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

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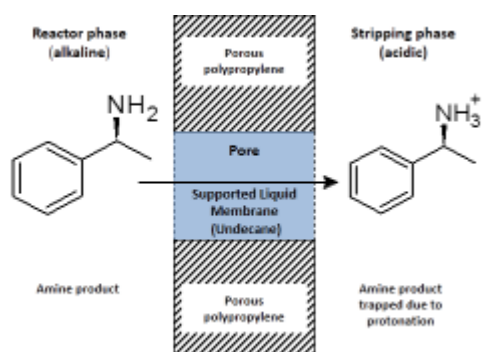
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## Graphical abstract:



**Highlights**

- The paper is focussed on discussing the important considerations for implementing the chiral amine extraction using supported liquid membrane.
- A very high product concentration (>1M) was reached employing the supported liquid membrane for *in situ* product removal.
- Product extraction was maintained by regenerating the liquid membrane, allowing the reaction to proceed continuously.

## Abstract

Chiral amines are important building blocks in the pharmaceutical industry, and the biocatalytic synthesis of these compounds using  $\omega$ -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) we reported on the successful use of a supported liquid membrane (SLM) for the *in situ* product removal (ISPR) of (S)- $\alpha$ -methylbenzylamine (MBA) produced by *Arthrobacter citreus*  $\omega$ -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 hours.

**Keywords:** Supported liquid membrane; in situ product removal (ISPR); Chiral amines; omega-transaminase

## 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 Dzygiel 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)- $\alpha$ -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 realized

(figure 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  $\omega$ -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 0.04x0.10  $\mu\text{m}$  and the surface areas of were specified to 100  $\text{cm}^2$  and 1800  $\text{cm}^2$

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 equation 1.

$$\frac{Flux_{amine\ prod}/[amine\ product]}{Flux_{IPA}/[IPA]} \quad (1)$$

## 2.5 Activity measurements

The activity of the  $\omega$ -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 0-100 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  $\mu$ l of cell suspension to 3.0 ml of substrate solution. The reactions were carried out in 4.5 ml glass vials heated to 40 °C in a thermoshaker (MKR13, HLC, Bovenden, Germany)

set to 600 rpm. Samples were diluted in 0.1 M HCl and analyzed by HPLC-UV (described below).

### 2.6 *E. coli* immobilization

The preparation of a packed-bed reactor containing a mixture of flocculated cells and celite was described previously [29]. *E. coli* cells (0.32 g) were suspended in 10 ml 100 mM sodium phosphate buffer, pH 7, containing 0.1 mM PLP. Chitosan was dissolved in 1 % acetic acid (0.5 mg/ml, pH 6). The cell suspension and chitosan solution (50 ml) were mixed and flocculation was induced by addition of NaOH to pH 9.0. The flocculated preparation was filtered off and then mixed with 0.90 g celite and packed in a glass column (5\*1 cm).

For the stirred tank experiment, the immobilization procedure was carried out inside the reactor using 1.35 g cells (suspended in 10 ml buffer) and 0.054 g chitosan in 100 ml 1 % acetic acid (pH 6).

Flocculation was induced by addition of NaOH to reach pH 9. After sedimentation of the flocs for 15 minutes, the supernatant was removed and substrate solution was added to start the reaction.

### 2.7 Production of (S)- $\alpha$ -MBA using a packed bed reactor

The system using the packed reactor is schematically presented in figure 2A. The substrate reservoir initially contained 20 mM acetophenone and 500 mM IPA dissolved in 0.50 L buffer borax-HCl (100 mM, pH 9.0). A pH control unit (Inventron AB, Mölndal, Sweden) connected to a peristaltic pump (Alitea, Stockholm, Sweden) was used to maintain pH 9.0 of the substrate reservoir by addition of IPA (5 M). At the start of the reaction, additional acetophenone (43 mmol) was added to the substrate reservoir to keep the solution saturated. The added acetophenone formed a separate phase at the bottom of the substrate reservoir. By stirring the reservoir gently, the acetophenone remained at the bottom of the reservoir ( $d = 1.030 \text{ g/cm}^3$  at  $25 \text{ }^\circ\text{C}$ ), thus avoiding pumping of the acetophenone phase into the reactor and preventing loss of acetophenone by evaporation.

The product reservoir initially contained 25 ml 3 M HCl. A two-channel peristaltic pump (Alitea, Stockholm, Sweden) was used to recirculate the two aqueous phases at a flow rate of 3 ml/min. A MiniModule® membrane contactor was prepared as described above and used for the extraction. The packed-bed contained 0.32 g (dry weight) of flocculated cells, blended with 0.9 g of celite.

## 2.8 Production of (S)- $\alpha$ -MBA using a stirred tank reactor

The system described above was modified in the following ways: A stirred tank reactor was used instead of the packed bed reactor, and the amount of cells was increased from 0.32 g to 1.35 g. The substrate solution (0.35L total volume) was recirculated between the reactor (0.25 L) and a separate vessel (0.1 L) containing a two-phase system of the aqueous substrate solution and acetophenone. Thus, acetophenone was continuously added to the reactor. The system is schematically presented in figure 2B. After every 22-24h, the SLM was rinsed with deionized water, dried by purging with nitrogen gas and then prepared as described in section 2.3. Also, part of the stripping phase was harvested and replaced by addition of new HCl (3M).

## 2.9 Analytical methods

All samples were analyzed using HPLC (Dionex Ultimate 3000) with UV detection. Acetophenone and MBA were analyzed using a Kinetex™ XBC18 column (50 x 2.10 mm, 1.7  $\mu$ m, 100 Å) (Phenomenex, Torrance, CA, USA) at 40 °C, with a mobile phase consisting of A: acetonitrile and B: 0.1 % trifluoroacetic acid in water. The composition of the mobile



phase was 0-1.5 min: 95% B, 1.5-2.5 min: gradient from 95% to 40% B, 2.5-5 min 95% B. The flow rate was 0.75 ml/min. Acetone, IPA, 1-aminoindane, 1-methyl-3-phenylpropylamine and 2-aminoheptane were analyzed using a Gemini®-NX column (100 x 2.0 mm, 3 $\mu$ m, 110 Å) (Phenomenex, Torrance, CA, USA) at 30 °C with a mobile phase consisting of A: acetonitrile and B: 10 mM ammonium bicarbonate (pH 11 adjusted with NaOH), and was used isocratically (95% B) with 0.2 ml/min flow rate. Samples from the extraction characterization experiments were analyzed isocratically using 35% acetonitrile (5% when analyzing IPA) in water (pH 11) at a flowrate of 0.4 ml/min.

### 3. Results and discussion

Implementing the SLM system involves several important considerations regarding its design and operation, which are discussed in the following sections.

To describe the system for extraction from the reactor to the strip phase we employ a simple model (equation 2).

$$J_n \times a = k \times \Delta C \times a \quad (2)$$

The flux ( $J_n$ , mol/(min\*m<sup>2</sup>)) is a function of the driving force ( $\Delta C = C_{\text{reactor}} - C_{\text{strip}}$ ) and a mass transfer coefficient ( $k$ ) which here represents several parameters such as temperature, molecular diffusion rate and distance through the membrane solvent, membrane contactor geometry and the flow rates of the recirculated liquids. The extraction rate (mol/min) is directly proportional to the liquid membrane area ( $a$ ).

### 3.1 pH of the reactor and stripping phase

The amines partition significantly to the hydrophobic membrane phase only in their uncharged state, thus the alkaline pH in the reaction medium is crucial for the extraction rate. While viewing both the charged and uncharged amines as the same species a maintained acidic stripping phase allows for an “uphill” amine extraction, i.e. the product concentration keeps increasing in the stripping phase despite the much lower concentration in the reactor, due to the charged state of the amines in acidic environment. Hence, accumulation of a high product concentration in the stripping phase does not diminish the driving force for the extraction ( $C_{\text{strip}}$  of the uncharged amines remains close to 0 at low pH), and extraction was readily carried out in presence of high

product concentrations (up to 3 M), as long as the stripping phase was acidic (data not shown).

The percentages of the uncharged amines (MBA and IPA) were calculated from the Henderson-Hasselbalch equation (equation 3) and plotted against the pH value (figure 3).

$$pH = pK_a + \log_{10} \left( \frac{A^-}{HA} \right) \quad (3)$$

While both species are extracted more effectively at higher pH due to the increased driving force ( $C_{\text{reactor}}$  increases), the  $[IPA^0]/[MBA^0]$  ratio increases with the pH, which negatively affects the selectivity. The compromise between extraction rate and selectivity is therefore an important consideration.

The flux of MBA was studied at pH values between 8 and 9.5 (figure 4). As expected the extraction rate increased with pH, in agreement with figure 3 and equation 2, and from this point of view it is desirable to run the reaction at a high pH. IPA has a considerably higher pKa and the extraction rate can be considered acceptably low below pH 9.5.

The possibility of running the reaction at a high enough pH is important for maximizing the benefit of the SLM technology for ISPR. In our previous study, activity was maintained between pH 7-9, and reasonable stability was observed at pH 9 [29]. Importantly, a pH optimum between 8-9 has been observed for many transaminases [31].

### 3.2 Choice of amine donor and the donor concentration

Because the extraction selectivity is largely determined by hydrophobicity and  $pK_a$  differences, the properties of the amine donor should preferably differ significantly from those of the amine product. Hence, IPA is suitable amine donor which facilitates the selectivity in the selected model reaction. In addition, IPA is readily used for controlling the pH in the reactor (figure 2). This simplifies the construction of a continuous system by combining pH control and a controlled substrate feed. Also, the build-up of a high ionic strength in the reactor is avoided, which may otherwise affect the biocatalyst.

Using an excess of the amine donor is the most straightforward strategy to shift the unfavourable equilibrium, although for strongly unfavourable reactions even a very large excess of the amine donor is insufficient for

reaching a high yield [19]. A high IPA concentration would enable the build-up of a higher MBA concentration before approaching the equilibrium concentration, thus allowing a faster product extraction. Although, by continuous product extraction, it is principally possible to reach a high conversion without the use of large excess of the amine donor.

The choice of IPA concentration is a balance between maximizing the reaction rate on the one hand and minimizing undesired IPA extraction on the other. However, it was decided to prioritize the MBA production and therefore a relatively high concentration of 0.5 M IPA was used in order to keep the reaction rate high, since the reaction rate was significantly reduced at lower IPA concentrations (figure 5).

### 3.3 Acetone concentration build-up

Using a relatively small volume of the strip phase, most of the formed acetone will be accumulated in the aqueous reaction phase, even when partitioning approaches equilibrium. Also, the low partitioning of acetone to the hydrophobic membrane phase hampers the flux of acetone across the liquid membrane. The transaminase activity, however, decreased only

slightly in presence of acetone up to 100 mM (figure 6), thus indicating that product inhibition by acetone is not a major problem. Nonetheless, at a relatively high concentration the removal of acetone by evaporation may offer further improvement and could be readily added to the current system, although possible implications in terms of substrate evaporation should then be taken into account [32]. Acetone and MBA are produced in stoichiometric amounts. Consequently, 100 mM acetone in the reaction phase here corresponds to a maximum product concentration of 1.5-2.5 M in the stripping phase, if the volume ratio of the aqueous phases is 15:1 to 25:1, as used in this study.

### 3.4 Membrane size and catalyst loading

In order to achieve both high productivity and good extraction selectivity, the balance between the extraction capacity (i.e. the membrane size) and the catalytic capacity should be considered. Previously, similar amounts of MBA and IPA were extracted despite the large excess of IPA present [29]. In an attempt to increase the productivity compared to our previous study, the SLM was prepared in a considerably larger hollow fibre membrane contactor (1800 vs.  $2 \times 100 \text{ cm}^2$ ), while the packed bed reactor

allowed only a slight increase of the amount of cells used (from 0.27g to 0.32g). Using the larger membrane in combination with the relatively small packed bed reactor, however, resulted in approximately a three-fold increase in the IPA extracted compared to that of MBA (figure 7).

Provided that the extraction rate is sufficient for efficiently removing the formed product, a further increased membrane area will essentially decrease the product purity, because it will unavoidably increase the undesired flux of the amine donor. On the other hand, a large enough membrane allows for good enzyme utilization by maintaining a low steady state concentration of the product, thus realizing a high reaction rate throughout the reaction.

In order to better exploit the greater extraction capacity of the large SLM contactor, the amount of cells was increased from 0.32 to 1.35 g. In order to fit the larger amount of cells, a larger sized reactor (stirred tank) was used. Compared to the packed bed reactor, the stirred tank reactor is also better suited for the recirculating system, since the catalyst is more accessible to the whole working volume and less susceptible to external mass transfer limitations. However, the packed bed reactor allowed for a simpler flow system configuration enabling feeding of the ketone substrate directly in the one reservoir (compare figures 2A and 2B).

### 3.5 SLM stability

The successful use of the SLM technology requires sufficient membrane stability. In this study, a partial loss of the membrane integrity was observed after ca 36 hours of operation. This was indicated by an increased volume of the stripping phase, which was larger than accounted for by the extracted amines (data not shown). In order to maintain the extraction performance, the membrane was regenerated at 22-24 hour intervals during the stirred tank reactor experiment (figure 8). This allowed the stripping phase product concentration to reach 1.0 M with similar concentration of IPA, despite the high IPA concentration in the reactor. Increased SLM stability by recirculating the membrane solvent into the membrane unit was previously reported by Teramoto *et al.*[33]. This strategy was attempted in the current study, but no stability improvement was observed.

Neplenbroek et al. showed that the SLM stability, primarily depends on the type of membrane solvent and the liquid membrane composition (e.g. presence of carrier) whereas viscosity was less important [34]. They also reported that the stability was improved by increasing the stripping phase NaCl concentration, thus showing that membrane failure is not due to osmotic pressure, which had previously been suggested [35].



Practical applications need to consider a trade-off between stability and extraction efficiency since a good extraction solvents for a given compound may prove insufficiently stable as a liquid membrane [36]. Strategies to improve the stability of SLMs include gelation of the liquid membrane and the application of a coating layer [37–39]. However, such techniques are likely to decrease the mass transfer rates through the membrane. The use of strip dispersion technique [40] is also impractical for the current process, first because the volume of the membrane phase should ideally be minimized and secondly because an additional phase separation step would make continuous operation comparatively more complex.

The stability requirement for an SLM-application should be highly dependent on the process. The membrane stability observed in the present study was above 30 h, which in our opinion is more than acceptable for several reasons. Chiral amines are high value products and the benefits of implementing an SLM-based ISPR strategy would most likely outweigh the increased cost and efforts required to regenerate the membrane. In addition, the procedure to prepare the membrane is simple and time consumption of about one hour is short compared to the operational lifetime.

### 3.6 Stripping phase volume

Minimizing the volume of the stripping phase has several benefits for the downstream processing, which often contributes significantly to the overall cost of biocatalytic processes. Compared to a regular batch type reaction, the SLM system offers significant advantages both because of the reduced volume *per se*, and by the much increased product concentration at the start of the downstream processing. Additionally, because the partitioning of the ketones is not influenced by pH, equilibrium concentrations between the three phases will eventually be reached. Thus, the amount of ketones extracted is proportional to the stripping phase volume, which should therefore be minimized. However, the minimum stripping phase volume required is set by the void volume of the membrane contactor.

### 3.7 Selectivity for different amine products

In order to assess the potential for ISPR of other amine products, the extraction of several such compounds was characterized (table 1). The results indicate that good selectivity can also be achieved between the amine donor (IPA) and several plausible amine products, including 1-

aminoindane, which is a more reactive compound compared to MBA, thus strongly depending on product removal for its asymmetric synthesis [7]. The results show the importance of both pKa value and particularly the partitioning of the amines to the organic solvent. For instance, compare the extraction rate of 2-aminoheptane and MBA where the former is considerably faster although MBA has a more favourable pKa value.

### 3.8 Potential for scale-up

The potential for scale-up of the liquid membrane technology depends strongly on the required membrane area. Therefore, the simple model (equation 2) for describing the flux was used, with the experimental data in figure 8, to estimate the area required for the extraction of 1 kg/day at different pH and steady state concentrations of each of the amine products (table 2). The results clearly show the benefits in terms of reduced membrane size with increased pH in the reactor phase and/or higher steady state amine product concentration (both affecting the driving force for the extraction). Furthermore, the results indicate that a membrane size in the range of 100-200 m<sup>2</sup> could be sufficient for extraction of 1 kg/day, provided that a pH close to 9 and a steady state

product concentration in the reactor of about 5 mM are acceptable process conditions.

#### 4. Conclusions

The use of SLM offers several benefits for ISPR in asymmetric synthesis of chiral amines. The technology enables shifting of the equilibrium while integrating selective product extraction, thus resulting in a highly concentrated and relatively pure product solution.

It is possible to operate the process in different ways, depending on which parameter is prioritized. The operating conditions can be altered in order to maximize either the product purity or the productivity and enzyme utilization, e.g. by adjusting the size of the membrane, the biocatalytic activity or the amine donor concentration (or choosing a different, non-extractable, amine donor). Also, developing a biocatalyst with a high pH tolerance and low  $K_m$  for IPA would improve the productivity and further enhance the product purity by allowing lower concentrations of IPA to be used.

The system presented in this work should allow for development of a continuous process, by continuously harvesting the product and replacing the stripping solution. The SLM lifetime, however, may be a limiting factor for some industrial applications. For synthesis of high value

products, such as the biocatalytic synthesis of chiral amines, the several important benefits offered can outweigh this problem. Also, although SLM stability is acceptable and regeneration is readily feasible, further development may lead to significant improvements.

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FIG:1

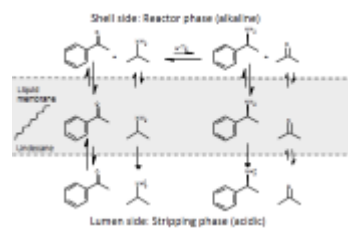


FIG:2

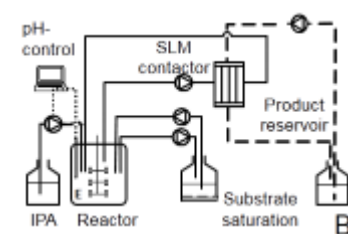
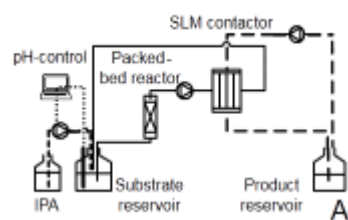


FIG:3

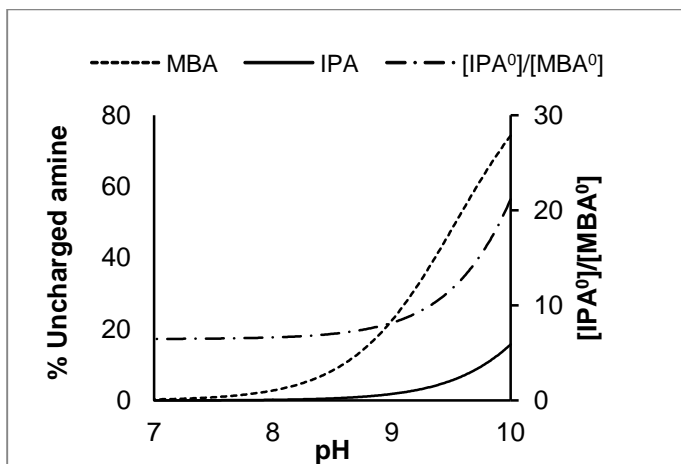


FIG:4

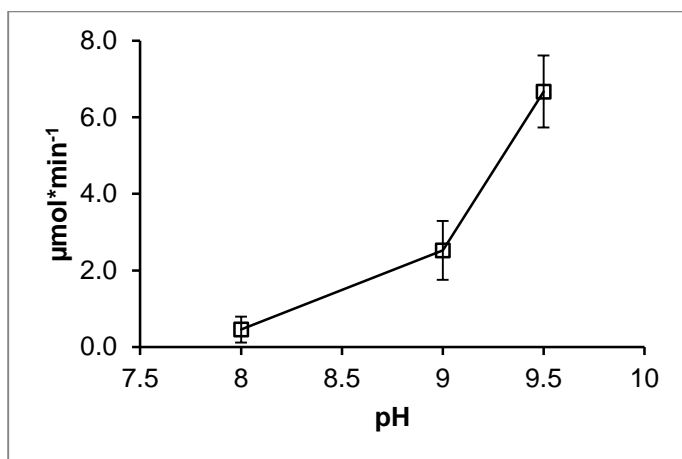


FIG:5

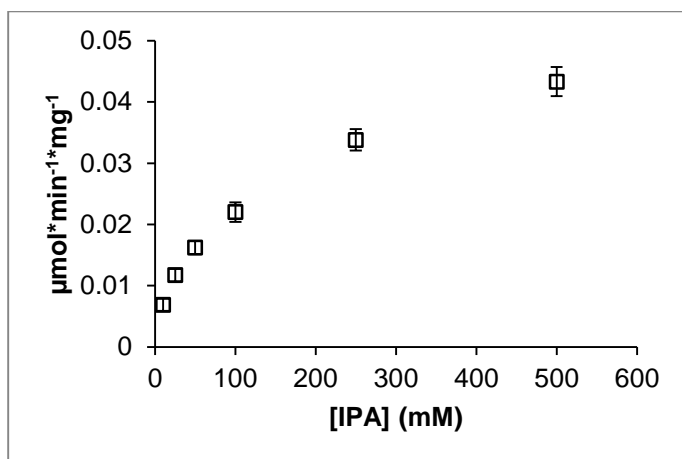


FIG 6:

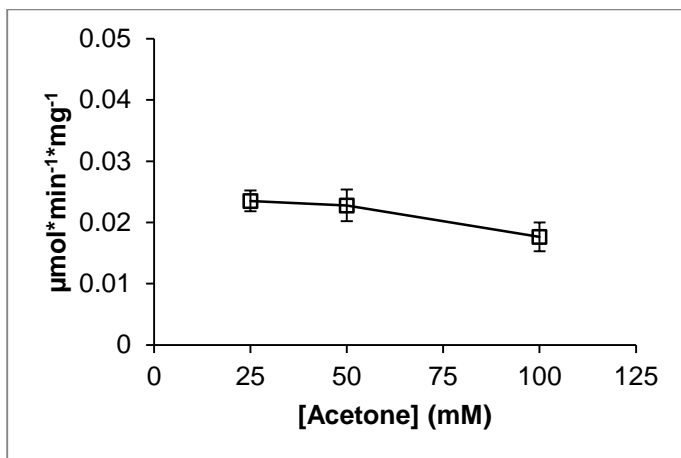


FIG 7:

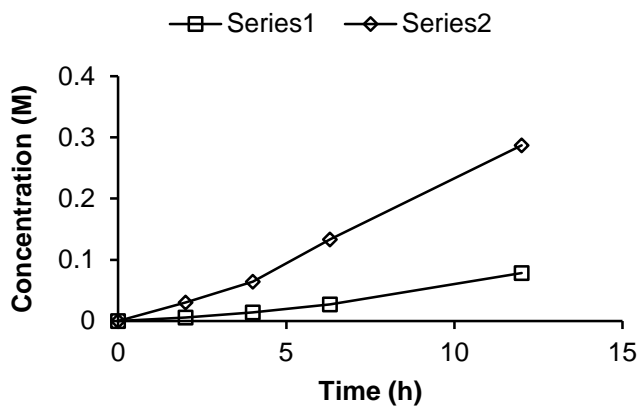
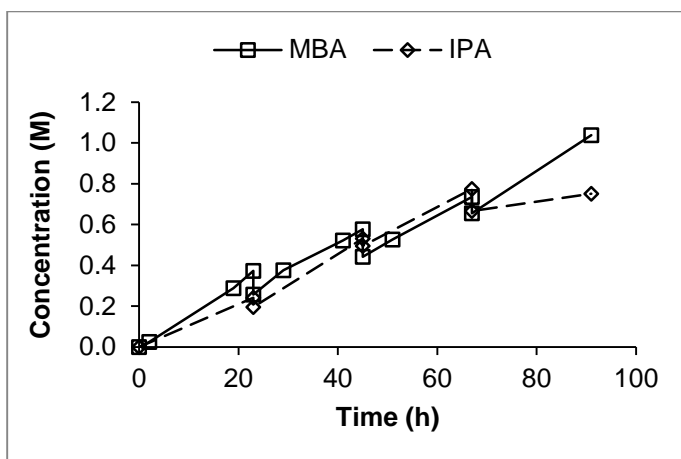


FIG 8:



**Table 1. Characterization of the SLM extraction for different amines.**

Amine	Concentration (M)	Flux ( $\mu\text{mol}/(\text{min}\cdot\text{m}^2)$ )	Selectivity <sup>+</sup> relative to IPA	Extraction rate relative to MBA
IPA	1.00	23 $\pm$ 8	1	0.005
MBA	0.02	92 $\pm$ 14	200	1.0
MPPA	0.02	72 $\pm$ 32	160	0.8
2-aminoheptane	0.02	138 $\pm$ 9	300	1.5
1-aminoindane	0.02	144 $\pm$ 27	310	1.6

Extractions were performed using a solution of IPA (1.0 M) and 0.02 M of MBA ( $\text{pK}_a$  9.5), 1-aminoindane ( $\text{pK}_a$  9.4), MPPA ( $\text{pK}_a$  10.6) or 2-aminoheptane ( $\text{pK}_a$  11.0) on the shell side (pH 9) and HCl (0.2 M) as the stripping solution. <sup>+</sup> Selectivity was defined in equation 1.



Table 2. Estimated membrane areas for realizing the ISPR of 1 kg/day of different chiral amine products. These estimations are based on the observed flux of MBA using the 1800cm<sup>2</sup> MiniModule® (figure 8). The flux for other amine products relative to that of MBA was assumed to be consistent with table 1.

Steady state total amine product concentration (mM)		1 mM	5 mM	10 mM
pH		Estimated membrane area (m <sup>2</sup> )		
MBA	8	10025	2005	1002
	9	1255	251	126
	10	378	76	38
1-aminoindane	8	5543	1109	554
	9	729	146	73
	10	248	50	25
MPPA	8	12796	2559	1280
	9	1306	261	131
	10	157	31	16
2-aminoheptane	8	8718	1744	872
	9	879	176	88
	10	95	19	10