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Production of 2-phenylethanol in hybrid system using airlift reactor and immersed hollow fiber membrane module

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ABSTRACT

To minimize capital and operative costs in many bioproductions of chemical specialities, where the product inhibits the bioreaction, a hybrid system based on the application of membrane extraction integrated in the bioreactor to remove the product is a suitable solution. Integration can be done by an external module for membrane extraction or, as a more effective solution, by an extraction membrane module immersed directly in the bioreactor. In this second case, it is not necessary to use microfiltration to prevent membrane fouling or to use another pump for shell flow in the membrane module. Moreover, the system is very compact, highly effective, resistant to failures and its mathematic simulation is also possible. These statements are proved in this paper where a hybrid system consisting of an airlift reactor and immersed extraction hollow fiber membrane module was used for the biotransformation of L-phenylalanine to the desired rose-like aroma, 2-phenylethanol, by yeasts *Saccharomyces cerevisiae*. Two biotransformation experiments were carried out using different feeding and aeration strategies. In both experiments, high conversion of L-phenylalanine (up 100%) and high volumetric production of 2-phenylethanol (up 18.6 g L^{-1}) were reached. Both biotransformation experiments were mathematically predicted with good agreement between experimental data and simulations.

1. Introduction

2-Phenylethanol (PEA) is a rose-like aroma produced naturally in many kinds of blossoms. It is used in food, cosmetics and perfumery industry, for which it is mainly produced by two different ways: a cheaper one by chemical synthesis or a more expensive one by direct extraction from blossoms. Another way that has been recently used with growing tendency is biotransformation production of 2-phenylethanol from L-phenylalanine (Phe) that can be realized by various breeds of yeasts [1]. Produced PEA is a strong inhibitor of the biomass growth and thereby of its own production [2–4]. To overcome the product inhibition (toxic PEA concentration of around 4 g L^{-1} for *Saccharomyces cerevisiae* [3]), continuous product removal from the fermentation medium is necessary. There are several possibilities of PEA removal from the bioreactor: two-phase extraction [5]; adsorption [6,7]; pervaporation [8]; immobilized solvent extraction [9]. A good review on recent advances in biotechnological production of PEA, with emphasis on the strategies used to increase the production and applications of in situ product removal, was made by Hua and Xu [2].

A special type of two-phase extraction is the membrane based solvent extraction, where the organic solvent is physically separated from the aqueous phase with a membrane in a membrane module and the contact between the phases is mediated only by the pores of the membrane. Membrane extraction is commonly used in many applications where product removal from the fermentation medium is required [10–14].

For in situ product removal during the bioprocess, the extraction membrane module can be reconnected with the bioreactor by several ways: (1) the fermentation medium is at first treated by microfiltration (to prevent fouling of the extraction module) and the permeate is led to the extraction module where the product is extracted to the organic phase and fermentation medium without product flowing back to the bioreactor [13,15]; (2) the fermentation medium with biomass is led directly to the extraction membrane module and then back to the bioreactor [16,17]; (3) the

Abbreviations: ALR, external loop airlift reactor; B1, B2, biotransformation experiments 1 and 2; EtOH, ethyl alcohol; Glu, glucose; HPLC, high pressure liquid chromatography; Phe, L-phenylalanine; PEA, 2-phenylethanol; X, biomass.

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Table 1
Characteristics of the immersed hollow fiber contactor.

Provider of fibers	MEMBRANA GmbH
Type of fibers	Accurel® PP S6/2
Dimensions of contactor	104 mm × 1310 mm
Fiber/fitting tube material	Polypropylene/polyethylene
Outer/inner fitting tube diameter	12/8 mm
Outer/inner rings diameter	104/80 mm
Overall contact area[a]	1.842 m^2
Number of fibers	169
Outer fiber diameter	2.7 mm
Inner fiber diameter	1.8 mm
Fiber wall thickness	0.45 mm
Effective fiber length	1285 mm
Pore diameter	0.2 μm
Porosity/tortuosity	0.8/2.25

[a] Based on outer diameter.

extraction membrane module is immersed directly in the fermentation medium in the bioreactor, which can save microfiltration membrane and a pump for microfiltration or extraction circuit [18].

Realization of the third process is the main aim of this paper, where a hybrid system for the PEA production (by *S. cerevisiae*) consisting of an airlift reactor (ALR) integrated with an immersed membrane module made from hollow fibers is proposed. The system is used for in situ continuous membrane extraction of the product from the fermentation medium to the organic solvent continuously regenerated in a distillation unit. In the previous work [19], extraction kinetics of PEA was studied in such a hybrid system (without solvent regeneration) at different operational conditions as well as hydrodynamics of the ALR with an integrated immersed membrane module. Mathematical model of membrane extraction was also presented and verified for various extraction kinetic data.

Within the presented work, extraction kinetics of PEA was measured in the proposed hybrid system using continuously regenerated pentane for the extraction. Two long-running biotransformation experiments were performed in the hybrid system at different aeration and feeding conditions with markedly different results in the PEA production. The course of the biotransformation experiments was mathematically predicted and compared with that of experiments applying a mathematical model of the hybrid system comprising the models of yeast growth, biotransformation, membrane extraction and PEA (ethanol) inhibition.

2. Experimental

2.1. Hybrid system

The hybrid system for 2-phenylethanol (PEA) production was created by a combination of an internal loop airlift reactor (ALR) with an immersed hollow fiber membrane module and a regeneration unit (Fig. 1a). Dimensions of the ALR are shown in Fig. 1b. Its maximum working volume was 17 L. It is the same ALR as that used in the paper by Juraščík et al. [20], where more detailed information can be found. Hydrodynamics and PEA extraction capability of this system (without the regeneration unit) were studied in a previous paper [19]. The membrane module was manually manufactured in our laboratory using polypropylene hollow fibers (Accurel® PP S6/2, 169 fibers, 1.84 m^2 outer area) and installed in the downcomer of the ALR extending from its bottom to its separator. Characteristics of the membrane module is shown in Table 1. The inlet and outlet of the membrane module were connected with the flexible and chemical resistant tubes leading from the ALR to the regeneration unit. The regeneration unit consisted of a boiling flask heated by a water bath, water cooler and a flask with the distillate. Organic solvent was sucked from the distillation flask to the boiling flask through the membrane module using a Teflon® membrane pump installed at the outlet of the membrane module. Temperature of the fermentation medium in the ALR was regulated using a countercurrent heat exchanger (tube in tube) connected to a thermostat and measured using a temperature probe embedded at the bottom of the ALR. The heat exchanger was linked with the ALR and the rotary lobe pump in a closed loop (2 L min^{-1} of liquid circulation). The ALR was bubbled by compressed air with the flow rate regulated and measured using a rotameter with a regulation valve. Oxygen saturation and pH value were measured using an oxygen probe and a pH probe installed at the bottom of the ALR (in the same way as temperature probe). Detailed characteristics of the hybrid system and the immersed membrane module can be found in our previous paper [19].

As the organic solvent, pure pentane was used (low boiling point: 36 °C, no destructive impact on the membrane module). The extracted product was accumulated in a boiling flask. The hold-up of the pentane in the hybrid system was about 3.4 L (1.4 L for the membrane module, 2 L for the regeneration unit). The average efficiency of the regeneration unit was 130 mL of regenerated pentane per minute.

2.2. Toxicity test for alkanes

To test the potential toxic impact of the extractants (pentane, heptane) on the microorganisms in the hybrid system, a set of toxicity experiments was carried out. At first, the inoculum was prepared by transferring 20 mg of dry vital yeasts to 100 mL of the fermentation broth (10 g L^{-1} of yeast extract, 20 g L^{-1} of glucose, distilled water, pH 6.8) in a 500 mL cultivation flask. The culture was grown at 30 °C and stirred at 180 rpm. After 14 h of cultivation, 1 mL of the culture was used to inoculate 100 mL of the fresh fermentation broth in 250 mL Erlenmayer flasks, one without stirring, the other one magnetically stirred, both tempered at 30 °C. In the first toxicity experiment, the cultures were grown with an addition of 0.5 mL of pentane or heptane into the medium at the beginning of the cultivation. In the second toxicity experiment, 0.5 mL of pentane or heptane was added into the medium after each sampling. The reference Erlenmayer flasks with no pentane or heptane addition were used in both experiments. Cell growth was determined spectrophotometrically as the change in the optical density (absorbance at 620 nm).

2.3. Extraction experiment

Extraction capability of the hybrid system to extract PEA from the fermentation medium using continual regeneration of pentane was tested in a 5 h long extraction experiment. The ALR was filled with 13.5 L of distilled water bubbled at the air flow rate of 5 L h^{-1} and tempered to 25 °C. Pentane flowed through the membrane module at 0.15 L min^{-1} and was continually distilled in the regeneration unit. The amount of 30 g of PEA was dissolved in 1.5 L of distilled water and then poured into the ALR at the beginning of the extraction to get 2 g L^{-1} of the initial PEA concentration in the ALR. Every 30 min, 1 mL of pentane was taken from the reboiler and analyzed. Concentration of PEA in the ALR was calculated from the measured mass of the extracted PEA in the reboiler using the material balance.

2.4. Biotransformation experiments

In the proposed hybrid system, two biotransformation experiments, B1 and B2, were carried out at different feeding and aeration conditions. At the beginning of both experiments, the bioreactor was filled with 13.8 L of the fermentation medium prepared as a water solution of dry corn-steep liquor, essential micro and macro components (according to medium L used by Stark et al. [3]),