Available online at www.sciencedirect.com
ScienceDirect
Journal of Membrane Science 297 (2007) 98–103
journal of MEMBRANE SCIENCE
www.elsevier.com/locate/memsci

Enzymatic membrane reactor involving a hybrid membrane in supercritical carbon dioxide

Tània Gumí, Delphine Paolucci-Jeanjean *, Marie-Pierre Belleville, Gilbert M. Rios

Institut Européen des Membranes, IEM/UMII-CC 047, Place Eugène Bataillon-34095 Montpellier Cedex 5, France

Received 10 October 2006; received in revised form 9 March 2007; accepted 12 March 2007
Available online 15 March 2007

Abstract

Enzymatic membrane reactors enable the performance of catalytic reaction, enzyme recovery and product isolation as a one-step process. Hybrid enzymatic membranes were obtained immobilizing *Candida antarctica* lipase B (CALB) onto a ceramic support, through a biopolymer layer, by means of a cross-linking agent (glutaraldehyde). Those membranes were applied in the synthesis of butyl laurate, from lauric acid and butyl acetate, in supercritical carbon dioxide ($scCO_2$), this medium showing well-known advantages over classical organic media, such as: being environmental friendly, allowing efficient mass transfer or having relatively low critical conditions. Several parameters, such as pore size of the membrane support, substrate entering flow rate, working pressure and temperature, were studied in batch mode. Continuous dead-end filtration which allows one to obtain attractive production rates thanks to an enhanced substrate supply to the enzyme surroundings was also investigated. The use of this operating mode for performing synthesis in an enzymatic membrane reactor in $scCO_2$ medium is reported here for the first time. System productivity was found to be dependant on the provided substrate amounts and still remaining attractive after five cycles in batch mode.

Keywords: Enzymatic membrane reactor; Butyl laurate synthesis; Supercritical carbon dioxide; *Candida antarctica* lipase B

1. Introduction

Enzymes are nowadays well appreciated as catalysts for many chemical processes occurring in non-aqueous environments. The use of such media is particularly advantageous to transform poorly water-soluble organic substrates, to avoid undesired side reactions, and to shift the thermodynamic equilibrium to a more favourable position in synthesis [1]. Particularly, lipases (E.C.3.1.1.3) show high stability in organic media, and they have been widely and successfully employed in non-conventional phases [2]. Lipases are able to accept a large number of substrates [3]. Among all lipases, *Candida antarctica* lipase B (CALB) is a very robust protein and exhibits specificity and stereoselectivity [4].

Among all non-aqueous media, supercritical fluids show the most attractive properties, as they have gas-like low viscosities and high diffusivities, and simultaneously, they possess attractive liquid-like solubilizing power. These properties increase the mass transfer and thus enhance reaction rates. Supercritical carbon dioxide ($scCO_2$) holds additional benefits, since it is environmentally benign, non-flammable, and exhibits low toxicity. Furthermore, its critical pressure is relatively low (73.4 bar) and it has an ambient critical temperature (31.3 °C). Besides, CO_2 can be easily removed from mixtures by simple depressurization [2,5]. However, $scCO_2$ has shown to have adverse effects on free enzyme activity [6]. Therefore, strategies for enzyme stabilization need to be investigated. Knez and Habulin [7] studied the enzymatic stability of various lipases both immobilized and free, and in all cases enhanced stability was encountered in the former case.

The use of membranes as supports for immobilizing enzymes is especially attractive since they can be placed in biocatalytic membrane reactors, which allows integrating in a single step, catalytic conversion, product separation and catalyst recovery [8].

The preparation of such active membranes can be achieved by different routes depending on the way the enzymes are immobilized on the membrane support (entrapment into membrane pores, adsorption, ionic binding, covalent linkage, etc.). The

* Corresponding author. Tel.: +33 467149186; fax: +33 467149119.
E-mail address: delphine.paolucci@iemm.univ-montp2.fr (D. Paolucci-Jeanjean).

0376-7388/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2007.03.015

latest option, enzyme covalent linkage, holds the advantage of leading to a highly stable catalyst [8].

During the last few years, a methodology has been developed at IEM, for enzyme immobilization on ceramic membranes, wherein enzymes were attached, covalently, by means of a cross-linker (glutaraldehyde), to a polymer layer previously deposited by filtration onto the ceramic membrane [9]. The choice of inorganic supports which are chemically and mechanically resistant, allows us to work under high pressure conditions and in conventional and non-conventional solvents. Recently, active membranes prepared according to this procedure, and containing CALB have been successfully used in aqueous solution [10], organic media [11], and also under high pressure [12,13].

In this work, enzymatic ceramic–hybrid membranes, containing immobilized CALB, have been prepared and applied for the synthesis of butyl laurate, from butyl acetate and lauric acid, in supercritical carbon dioxide. Several membrane reactor operating modes have been considered. The membrane stability, as well as the influence of both pressure and temperature on membrane performance, have been checked. Butyl laurate synthesis was selected as a model reaction leading to added value esters.

2. Experimental

2.1. Materials

A commercial preparation of *Candida antarctica* lipase B, namely Novozym 525F was supplied by Novo Nordisk (Denmark). The enzyme solution was stored at −20 °C and used without further purification.

Alpha-alumina ceramic tubular supports (130 mm length, 7 mm internal diameter, 10 mm external diameter, 28 cm^2 effective area, and pore diameter between 0.2 and 1 μm) were purchased from Inocermic (Germany). Carbon dioxide (CO_2 N45 TP) was supplied by Air Liquide (France).

Substrates, solvents and other reagents were purchased from Sigma–Aldrich and were of the highest purity available.

2.2. Preparation of enzymatic membranes

Enzyme immobilization on ceramic membranes was performed in three steps, as described previously: formation of a dynamic biopolymer layer on the membrane surface, its activation with a cross-linking agent (glutaraldehyde), and finally the attachment of the enzyme. Further details on each preparation step can be found elsewhere [10]. Enzymatic membranes containing immobilized lipase were dried in presence of P_2O_5 under vacuum conditions and stored at room temperature until being used.

After membrane use, ceramic supports were regenerated following a severe cleaning procedure [10], by means of sodium hypochlorite, sodium hydroxide and nitric acid. The correct regeneration of membrane supports was confirmed by determination of pure water permeation fluxes across the membranes.

Blank membranes, to be used as controls were also obtained. To prepare them, an identical procedure was carried out, but no enzyme was added to the membrane support.

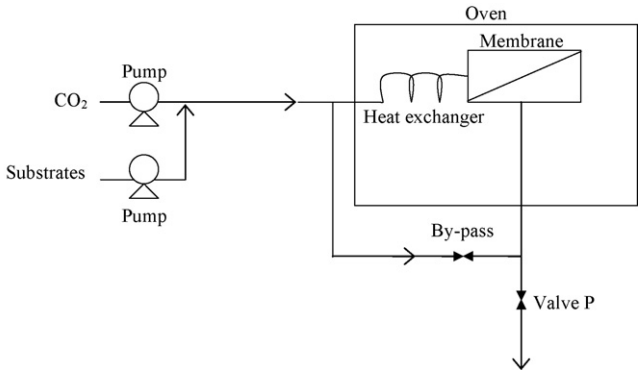

Fig. 1. Scheme of the catalytic membrane pilot.

2.3. Membrane reactor set-up

Enzymatic membranes were tested in the membrane reactor shown in Fig. 1, which can operate in different modes: continuous dead-end filtration, semi-batch and batch mode.

The substrate solution (15 mM butyl acetate and 15 mM lauric acid in hexane), was pumped to the reactor via an HPLC pump (Gilson 307) at a constant rate and mixed with liquid CO_2. Next, the blend entered to the thermostatized membrane cartridge after passing through a spiral tube (also thermostatized) where the mixture was heated and turned in supercritical conditions. Carbon dioxide flow rate was generally ca. 0.5 kg h^{-1}.

When working in continuous mode, substrate solution was pumped at constant rate (2 or 7.5 ml min^{-1}) and valve P was maintained opened for the whole duration of the experiment.

Batch mode involved 1 h of continuous filtration of feed solution (at 2 ml min^{-1}) maintaining the permeate outflow opened. Then, the feed was stopped, the valve P was closed and the reaction took place for 2 h in batch mode. Afterwards all substrates and products accumulated in the pilot were recovered. This was achieved by pumping pure hexane at 2 ml min^{-1} during 1 h and collecting all exiting permeate.

Semi-batch mode consisted of the continuous entering of substrate solution into the membrane reactor at a constant flow rate (0.5 or 2.0 ml min^{-1}) maintaining the permeate exit (valve P) closed, except for sample withdrawing.

The membrane reactor was operated at pressures and temperatures between 80 and 100 bar and 50 and 60 °C, respectively, with permeate flux regulated by valve P. By-pass way was used when pressurizing–depressurizing the membrane pilot. By-pass valve was kept closed for each experiment. Permeate samples were collected during experiments to monitor butyl laurate production in supercritical CO_2.

2.4. Reaction and analytical method

The interesterification between lauric acid and butyl acetate, catalysed by CALB takes place by the following reaction:

lauric acid + butyl acetate ⇆ butyl laurate + acetic acid

Withdrawn samples were analysed by gas chromatography using a flame ionization detector, a J&W Scientific column (DB-