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Enhancing the enzymatic synthesis of alkyl esters by coupling transesterification to an efficient glycerol separation system

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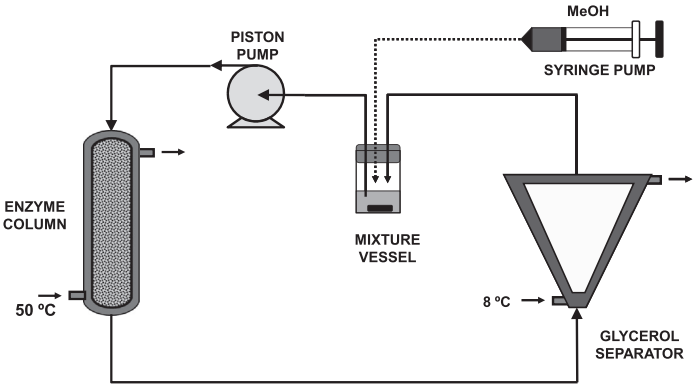
HIGHLIGHTS

- The enzyme synthesis of biodiesel was coupled to glycerol separation steps.
- A cold trap combined with a resin adsorption column were combined.
- Only 0.4% of the total glycerol produced was retained by the enzyme.
- The reaction mixture transported the glycerol from the enzyme to the separation system.
- Enzyme was preserved from the negative blocking effect of glycerol.

GRAPHICAL ABSTRACT



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ABSTRACT

Major drawbacks for enzyme-catalyzed transesterification of vegetable oil triglycerides to produce biodiesel (esters of short chain alcohols) are the high concentration of alcohol in the reaction mixture and the deposition of the produced glycerol on the enzyme support. Although stepwise addition of alcohol to the reaction mixture solves the first problem, an effective way to remove glycerol from the reaction system is needed. In the present study the efficiency of three systems for removal of glycerol from the reaction mixture during the enzymatic synthesis of sunflower oil methyl esters was investigated. By coupling a single cold column to the reactor 87.6% of total glycerol produced by the reaction was removed, while 10.9% was still retained by the enzyme. The percentage of glycerol separated from the reaction system increased to 93.1% when a second separation column packed with Amberlite BD10DRY resin was included, reducing the percentage of glycerol retained by the enzyme to 5.8%. Finally, changing the geometry of cold separation column in which a conical decanter was used, the percentage of glycerol separated increased to 99.2%, while only 0.4% was retained by the enzyme. Results demonstrated that use of a convenient separation system such as those described before, glycerol produced during transesterification of sunflower oil can be efficiently removed from the reaction mixture. In this way the well-documented inhibitory effects of the glycerol product are controlled and enzymatic catalysis remains fully functional.

1. Introduction

It is well-known that biodiesel, the product of transesterifying triglycerides with short chain alcohols, has gained in importance

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due to diminishing petroleum reserves and the environmental consequences of gas emissions in exhaust from engines using petroleum-based fuels [1,2]. Additionally, developing this process is considered to be the most convenient alternative for petroleum-based fuel for diesel engines due to similarities in physicochemical properties and high compatibility with traditional diesel fuel [3].

Although chemically-catalyzed transesterification offers a practical and relatively simple method for industrial scale conversion of vegetable oils to biodiesel, there are several drawbacks associated with this type of catalysis [1]. The careful removal of catalyst residues from the finished product is required to achieve quality control standards, and often involves large volumes of effluents from final washing steps [4]. Additionally, purification of glycerol produced as by-product (about 10 wt% of biodiesel products) critically depends on the removal of inorganic material and, occasionally, on the removal of undesirable by-products requiring expensive multiple-stage separation procedures [1,4].

Enzyme-catalyzed transesterification has been proposed as a cleaner and greener alternative conversion method [5,6]. Highly purified glycerol can also be obtained using this processing alternative, which is indicated to be essential for developing a sustainable biodiesel industry [7]. Additionally, enzyme processes can be efficiently performed under much milder reaction conditions, and results in improved integrity of the alkyl esters produced [8,9]. However, due to the high cost of the enzymes needed and the loss of catalytic activity, e.g. due to product inhibition during the process, enzyme-catalyzed biodiesel production has not been widely pursued or developed in scale-up research [10,11].

It is well-known that the enzyme inactivation during the progress of the alcoholysis very much depends on reaction conditions. If high concentrations of short-chain alcohols such as methanol or ethanol are used, complete inactivation of the enzyme occurs rapidly. This has been associated with heterogeneity of the reaction mixture and the separation of two phases due to low miscibility of alcohols and oils [12].

Furthermore, the low solubility of the glycerol in the mixture has a strong negative effect, expressed as a blocking effect, on enzyme activity [13]. This effect has been attributed to deposition of the glycerol on the enzyme surface, leading to hydrophilic hindrance of the enzyme, thus causing diffusion constraints for the transfer of the hydrophobic substrate from the organic phase to the enzyme's active site [14].

In order to improve the utility of the enzymatic process, research has focused on alternative methods for preserving enzyme activity during the process and extensive work has been done using a variety of methods. One obvious improvement was the use of appropriate organic solvents, and this was demonstrated to improve the efficiency of the process by increasing the solubility of alcohol and glycerol in the reaction medium [15]. An example reported was the use of methyl acetate instead of methanol as acyl acceptor to avoid both enzyme denaturation by methanol and the production of glycerol as by-product [16]. The slow addition of methanol to the reaction mixture in multiple steps led to higher conversion yields in both batch and continuous transesterification of vegetable oil to methyl esters [17,18]. Also the addition of fatty acid alkyl esters at the start of the reaction may prove useful in modifying the intersolubility between the oil and the alcohol. As such, the composition of the transesterification reaction mixture has been a major focus of attention in efforts to improve enzyme performance [19].

The intersolubility of transesterification reaction mixture substrate and product components was demonstrated to have strong influence on efficiency of the enzyme-catalyzed process in our previous study [19]. These results showed that in order to have effective enzyme activity careful design of reaction mixture composition is necessary. It is important to ensure that both a low concentration of alcohol is maintained and that the glycerol produced during the reaction remains soluble to retain enzyme activity. Despite efforts to optimize such a strategy, adsorption of glycerol on the enzyme support remained difficult to avoid, and thus continued to result in a strong negative effect on enzyme activity. Previous work suggests that a key for success in the enzyme-catalyzed conversion of oils to biodiesel should necessarily include an efficient glycerol separation step, able to reduce the concentration of glycerol in the reaction mixture to the minimum possible [19]. Thus, the purpose of the present work is the study of different systems for the removal of glycerol from the reaction mixture, which were "coupled" to the reaction step. This strategy also avoids the use of exogenous organic solvents to achieve an efficient enzyme-catalyzed transesterification.

2. Materials and methods

2.1. Materials

Refined sunflower oil was provided courtesy of COUSA S.A., Montevideo, Uruguay, and had a fatty acid composition of: 5.3% of 16:0; 3.8% of 18:0; 29.3% of 18:1; 60.7% of 18:2. Total free fatty acids in the oil were 0.04% (as oleic acid) and the moisture content was less than 0.05%. Commercial lipase from *Candida antarctica* (Novozym 435) immobilized on a macroporous anionic resin (Novozym 435) was provided courtesy of Novozymes Latinoamerica, Curitiba, Brazil. Solvents, standards and reagents used were also purchased from Sigma–Aldrich. Amberlite BD10DRY resin was kindly provided by Urupema S.A.-Petrosul, Canelones, Uruguay.

2.2. Continuous enzyme-catalyzed transesterification

Fig. 1a shows a diagram of the apparatus used for the enzyme-catalyzed methanolysis process. Fluid from the reaction mixture vessel was pumped at a constant flow rate of 1.5 mL/min by a piston pump through the different components of the system: first the enzyme column and then the glycerol separator system (alternatively A, B or C). The eluent from the separator was then returned to the reaction mixture vessel.

At the beginning of a typical reaction process, the fluid was 100% oil (from 70 to 100 g, depending on the type of system used). Fluid was continuously recirculated throughout the unit at the target temperatures in the different sectors of the system. The starting time for a given reaction was considered to be when the syringe pump was activated and the addition of alcohol to the system was initiated. Alcohol was added in order to achieve a final amount equal to 110% of the stoichiometric requirement, and at an appropriate constant flow rate such that addition of total alcohol amount took 24 h. The final alcohol excess of 10% was used in order to "push" the reaction toward completion.

The reactor consisted of a vertical jacketed glass column (300 mm long × 10 mm internal diameter) with a PTFE bed support (20 μm) at the bottom, packed with the immobilized enzyme Novozym 435 (5 wt% of the initial amount of oil in the system). Reactor temperature was maintained at 50 °C by recirculation of thermostated water through the jacket. Samples of reaction product were collected in triplicate at reactor output at different periods and processed as described below.

Fig. 1b–d shows the three types of glycerol separation systems that were connected alternatively to the reaction unit. Separation system A (Sep-A, Fig. 1b) operates as a cold trap and consisted of an empty column identical to that described for the reactor, but its temperature was maintained at 8 °C. Separation system B (Sep-B, Fig. 1c) consisted of a cold trap (SB_1) identical to that described previously for Sep-A, plus a second column packed with Amberlite BD10DRY resin and thermostated at 50 °C (SB_2, 5 wt% of resin with respect to the initial amount of oil). This "dual"