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Three-liquid-phase Spinning Reactor for the Transaminase-catalyzed Synthesis and Recovery of a Chiral Amine

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A device for the transaminase-catalysed synthesis combined with continuous recovery of chiral amines was designed. The system enabled the separation of the reaction components in three liquid phases: a reaction phase, an organic solvent phase (where the poorly water soluble ketone substrate was supplied), and an aqueous extraction phase for continuous product recovery. The transaminase-mediated asymmetric synthesis of (*S*)-1-methyl-3-phenylpropylamine was employed as model reaction. Factors influencing the performance of the system, such as reactor geometry, working volumes and operating parameters, were investigated. Specifically, reaction yield and product recovery were enhanced by i) reducing the thickness of the reaction phase, while continuously stirring and ii) reducing the volume of the extraction phase. Under the optimal condition tested, 85% of the product formed was extracted and a product concentration value of 9 g/L was reached. However, co-extraction of the unreacted amine donor (17%) was observed. Advantages and drawbacks of this process compared to existing technologies, as well as possible optimization strategies are discussed.

Biocatalytic transamination has attracted significant interest in recent years as an efficient method for the synthesis of chiral amines. These compounds are key building blocks in the agrochemical, fine-chemical and pharmaceutical industries. Transamination can be carried out either by direct amination of prochiral ketones (asymmetric synthesis mode) or by the thermodynamically favourable kinetic resolution of racemic amines. Due to a theoretical yield of 100%, the asymmetric synthesis is often preferred.[1–3] However, physical and chemical strategies for counteracting the unfavourable thermodynamic equilibrium and/ or product inhibition are needed.[4,5] Besides the use of an excess of amine donor (AD), methods such as co-product or product cyclization[6–8] or polymerization,[9,10] evaporation of the volatile co-product[11,12] and enzymatic cascades[4,13] for co-product removal have been developed. Membrane-assisted techniques for *in situ* product removal have been also investigated.[14–17] Specifically, membrane-based three liquid phase (3LP) systems were developed by filling the pores of an hydrophobic hollow fibre membrane contactor with an hydrophobic solvent. This operation allowed to physically separate the reaction and the extraction aqueous solutions by using a supported liquid membrane.[18–20] The only 3LP system developed without membranes was reported by Yun and Kim, 2008. They employed isooctane as an organic solvent bridge for the selective extraction of the inhibiting (*S*)-α-methylbenzylamine product. More general, 3LP systems have been mainly reported for the separation and recovery of metals from complex mixtures.[21] In addition, working with three phases offers relevant technological solutions in oil recovery processes, in industrial processes such as ε-caprolactam production,[22] for the rapid isolation of organic macromolecules such as cellulose enzymes and proteins[23] and for the straightforward separation of organic compounds e.g. during extraction of natural products from plants.[24]

We have previously demonstrated the feasibility of performing the asymmetric synthesis of (*S*)-1-methyl-3-phenylpropylamine using high molecular weight (HMW) donor amines in aqueous,[15] organic solvent and solvent-free media.[16] With a molecular weight (MW) between 400 and 1500 g/mol, these large molecules were effectively retained by commercial nanofiltration membranes, when employed in an aqueous environment.[15] Transamination using HMW ADs was also performed in the presence of a non-polar organic solvent (*n*-heptane). Specifically, the HMW AD Jeffamine ED-600 (MW of 600 g/mol), commercialized by Huntsman corporation, was insoluble in n-heptane, thus, resulting in a two-liquid-phase system.[16] Coupling the two-liquid phase reaction system with membrane-assisted product extraction, the reaction equilibrium was successfully shifted to reach 60% conversion compared to 15% without product extraction. Although product extraction without consistent contamination of the unreacted substrates was proven, the long term operational stability of the

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membrane set-up was found to be one of the main limitations for further process optimization.

As alternative to the mentioned membrane-assisted strategy, in this study we propose a 3LP spinning reactor (Figure 1) for the synthesis and recovery of chiral amines. The 3LP spinning reactor can be considered an evolution of a standard stirred 1.5 L double-jacketed glass reactor. The motor driven central shaft supported a stainless steel inner tubular cylinder and one radial flow impeller. Being mounted on the shaft, the designed inner tubular cylinder rotated together with the impeller. The feasibility of developing a three-liquid-phase system, employing the aforementioned device, relies on the selection of a suitable phase **A**, non-miscible with either phase **B** or **C** (Figure 1). The transaminase-mediated synthesis of (*S*)-1-methyl-3-phenylpropylamine (MPPA) was employed as model reaction (Scheme 1).

Based on the previous studies,[14,16] *n*-heptane was selected as organic solvent phase **A**. The substrate 4-phenyl-2-butanone (BA), initially supplied to phase **A**, progressively moved to phase **C**. The enzymatic reaction occurred in phase **C**, (reaction phase), consisting of the enzyme TA-v2[25] and the AD (Jeffamine ED-600), not soluble in n-heptane. Once formed, the product moved from the reaction phase **C** to the extracting phase **B** via diffusion through *n*-heptane, due to partitioning. The acidic pH of the extracting phase traps the amines in their charged state, thus preventing back extraction into the organic phase and allowing the enrichment of amine product. Depending on the design of the system, and on the working volumes employed, the device was tested in two different configurations, depicted in Figure 1.

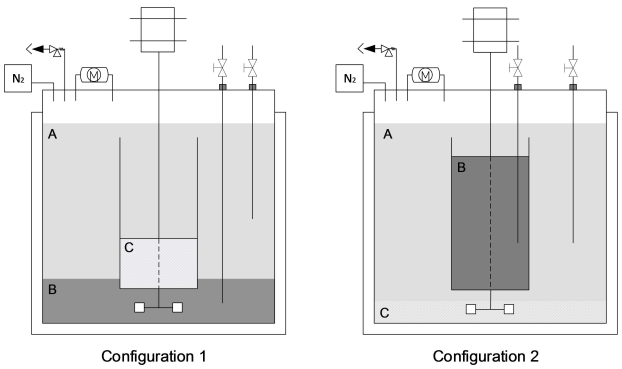
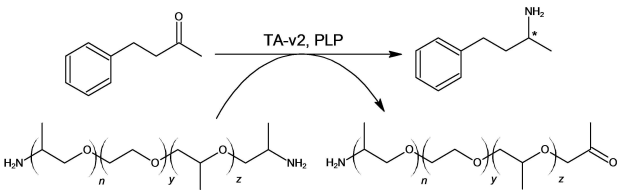


Figure 1. Principle of the three-liquid-phase (3LP) spinning reactor. A, B and C are the organic solvent phase, the reaction phase and the extraction phase, respectively. Depending on the design of the system, the device can operate in two configurations. The transaminase-mediated synthesis of (*S*)-1-methyl-3-phenylpropylamine (MPPA) was employed as model reaction (Scheme 1).



Scheme 1. The transaminase-mediated synthesis of (*S*)-1-methyl-3-phenylpropylamine (MPPA) from 4-phenyl-2-butanone (BA), using Jeffamine ED-600 amine donor.

To investigate the performances of the 3LP spinning reactor, a preliminary partitioning experiment using a synthetic solution was carried out. The device was tested in configuration 1, (Figure 1). In 6 h of operation, 3.5 g of MPPA, equivalent to 71 % of the initial amount added to the system, was extracted (Figure 2). The content of Jeffamine co-extracted amounted to 9.7 %. When the stirring rate, initially set to 150 rpm, was increased to 200 rpm, the rotation rate of the inner tubular cylinder, fixed to the agitator shaft, increased. The AD phase, placed in the inner tubular cylinder, spilled over the inner tubular cylinder, where it diffused through the *n*-heptane phase and was extracted into the buffer phase. Consequently, the pH of the buffer increased (Figure 3) and back extraction of MPPA was observed (Figure 2).

The first transaminase-mediated synthesis combined with continuous product recovery was performed employing the same configuration of the preliminary partitioning experiment (Figure 1, configuration 1). Having already proven in our recent work the reproducibility of the reaction system,[16] a single

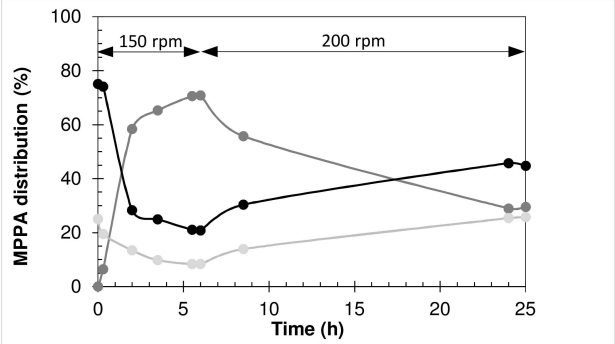


Figure 2. Preliminary partitioning experiment: distribution of 4-phenyl-2-butylamine (MPPA) in Jeffamine ED-600 (reactor phase, ●), *n*-heptane (●) and citric acid buffer solution (extraction phase, ●) over the time. The three-liquid-phase (3LP) spinning reactor was tested in configuration 1 (Figure 1). Experimental details are provided in the supplementary information, section 1.3.

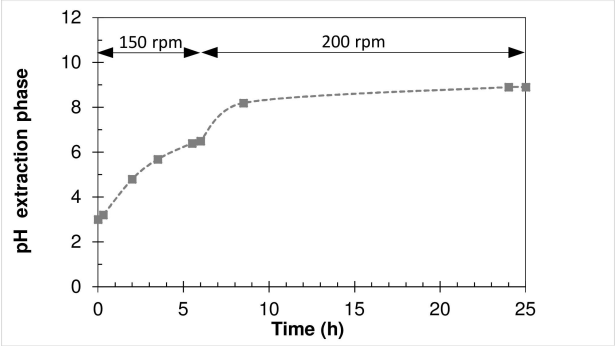


Figure 3. Preliminary partitioning experiment: pH of the extraction phase over the time. The three-liquid-phase (3LP) spinning reactor was tested in configuration 1 (Figure 1).

experiment at 1L scale was carried out. Out of 5 g of ketone substrate, initially added in *n*-heptane, only 0.8 g of MPPA was extracted and isolated after 17 days operation (Figure 4). It is possible that more MPPA could be formed in the reactor phase but was not released into the *n*-heptane thus escaping extraction. Remarkably, the diffusion of product and therefore product extraction increased when continuously stirring the system at 100 rpm (Figure 4, day 8–14). A continuous motion of the inner tubular cylinder, imparted by the stirrer, enhanced product release and thus improved the product extraction. As demonstrated in our previous study,[16] the geometry of the reaction system affects the rate of product diffusion from the AD to the upper *n*-heptane phase. Working with a larger diameter cylinder would decrease the thickness of the AD layer thus facilitating the diffusion of the formed product from the reactor phase to the *n*-heptane phase. In accordance with the partitioning experiment, less than 2% of unreacted substrate BA was co-extracted in the acidic buffer. AD co-extraction could not be avoided and depended on the stirring. Less than 1% of the initial AD Jeffamine added in the inner tubular cylinder was found in the *n*-heptane middle phase during the entire test. However, the stirring and the constant contact/mixing between *n*-heptane and the acidic extracting buffer resulted in AD co-extraction, thus affecting the product purity of the buffer phase. After 6 days of operation, the concentrations in the extracting phase of MPPA and Jeffamine ED-600 were 8 mM and 44 mM, respectively. Although 25% of the AD was co-extracted, the AD was present in lower concentrations compared to that would be the case if performing a batch reaction in aqueous environment, without any product removal strategy.

High reaction rates combined with fast product release from the reaction phase to *n*-heptane are essential for optimizing the ratio between the extracted amines. With this aim, the second experiment was carried out in a different configuration (Figure 1, configuration 2). By placing the enzyme on the bottom of the vessel (diameter 2.6-fold larger than the inner tubular cylinder), the AD thickness was considerably reduced. As expected, in this configuration product release from the AD layer to *n*-heptane was faster. In 5 days of continuous operation, a product yield of 52% was achieved. Compared to the control, a conventional batch experiment, where no MPPA extraction was applied, 2.6-fold higher product yield was achieved. Moreover, 85% of the formed product was extracted from the *n*-heptane into the buffer. The MPPA concentration constantly increased in the stripping phase, despite the much lower concentration in the reactor (0.1 g/L). Minimizing the volume of the stripping phase has several benefits for the downstream processing (i.e. higher product concentration). Having reduced the buffer volume by a factor of 3.5, a product concentration of 9 g/L was achieved in 5 days (Figure 5, ●). A higher product concentration could probably be achieved by prolonging the reaction time. On the one hand, stirring enhanced product release from the reaction phase to n-heptane and thus proved beneficial for simultaneous product extraction. On the other hand, stirring led to increased AD co-extraction into the extracting buffer (17% of the initial amount added was found in the extracting phase). Therefore, the system was stopped after 5 days of operation.

The main limitation of the extraction strategy for product removal (using solvents, membranes or resins) is often the poor selectivity between substrates and products.[26] Higher product purity is probably achievable by performing the reaction in an aqueous environment, using alanine or another zwitterionic AD, as neither the AD nor pyruvate co-product would partition to the hydrophobic organic solvent phase.[20,27]

The major difficulty to tackle for a 3LP process is the physical separation of the three different phases. This can be achieved with the classical separation funnel, for batch applications. For conducting countercurrent and continuous operations, more complex devices have been developed. The recently proposed mixer-settler-mixer three chamber integrated extractor was used for the separation of *p*-nitrophenol and *o*-nitrophenol. The separation of the two isomers was achieved by continuous mixing and separation of three non-miscible liquid phases: nonane (organic top-phase); polyethylene glycol (PEG 2000), (polymer middle-phase); and $(NH_4)_2SO_4$ aqueous solution, (aqueous bottom-phase).[28] The 3LP spinning reactor here proposed, does not require special laboratory equipment, and allows separations of multicomplex mixtures between two

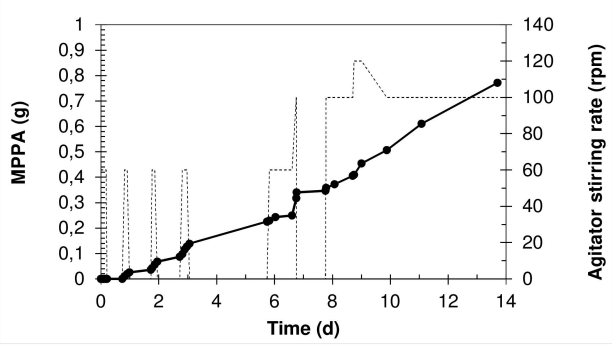


Figure 4. Effect of intermittent/discontinuous stirring rate (right axis —) on product extraction (left axis ●) in citric acid buffer, using the 3LP spinning reactor in configuration 1. Experimental details are provided in the supplementary information, section 1.4.

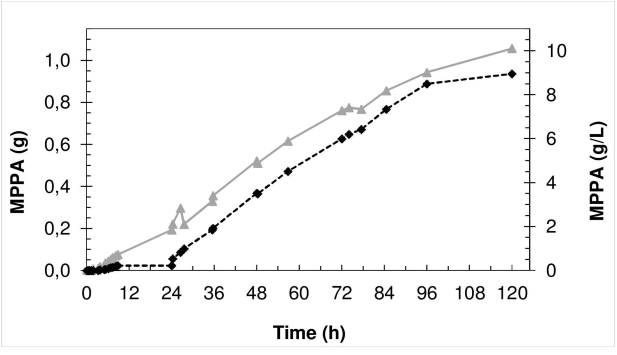


Figure 5. Production of MPPA for 4 days using the 3LP spinning reactor, configuration 2. Total product amount (g) detected in n-heptane and in the extracting buffer, (▲, left axis) and product concentration in the extracting buffer (●, right axis) are shown. Experimental details are provided in the Supplementary information, section 1.4.

miscible phases separated by a third immiscible phase to be performed. Additionally, it introduces more freedom for the selection of the three phases. The performance of the 3LP device can be exploited by varying the rotor speed, and the position, type, size and numbers of the impellers. Moreover, the geometry, the size and the position (height) of the tubular cylinder inside the reactor can be changed, depending on the working volumes of each phase.

In conclusion, the 3LP reactor concept, employed for transaminase-mediated synthesis of (*S*)-1-methyl-3-phenylpropylamine, was shown to be superior to the conventional set-up, where the reaction without product extraction was performed. Process engineering strategies for chiral amine synthesis have proven to enhance physical and chemical properties of transaminase-catalysed systems, such as low solubilities of reactants within aqueous media or undesired unfavourable thermodynamics.[26] Hereof, this study presents a step towards process intensification. Furthermore, the device should allow for the development of a continuous process, overcoming the limited lifetime of existing membrane-assisted three-liquid phase systems. The use of our device can be potentially extended to other product inhibited or thermodynamically unfavourable reaction systems or to different applications (e.g. separation of multi-component mixtures).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Chiral amines · transaminases · biocatalysis · liquid-liquid extraction · asymmetric synthesis

[1] M. Höhne, S. Kühl, K. Robins, U. T. Bornscheuer, *ChemBioChem* **2008**, *9*, 363–365.
[2] A. W. H. Dawood, R. O. M. A. de Souza, U. T. Bornscheuer, *ChemCatChem* **2018**, *10*, 951–955.
[3] I. V. Pavlidis, M. S. Weiß, M. Genz, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, *Nat. Chem.* **2016**, *8*, 1076–1082.
[4] F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333–360.
[5] I. Slabu, J. L. Galman, R. C. Lloyd, N. J. Turner, *ACS Catal.* **2017**, *7*, 8263–8284.
[6] J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd, N. J. Turner, *Green Chem.* **2017**, *19*, 361–366.
[7] S. E. Payer, J. H. Schrittwieser, W. Kroutil, *Eur. J. Org. Chem.* **2017**, *2017*, 2553–2559.
[8] A. Gomm, W. Lewis, A. P. Green, E. O'Reilly, *Chem. Eur. J.* **2016**, *22*, 12692–12695.
[9] A. P. Green, N. J. Turner, E. O'Reilly, *Angew. Chem. Int. Ed.* **2014**, *53*, 10714–10717; *Angew. Chem.* **2014**, *126*, 10890–10893.
[10] A. Gomm, S. Grigoriou, C. Peel, J. Ryan, N. Mujtaba, T. Clarke, E. Kulcinskaja, E. O'Reilly, *Eur. J. Org. Chem.* **2018**, *2018*, 5282–5284.
[11] C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, *Science* **2010**, *329*, 305–309.
[12] Y. Satyawali, D. F. del Pozo, P. Vandezande, I. Nopens, W. Dejonghe, *Biotechnol. Prog.* **2019**, *35*, e2731.
[13] R. C. Simon, N. Richter, E. Busto, W. Kroutil, *ACS Catal.* **2014**, *4*, 129–143.
[14] Y. Satyawali, E. Ehimen, L. Cauwenberghs, M. Maesen, P. Vandezande, W. Dejonghe, *Biochem. Eng. J.* **2017**, *117*, 97–104.
[15] C. Matassa, D. Ormerod, U. T. Bornscheuer, M. Höhne, Y. Satyawali, *Proc. Biochem.* **2019**, *80*, 17–25.
[16] C. Matassa, A. Romani, D. Ormerod, U. T. Bornscheuer, M. Höhne, Y. Satyawali, *J. Chem. Technol. Biotechnol.* **2019**, jctb.6241.
[17] Y. Satyawali, K. Vanbroekhoven, W. Dejonghe, *Biochem. Eng. J.* **2017**, *121*, 196–223.
[18] G. Rehn, P. Adlercreutz, C. Grey, *J. Biotechnol.* **2014**, *179*, 50–55.
[19] G. Rehn, B. Ayres, P. Adlercreutz, C. Grey, *J. Mol. Catal. B* **2016**, *123*, 1–7.
[20] T. Börner, G. Rehn, C. Grey, P. Adlercreutz, *Org. Process Res. Dev.* **2015**, *19*, 793–799.
[21] B. Braibant, D. Bourgeois, D. Meyer, *Sep. Purif. Technol.* **2018**, *195*, 367–376.
[22] G. H. Van Bochove, G. J. Krooshof, T. W. De Loos, *Fluid Phase Equilib.* **2002**, *194–197*, 1029–1044.
[23] R. Dutta, U. Sarkar, A. Mukherjee, *Ind. Crops Prod.* **2015**, *71*, 89–96.
[24] S. Shen, Z. Chang, J. Liu, X. Sun, X. Hu, H. Liu, *Sep. Purif. Technol.* **2007**, *53*, 216–223.
[25] T. Börner, S. Rämisch, S. Bartsch, A. Vogel, P. Adlercreutz, C. Grey, *ChemBioChem* **2017**, *18*, 1482–1486.
[26] P. Tufvesson, J. Lima-Ramos, J. S. Jensen, N. Al-Haque, W. Neto, J. M. Woodley, *Biotechnol. Bioeng.* **2011**, *108*, 1479–1493.
[27] H. Yun, B.-G. Kim, *Biosci. Biotechnol. Biochem.* **2008**, *72*, 3030–3033.
[28] X. He, K. Huang, P. Yu, C. Zhang, K. Xie, P. Li, J. Wang, Z. An, H. Liu, *Chin. J. Chem. Eng.* **2012**, *20*, 27–35.

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