Introduction

In the last years, enzymes have shown to be powerful catalysts for a number of reactions. In comparison with chemical catalysts, enzymes show higher selectivity, they work in milder conditions, and they are environmentally friendlier. Lipases (EC 3.1.1.3) are a family of enzymes that in their natural environment catalyze the hydrolysis of fats. However, in the appropriate reaction media, lipases have shown to be very active in synthetic bio-catalysis of reactions of esterification of fatty acids, alcoholysis and *trans*-esterification [1–4].

Synthetic reactions catalyzed by lipases may be performed in aqueous media, in organic solvents, in supercritical fluids [5], in ionic liquids [6] or, alternatively, in solvent-free systems [7–9]. Solvent-free systems (SFS) are highly concentrated media, economically and operationally interesting for industrial processes. In this kind of systems not only the cost of the solvent itself is avoided, but also its separation from un-reacted substrates and products, and the cost of recycle as well.

In the current work, the enzymatic synthesis of ethyl oleate has been studied. Ethyl oleate is a fatty ester which finds wide application in cosmetic and food additives industries, in the production of tailored triglycerides or in diesel fuel additives [10–11]. No solvent has been added to reaction medium consisting only of oleic acid, ethanol, variable percentages of added water, and native or immobilized lipase B from *Candida antarctica* (CALB). CALB is an interesting lipase with potential application in a number of industrial processes such as the synthesis of optically active compounds in the pharmaceutical industry [12], in the pulp and paper industry for pitch removal and de-inking processes [13] or in the synthesis of esters used in the flavor industry [14]. CALB has been mostly used in an immobilized form, commercially available from Novo Nordisk (*Novozyme 435*). However, this report concerns with the solvent-free synthesis of ethyl oleate catalyzed by CALB immobilized on a cheaper support such as polypropylene (PP) powder. CALB lipase has been previously adsorbed on EP100 with interesting results in the synthesis of 6-*O*-glucose palmitate [15].

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With the aim of optimizing the conditions for ethyl oleate synthesis, the influence of reaction temperature, mass of catalyst and water was carefully studied. The trends obtained in CALB-mediated ethyl oleate synthesis have been compared with a previous work performed using *Candida rugosa* lipase, native and supported on PP.

2. Experimental

2.1. Materials

Native lipase B from *Candida antarctica B* (5000 U/ml) was kindly supplied by Novozyme. Oleic acid (99%) was purchased from J.T. Baker. Absolute ethanol (99%) and sulphuric ether (99%) were both purchased from Dorwil. Buffer solution of pH 7 (di-sodium hydrogen-phosphate) and potasium hydroxide were both from Merck. Octane used in pre-treatment experiments was provided by Laboratory Ceblaco.

Low-molecular-weight polypropylene powder (30,000g/mol, BET area: 23 m^2/g) was obtained by polymerization using metallocenes. Commercial PP obtained with Ziegler-Natta catalysts (controlled particle diameter between 590 and 1190 um), and glass spheres of 1 mm diameter (purchased from Científica Nacional, Argentina) covered with PP, were both used as supports.

2.2. Immobilization procedure

The immobilization of lipase B from *Candida antarctica* was performed at room temperature for 7 h with 350 rpm stirring. 2.4 ml of the enzyme commercial solution (12,000 U) were diluted up to 50 ml with standard buffer of pH 7 and contacted with 1 g of ethanol pre-treated polypropylene (PP), following the method previously described in the immobilization of *Candida rugosa* lipase [16]. After the desired contact time, the insoluble material was recovered by filtration and washed with distilled water. Finally, the biocatalyst (CA/PP) was dried to constant weight at 45 °C. The same batch of CA/PP was used in the whole range of conditions tested.

2.3. Esterification reaction

In all experiments performed, reaction medium consisted of the stoichiometric mixture of substrates (N = moles of ethanol/moles of oleic acid), and different percentages of added water. Reaction was started by the addition of the biocatalyst (CALB or CA/PP) to reaction mixture, kept at 45 °C in 10 ml vials stirred at 350 rpm. During reaction (7 h) several samples were withdrawn and analyzed by titration for the residual acid content with a basic solution of potassium hydroxide. Phenolphthalein was used as the end-point indicator. The percentage of conversion of fatty acid at a definite t, was determined according to:

$$X(\%) = \frac{\text{initial moles of oleic acid} - \text{moles of oleic acid at time } t}{\text{initial moles of oleic acid}} \times 100 \quad (1)$$

2.4. Parameter study

2.4.1. Influence of the water content

When SFS are analyzed, low contents of water such as 1% (g of water/g of fatty acids × 100) reach water activities (a_w) near 1 [17]. Therefore, since for the water percentages used in this contribution a_w is near 1 in all cases, we have chosen to present data in terms of concentration of water (W: initial mass of water/initial mass of fatty acid × 100) instead of using water activities. The effect of increasing the initial water content of reaction medium was studied using 300 U of native lipase and 50 mg of CA/PP, at 45 °C and 350 rpm.

2.4.2. Influence of temperature on reaction

The effect of temperature on conversion achieved in ethyl oleate synthesis was examined at the temperature range of 35–75 °C with both free and immobilized lipase. For all reactions performed, W was kept in 20%.

2.4.3. Influence of the amount of lipase

In the case of the immobilized lipase (CA/PP) the amount of biocatalyst added to reaction medium was varied from 50 to 150 mg. In both experiments temperature was kept at 45 °C and W was 20%. Agglomeration effects were analyzed.

2.4.4. Pre-treatment of CA/PP with "oil–water" interfaces

Immobilized lipase (50 mg) were typically contacted with 5 ml of octane/buffer of pH 7 mixture for 30 min, at 45 °C and 1000 rpm. According to previous results obtained for *Candida rugosa* lipase adsorbed on PP, the mixture of octane/buffer in a volumetric ratio of 5/95 provided the immobilized catalyst with an "oil–water" interface that induced lipase activation [18]. After 30 min, the pre-treated catalyst was recovered by filtration and dried at 45 °C for 1 h. Increasing amounts of the pre-treated biocatalyst were used in ethyl oleate synthesis preformed at 45 °C with W = 20%. Activation of lipase was analyzed.

2.5. Desorption of lipase

Being CALB immobilized upon PP by simple adsorption, the desorption of lipase was checked. Reuse of CA/PP was assayed in order to determine if huge activity of CA/PP was due to the adsorbed lipase, or if it was actually caused by desorbed lipase catalyzing reaction in its native form. Several experiments using CA/PP, CALB adsorbed on PP obtained using commercial Ziegler-Natta catalysts, and CALB adsorbed on PP linked to glass spheres, were