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The centrifugal partition reactor, a novel intensified continuous reactor for liquid–liquid enzymatic reactions

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ABSTRACT

Implementation of continuous processes for production of fine chemicals and pharmaceuticals is an efficient way for process intensification. This study aims at demonstrating the potential of a Centrifugal Partition Chromatography (CPC) apparatus as a novel type of intensified reactor (termed Centrifugal Partition Reactor, CPR) for biphasic (water-organic solvent) enzymatic reactions. The reaction of esterification of oleic acid with n-butanol catalyzed by the *Rhizomucor miehei* lipase was tested as the model reaction.

The influence of rotation speed, flow rate, enzyme and substrate concentrations on esterification reaction were studied. The CPR proved to be efficient to generate sufficient interfacial area (weakly dependent of the flow rate) and sufficient residence time (30 min) to achieve good conversion (85%). Also, increasing rotation speed of the CPR surprisingly decreased performances, probably due to very specific inner hydrodynamics. For a given configuration, the productivity of the CPR (40.5 g h^{-1} L^{-1}) was found to be more favorable than the conventional batch process (21.6 g h^{-1} L^{-1}). Steady state operation of the reactor at 22 °C, (i.e., constant conversion at the output, see Fig. 8), was reached after about 2 residence times and lasted for 24 h. After 24 h, the output conversion slowly decreased due to the low intrinsic stability of the enzyme at room temperature.

The promising results obtained in this study are a good incentive to promote the CPR as a competitive innovative technology for operating continuous two phase enzymatic reactions.

1. Introduction

Lipases (EC 3.1.1) catalyze hydrolysis, esterification, inter- and trans-esterification reactions in aqueous or non-aqueous media [1]. Lipase-catalyzed esterification reactions have gained growing interest during the last decades due to an increased use of organic esters in the biotechnology and chemical industry (food, detergents, plasticizer, lubricant, etc.) [1–2]. Furthermore, some studies have been published on enzymatic esterification with the aim to improve biofuel production [3–5]. The lipase esterification in two phase media (water-organic system) offers several advantages:

1. the thermodynamic equilibrium of the reaction is switched towards synthesis [4],
2. the solubility of non-polar substrates and products is high,
3. the enzyme is located in a favorable aqueous environment at the interface,
4. it is possible to directly use an aqueous enzyme solution, avoiding the need of immobilization onto a solid support,
5. the separation between catalyst and products is easy [6–7].

Therefore, lipase-catalyzed esterification in such systems may gain considerable industrial potential and there is a need to propose intensification of this process to increase productivity and to ensure economic viability. In this case intensification is achieved by implementing continuous operation of a reactor with efficient mixing, high interfacial area and robustness of operation. Recently, some attempts were done to perform this kind of reaction using different novel technologies of intensified continuous reactors. Elgue et al. [8] successfully tested the Corning type and Chart type reac-

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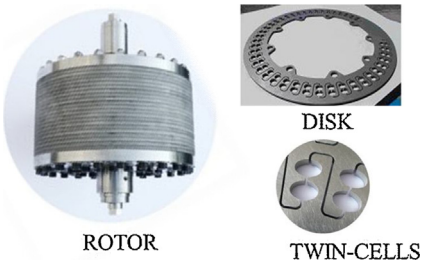

Fig. 1. The rotor and an engraved disk composed of twin-cells connected by ducts. The pictures were from Armen Instrument catalog [15].

tors. Such continuous reactors have the advantage to promote mass transfer and to generate high interfacial area. However, their performances depend on flow rates and maximizing them implies an increase in flow-rates, which correlatively shortens the residence time. This could be a problem for enzyme operating at liquid–liquid interface where global kinetics is highly dependent upon interfacial area. In addition, in this type of reactor, the aqueous enzymatic phase is continuously fed simultaneously with the substrate containing phase. Thus, the enzymatic aqueous phase needs to be separated at the output of the reactor in order to be recycled. This requires an inconvenient operation of decantation which is not always easy to operate in the continuous mode. So an intensified reactor able to "immobilize" the catalytic aqueous phase and where mass transfer performances would be weakly dependent upon flow rates, would be very useful to develop efficient intensified enzymatic processes.

Such a concept is investigated here through the use of an existing apparatus, diverted from its original application, the Centrifugal Partition Chromatograph (CPC, also called Hydrostatic CounterCurrent Chromatography). This system is originally and conventionally used for separation purposes (support-free liquid chromatography or extraction). Basically, a CPC apparatus consists in a series of cells engraved on a disk and connected in cascade by ducts. Disks are stacked to form a column called "rotor" (Fig. 1). This latter is rotated and this assembly is then submitted to a constant centrifugal field (several hundreds of g) [9]. This centrifugal field enables maintaining one of the liquid phases inside the cells (the stationary phase) while the other one (the mobile phase) percolates this stationary phase in each cell as a jet. Depending on the physico-chemical properties of the two phase system, the jet may disintegrate as a film along the cell wall or as very tiny droplets [10]. It then coalesces to leave the cell and flows to the next cell via a tiny duct. When this apparatus is operated in the so-called "ascending" mode, the heaviest phase (here the aqueous phase containing the enzyme) is retained in the column as the stationary phase. The understanding of the CPC functioning, especially the influence of rotational speed and mobile phase flow-rate, upon the liquid–liquid dispersion and the value of the stationary phase hold-up, is complex and its exhaustive description is out of the scope of this paper. The reader is advised to refer to some dedicated papers such as the book of Foucault [9] or the paper of Marchal et al. [10].

In this work, it is proposed to operate this system as a two liquid phase reactor (termed Centrifugal Partition Reactor, CPR) as already proposed as a continuous reactor for catalysis [11–12] and as a chromatographic reactor for enzymatic reactions [13–14]. Indeed, in the CPC apparatus, one of the phases, containing the enzyme catalyst, can be maintained in the reactor while the other one, carrying substrates and products, is continuously fed and extracted from the system. Basically, it can be said that the whole cascade of liquid–liquid contacts in each cell (droplet (or film) generation then coalescence) is equivalent to a continuous reactor with an immobilized catalytic phase. Because the very high number of contact cells (usually in the range 200–2000), the back mixing is reduced and the CPR can be easily assimilated to a continuous plug flow reactor [12].

Fig. 1 presents pictures of the elements of a CPR: the rotor and one of the engraved disks composed of twin-cells connected by ducts.

In the present case, the main advantages of this type of device lies in its ability to immobilize a liquid phase containing the enzyme while generating a large interfacial area with the organic phase containing the substrates. In this sense, the system behaves similarly to a conventional fixed bed reactor of porous particles where enzyme is immobilized onto the inner surface of particles. Note that when efficient immobilized enzymes are easily available the fixed bed reactor is undoubtedly a very good reactor technology. It is important to note that it has been shown that in CPC the interfacial area is only slightly dependent upon the flow rate of the mobile phase [9] and therefore, in such a system, a suitable residence time can be achieved by adapting the mobile phase flow rate and makes it possible to operate moderately fast enzymatic reactions. In this work, the potential of CPC apparatus to perform two phase enzymatic esterification was investigated using esterification of oleic acid with *n*-butanol, catalyzed by lipase from *Rhizomucor miehei*, as a model reaction. Heptane was used as the organic phase containing oleic acid and *n*-butanol substrates and the immobilized aqueous phase consisted of a solution of *R. miehei* lipase in a phosphate buffer (pH 5.6). The aim of this work was to prove the feasibility of operating this reaction in such a continuous liquid–liquid centrifugal reactor. The main parameters usually influencing enzymatic esterification (oleic acid/*n*-butanol molar ratio, lipase concentration, substrate concentrations) and operating parameters of the CPR (rotation speed and flow rate) were evaluated. A comparison between this novel CPR technology and conventional batch agitated vessel reactor is also reported.

2. Methods

2.1. Materials

The *R. miehei* lipase, produced in *Aspergillus oryzae* was purchased from Sigma–Aldrich Chemie (Saint-Quentin Fallavier, France). Lipase activity (918 AU mL^{-1}) was spectrophotometrically determined by following the hydrolysis of *p*-nitrophenyl butyrate (*p*NPB) at 405 nm. For this purpose, an aliquot of the enzyme solution (20 μL) is added to a reaction mixture composed by 175 μL of 100 mM of phosphate buffer (pH 7.2) and 5 μL of *p*NPB (40 mM in 2Methyl2Butanol). The mixture is incubated at 25 °C for 10 min. One unit of lipase activity (U) is defined as the amount of lipase required to release 1 μmol of *p*NPB per minute, under the specified conditions (25 °C and pH 7.2).

Oleic acid (purity 95%) was obtained from the same provider. All other chemicals were of analytical grade (heptane, *n*-butanol, methanol and ethanol) and purchased from Fisher Scientific (Illkirch, France). The standard for HPLC analysis of *n*-butyl ester (purity > 95%) was obtained from Combi-Blocks, Inc. (San Diego, CA, USA).

2.2. Experiments and conditions

2.2.1. CPR experiments

The experiments were performed in a CPC-250-F apparatus, manufactured by Armen Instrument (Saint-Avé, France). The column consisted of 21 disks stacked as a rotor. Each disk is composed of 90 twin-cells (100 μL volume for each cell) linked in cascade by tiny ducts. From tracer experiments, the actual column volume (cell and ducts) was estimated at 200 mL. The liquid–liquid system consisted of an organic phase composed of *n*-butanol dissolved in