Journal of Molecular Catalysis B: Enzymatic 123 (2016) 1–7

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

An improved process for biocatalytic asymmetric amine synthesis by *in situ* product removal using a supported liquid membrane

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ARTICLE INFO

Article history:
Received 7 March 2015
Received in revised form 14 October 2015
Accepted 14 October 2015
Available online 28 October 2015

Keywords:
Supported liquid membrane
In situ product removal (ISPR)
Chiral amines
Omega-transaminase

ABSTRACT

Chiral amines are important building blocks in the pharmaceutical industry, and the biocatalytic synthesis of these compounds using ω-transaminases has been increasingly studied in recent years. In principal, asymmetric synthesis of chiral amines from a prochiral ketone is the preferable route, but it is often hampered by an unfavourable equilibrium position and product inhibition. An effective method for product removal is therefore necessary to drive the reaction towards product formation. In a recent study (Rehn et al., 2014) [29] we reported on the successful use of a supported liquid membrane (SLM) for the *in situ* product removal (ISPR) of (S)-α-methylbenzylamine (MBA) produced by *Arthrobacter citreus* ω-transaminase present in immobilized *Escherichia coli* cells.

In the present work, we thoroughly discuss the factors influencing the performance of the SLM system and considerations for its successful use. Moreover, the system is further improved by implementing continuous control of the reactor pH using the amine donor substrate, and regeneration of the SLM unit at regular intervals to maintain the extraction performance, allowing the accumulation of 1.0 M (121 g/l) product in the stripping phase during operation for 91 h.

1. Introduction

Biocatalytic transamination has received increasing attention in recent years. The products, chiral amines, are key building blocks in the pharmaceutical industry [1–3]. Transamination reactions can be either carried out by kinetic resolution of a racemic amine, or by asymmetric synthesis of the desired enantiomer from a prochiral ketone. Because of the higher theoretical yield, the latter method is often preferable. Due to thermodynamic limitations and/or product inhibition, it is often required to drive the reaction towards the product side in order to obtain high yields. Methods include the use of excess concentration of the amine donor, evaporation of a volatile by-product, by-product removal via enzymatic cascades, formation of a cyclizing or polymerizing product or by-product and extraction methods [4–14]. The methods for shifting the equilibrium have also been addressed in several reviews [15–19].

A supported liquid membrane (SLM) typically consists of a hydrophobic porous polymeric support material, such as a flat sheet or hollow fibre membrane, in which the pores contain a hydrophobic liquid. The liquid constitutes a membrane, separating two aqueous phases, whereas the polymeric material acts as a supporting matrix. Many possible variants of SLMs can be constructed, and the topic has been comprehensively reviewed by Dżygiel and Wieczorek [20]. The use of supported liquid membranes in hollow fibre membrane contactors has gained increasing interest in recent years, predominantly for applications such as the extraction of metal ions and organic acids from waste water [21–24]. However, SLMs have also been used for *e.g.* extraction of acids or fructose from fermentation processes [25,26]. Integrated with a biotransformation reaction, a variant of SLM was used for ISPR of the antibiotic cephalexin [27]. As such, hollow fibre membrane contactors are beneficial for creating a large surface area for extraction. Straightforward scale-up and modular design are advantages in comparison with other conventional mass transfer equipment [28].

In a recent study, we demonstrated the successful use of an SLM system for *in situ* product removal (ISPR) of (*S*)-α-methylbenzylamine (MBA) produced by asymmetric synthesis, which is a commonly studied model reaction strongly hindered by its unfavourable equilibrium position [29]. The liquid membrane consisted of undecane kept within the pores of a polypropylene hollow fibre membrane contactor. By recirculating the aqueous reaction medium (alkaline) and an acidic stripping phase on the respective sides of the liquid membrane, a selective three-phase extraction (aqueous:organic:aqueous) of the amine product was

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http://dx.doi.org/10.1016/j.molcatb.2015.10.010
1381-1177/

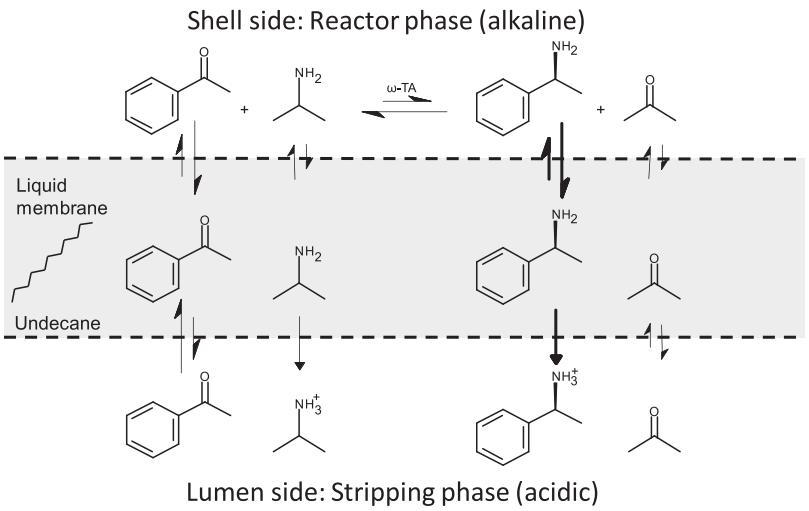

Fig. 1. Principle of the SLM extraction for *in situ* product removal. The amines are trapped by protonation in the acidic stripping phase, allowing "uphill" extraction. The ketones are extracted to equilibrium concentrations. Selectivity between the two amines depends on several factors, such as the difference in hydrophobicity, their respective pK_a values, pH and concentrations on the reactor side. The figure is reproduced from [29] with permission from the publisher.

realized (Fig. 1). The extraction selectivity is dependent on difference in pK_a values and hydrophobicity between the different components. The equilibrium was successfully shifted to reach 98% conversion compared to ca 50% without the SLM. Also, the amine product was highly enriched in the stripping phase whereas the other components were present in very low concentrations compared to what would be the case if employing a batch reaction without the SLM. The two ketones distribute between the three liquid phases to reach equilibrium, hence equal concentrations in the two aqueous phases. In contrast, due to protonation in the acidic stripping phase the amines are effectively trapped, thus preventing back extraction while allowing build-up of a high product concentration in the stripping phase. While our previous study was concerned with the proof of concept for ISPR using the SLM system, the present work aims to provide a more in depth discussion concerning important considerations for its successful implementation. For example, how choices regarding the reaction pH, the size of the membrane unit and the stripping phase volume all influence the performance of this ISPR system. Also, this work aims to refine the SLM system through several improvements, first by employing feedback control of the reactor pH using the amine donor, secondly by regenerating the SLM in order to maintain its performance and thirdly by using a strongly acidic stripping phase which allows product accumulation to high concentrations without the need for controlling the pH of the stripping phase.

2. Material and methods

2.1. Chemicals

Escherichia coli cells containing a recombinant ω-transaminase from *Arthrobacter citreus* were provided by Cambrex Karlskoga AB in spray dried form. The development of this biocatalyst was reported by Martin et al. [30]. Celite (0.2–0.5 mm, 30–80 mesh) was purchased from BHD Laboratory supplies (Poole, England). All other chemicals were purchased from Sigma–Aldrich. All weights are given as dry weights.

2.2. Hollow fibre membrane contactors

Liqui-Cel® MicroModule® and MiniModule® hollow fibre membrane contactors were purchased from Membrana® (Charlotte, NJ, USA). The pore dimensions of the polypropylene hollow fibres were 0.04 × 0.10 μm and the surface areas of were specified to 100 cm^2 and 1800 cm^2 respectively. A peristaltic pump, (Alitea, Stockholm, Sweden) was used to circulate the aqueous phases at 3 ml/min.

2.3. Preparation of the supported liquid membrane

The SLM was prepared by pumping undecane through the lumen side of the dry hollow fibre for 10 min. Before the start of an experiment, excess undecane was rinsed from the contactor with the solutions chosen for the experiment. Generally 1 M HCl was used on the lumen side and 0.1 M borax–HCl or borax–NaOH (pH 7.0–10.0) on the shell side.

2.4. Extraction experiments

The extraction of MBA was investigated using the MicroModule® unit. On the shell side 0.25 l of borax-buffer (0.1 M, pH 8.0, 9.0 or 9.5) containing 20 mM of MBA was recirculated. On the lumen side, 25 ml HCl (1 M) was recirculated to extract the amine. Extractions of MBA were also carried out at elevated stripping phase concentrations of MBA (up to 3 M), using 4 M HCl as the stripping phase.

The extraction of IPA, MBA, 1-methyl-3-phenylpropylamine (MPPA), 1-aminoindane and 2-aminoheptane was investigated by recirculating 0.5 l of buffered solution (borax–HCl, 0.1 M, pH 9.0) containing 1.0 M IPA and 20 mM of MBA, MPPA, 1-aminoindane or 2-aminoheptane on the shell side, using 25 ml of 0.2 M HCl as the stripping phase (lumen side). The selectivity for each amine product was determined according to Eq. (1)

$$\frac{\text{Flux}_{\text{amine prod/[anime product]}}}{\text{Flux}_{\text{IPA}}/[\text{IPA}]} \quad (1)$$

2.5. Activity measurements

The activity of the ω-transaminase was measured using the conversion of acetophenone and isopropylamine (IPA) to MBA and acetone. Acetophenone (20 mM) and IPA (10–500 mM) were dissolved in borax–HCl buffer (0.1 M, pH 9.0) containing pyridoxal-5′-phosphate (1.0 mM). The IPA concentration was 100 mM when varying the acetone concentration between 0 and 100 mM. The *E. coli* cells were suspended in the same buffer (10 mg/ml), also containing PLP (1.0 mM). Reactions were started by the addition of 100 μl of cell suspension to 3.0 ml of substrate solution. The