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Asymmetric synthesis of chiral amine in organic solvent and *in-situ* product recovery for process intensification: A case study

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ABSTRACT

Membrane assisted extraction in a contactor, which allows for the separation of the reaction components between two immiscible liquid phases, was applied for transamination. The reaction was conducted in an organic solvent which resulted in two benefits: higher solubility of the poorly water soluble substrate benzyl acetone, and the possibility of product removal in an aqueous phase. The aqueous phase was circulated across the shell of a hollow fiber membrane module and the organic phase (*n*-heptane) through the lumen. Approaches to extract the product amine, 1-methyl-3-phenylpropylamine proved beneficial to reduce its inhibitory effects, while providing an equilibrium shift aiding in higher product formation. After 72 h of operation in membrane contactor, 99% substrate conversion was observed.

1. Introduction

Chiral amines are important molecules in agrochemical, chemical/fine-chemical and pharmaceutical industry. They are used as intermediates and end-products in the synthesis of cardiovascular, antihypertensive, and antiemetic drugs. Furthermore, they also find applications as chiral-resolving auxiliaries for the preparation of optically pure carboxylic acids [1]. The use of ω-transaminases has been identified as a green and sustainable method for chiral amine production compared to the chemical methods which mainly include hydrogenation of imines and enamines, alkylation of imines, aminohydroxylation, and reductive amination. Besides kinetic resolution and deracemization, ω-transaminase (ω-TA) catalyses the asymmetric synthesis of chiral amines which includes the transfer of an amine group from an amine donor to an amine acceptor (substrate bearing a carbonyl group), yielding an optically active amine target product and a non-chiral carbonyl co-product. The reaction is conducted in the presence of co-factor pyridoxal 5′-phosphate (PLP) [2]. ω-TA has significant potential for industrial processes due to high turnover rate, excellent enantioselectivity, and no requirement of external cofactor regeneration [3]. Moreover, it is possible to synthesize both enantiomers of the amines due to the recent identification of numerous ω-TAs with (S)- as well as (R)-stereoselectivity [4].

The wide applicability of this enzyme is frequently hampered by a few challenges, which include, unfavourable thermodynamic equilibrium or enzyme inhibition by substrates and/or products or instability of the enzyme. For countering enzyme related issues, significant advances have been made in the field of protein engineering leading to enzyme modification. However, the thermodynamics of the reaction, which is very often found to be the primary limitation, is independent of the enzyme [5]. This situation leads to the requirement of process engineering interventions aiming at intensified processing. Though membrane based technologies exhibit immense potential to play an important role in intensifying transamination for chiral amine production, there are only limited number of reported investigations in this field. The use of membrane assisted extraction has been reported about 15 years ago but in kinetic resolution (not asymmetric synthesis) of α-methylbenzylamine (α-MBA) and 1-aminotetralin [6]. Looking at recent years, a few examples in asymmetric synthesis have been reported using supported liquid membranes (SLM) [7,8]. The factors affecting the SLM system were discussed in detail where the trade-off between the membrane stability and extraction efficiency is considered for practical applications [9]. In the present case-study, process intensification in ω-TA mediated asymmetric synthesis was conducted in an organic solvent by using a hollow fiber membrane contactor for *in-situ* product recovery. The transamination of benzyl acetone (BA) was conducted in *n*-heptane,

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to increase the solubility (10 mM BA solubility in aqueous phase), using isopropyl amine (IPA) as the donor amine and a non- miscible acidic aqueous phase to extract the product amine, 1-methyl-3-phenylpropylamine (MPPA). Since co-extraction of the donor amine (IPA) into the aqueous phase could not be prevented, intermittent extraction was conducted at predetermined time points. Furthermore, nanofiltration (NF) was investigated with an aim to purify the product.

2. Materials and methods

2.1. Chemicals and enzyme

The chemicals used in this study including 4-phenyl-2-butanone (benzyl acetone, BA) (98% purity), 4-phenyl-2-butylamine (1-methyl-3-phenylpropylamine, MPPA) (98%), 2-aminopropane (isopropyl amine, IPA) (99.5%), acetone (99.9%), pyridoxal 5′-phosphate (PLP), *n*-heptane (>97%), toluene (99.9%) and methyl *tert*-butyl ether (MTBE) (99.9%) were purchased from Sigma-Aldrich Corporation. The amine transaminases (ATA-wt and ATA-v2) were kindly provided by c-LEcta GmbH, Leipzig, Germany. The purified enzymes were supplied as freeze dried powder. The activity of ATA-wt and ATA-v2 was 1.73 U/mg and 1.02 U/mg, respectively. One unit is the amount of enzyme that liberates 1 μmol acetophenone per minute from α-methyl-benzylamine (MBA) at 30 °C in 50 mM potassium phosphate buffer pH 7.4, 0.1 mM PLP, 10 mM sodium pyruvate and 10 mM racemic MBA. A manuscript detailing the amino acid sequence of the enzyme, its crystal structure and kinetics is under preparation.

2.2. Batch scale reactions

2.2.1. Solvent screening

Three organic solvents, *n*-heptane, toluene and MTBE, were selected for transamination by the enzyme ATA-wt at various water activities. The selection of solvents was based on their water immiscibility and literature information on possibly viable transamination. Moreover, it was also ensured that the solvents do not belong to the hazardous or highly hazardous categories [10]. Screening tests were conducted in glass vials at 1 mL scale with 10 mM BA, 100 mM IPA and 10 mg/mL ATA-wt at 30 °C. Afterwards the A predetermined amount of water was added to each solvent. The details of water activity (a_w) calculations and corresponding amount of added water are provided in Supplementary information.

2.2.2. Transamination in selected solvent and partitioning

Transamination was conducted at 1 mL scale in *n*-heptane at 30 °C to investigate the optimum operational parameters to be subsequently used in membrane assisted extraction. The use of 10 mM BA concentration was used based on its solubility in aqueous solutions. Since organic solvent is used as a reaction medium in this study; therefore BA concentration up to 50 mM was examined. The maximum IPA concentration was kept 10-times of the BA concentration based on previous studies with subsequent investigation of 1:1 and 1:5 BA:IPA ratios [11], [12]. For solvent screening, the wild-type enzyme ATA-wt was used (with 0.5 mM PLP as the co-factor), however during the course of the study, mutant enzyme ATA-v2 was made available (produced by c-LEcta GmbH, Leipzig, Germany). The enzymes ATA-wt and ATA-v2 are both ω-aminotransferases (EC 2.6.1.18). ATA-v2 is thermostable variant of ATA-wt and shows high performance in terms of substrate concentration, and co-(solvent) tolerance. Therefore the subsequent tests were conducted with the mutant enzyme ATA-v2 with 0.5 mM PLP as the co-factor, to achieve better process performance. For partitioning tests, 4 mL of aqueous phase (100 mM sodium citrate/acetate buffer with pH ranging from 3 to 5.15) and 4 mL *n*-heptane were added together in glass tubes. Approximately 0.5 g/L of each component BA, IPA, MPPA and acetone was added to the *n*-heptane-aqueous mixture and equilibrated for 24 h on a rotatory shaker. After 24 h, samples were collected from solvent and aqueous phase and analysed for these four compounds on HPLC and GC, respectively.

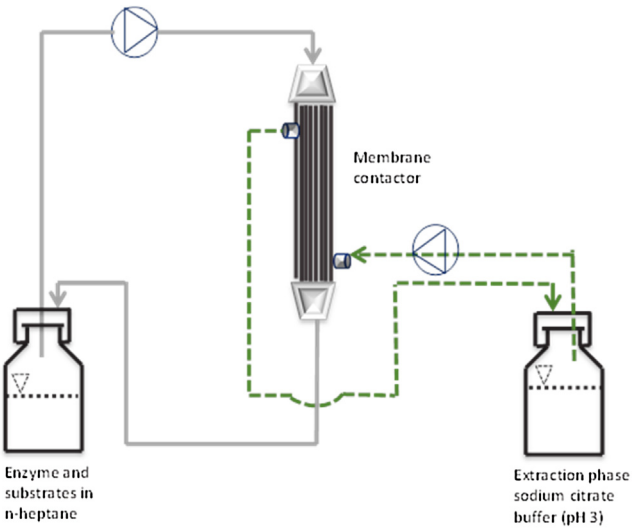

Fig. 1. Schematic of the experimental set-up used for membrane assisted extraction.

2.3. Membrane assisted extraction

For membrane assisted extraction, the enzyme ATA-v2 (5 mg/mL) was used with 25 mM BA and 125 mM IPA in the presence of 0.5 mM PLP for transamination in *n*-heptane. Before adding the solvent, the enzyme and PLP were wetted with 2 mL demineralized water. The *n*-heptane phase (0.35 L) containing the substrates, wetted enzyme and PLP was kept in a Schott Duran bottle in a thermoshaker at 30 °C. This bottle was prepared in duplicate, out of which, one was used for intermittent extraction of product amine into aqueous phase and the other was used as a control without extraction. The aqueous phase (extractant), sodium citrate buffer (pH 3), was kept in another Schott Duran bottle with 0.35 L working volume. Due to the fact that the donor amine (IPA) was co-extracted with product amine into aqueous phase, intermittent extraction was conducted every 7 to 17 h until a total duration of 72 h and after extraction 5-times IPA was provided. Extraction was done for 30 min on each instance in a hollow fiber membrane contactor (1 × 5.5 Mini Module, Liqui-Cel®, purchased from Membrana, GmbH). The contactor consisted of bundles of polypropylene hollow fibres with a total membrane surface area of 0.2 m^2. For each extraction (30 min duration), the reaction mixture (in *n*-heptane phase) was circulated at the rate of 25 L/h through the lumen of the membrane module. In addition, the acidic aqueous extraction phase was circulated across the shell of the module (Fig. 1). The pressure on the solvent side was 1.23 bar and the aqueous side was kept at a slightly higher pressure, 1.39 bar, to avoid wetting of the hydrophobic membrane fibres and leaching of organic phase into the extractant phase. The contactor was operated at 30 °C.

2.4. Nanofiltration

To remove the co-extracted IPA substrate from the MPPA rich aqueous phase, three commercial pH-stable nanofiltration (NF) membranes, B-4022 (AMS Technologies, Israel), SelRO MPF-34 (Koch, USA) and Nadir NP030 (Microdyn-Nadir, Germany), were