Rotating bed reactor technology for immobilized enzymatic reactions

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Introduction

The use of immobilized enzymes combined with novel reactor technologies holds promises for more efficient, economical and environmentaly friendly industrial scale synthesis processes in the pharmaceutical, fine chemicals, flavor and fragrances, vitamins, food and textile industries. This poster presents data from an article recently accepted for publication in the scientific journal Organic Process Research & Development. In this work, we performed a case study of applying rotating bed reactor (RBR) technology for the lipase-mediated stereoselective acetylation of a racemate amine as a model reaction for the manufacturing of pharmaceutical building blocks (Fig 1). After reaction optimization, the results showed that enzyme recycling and synthesis scaleup was easy to achieve with preserved yield, enantioselectivity and catalytic activity.

Fig 1

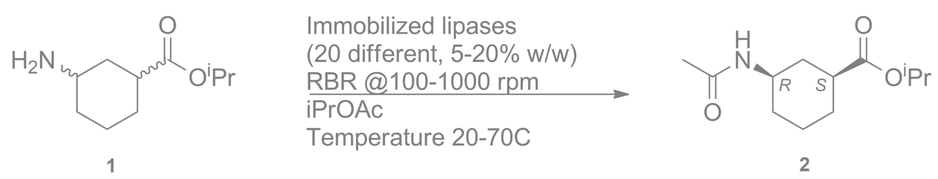


Fig 1. Schematic drawing of the model reaction used to study the implementation of rotating bed reactor (RBR) technology. This enzyme catalyzed acetylation of (cis)-isopropyl 3-aminocyclohexanecarboxylate (1) by isopropyl acetate (iPrOAc) into the chiral amide 2 was previously reported to give high yield and enantiomeric purity in traditional stirred tank batch reactor setup (Org. Process Res. Dev., 2016, 20:1336-1340). Optimization of reaction parameters was performed with 10% loading (1.5 g enzyme) of Novozymes 435 in a SpinChem® RBR S2 at 20 °C and 500 rpm agitation speed in 150 mL anhydrous iPrOAc (200 ppm water) containing 15 g (77.73 mmol) of amine 1 under a nitrogen atmosphere in a SpinChem® flower-baffled reaction vessel V2 unless otherwise specified. All conversions were recorded using ^{1}H NMR analysis of the crude reaction mixtures. Enantiomeric excess purity (ee) of compound 2 was determined by chiral HPLC with UV detection using 15% 2-propanol in supercritical carbon dioxide at 120 bar as eluent. This poster presents data from an article by S. Pithani, S. Karlsson, H. Emtenäs, and C. T. Öberg, recently accepted for publication in the scientific journal Organic Process Research & Development.

Reaction optimization

We studied the influence of enzyme resin (Fig 2a), enzyme loading (Fig 2b), agitation speed (Fig 2c), and reaction temperature (Fig 2d) to optimize the biocatalytic reaction.

Fig 2a

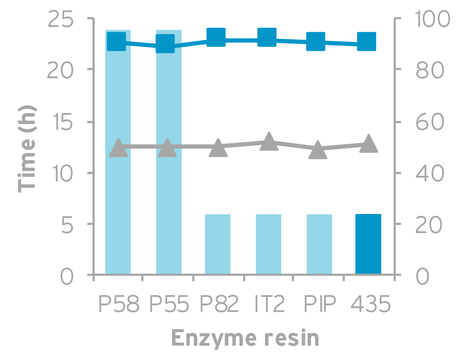


Fig 2b

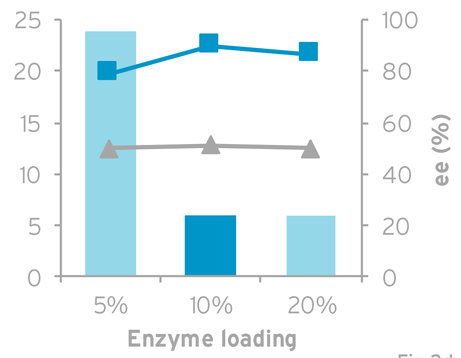


Fig 2c

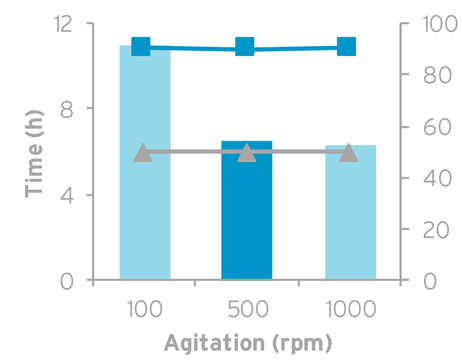


Fig 2d

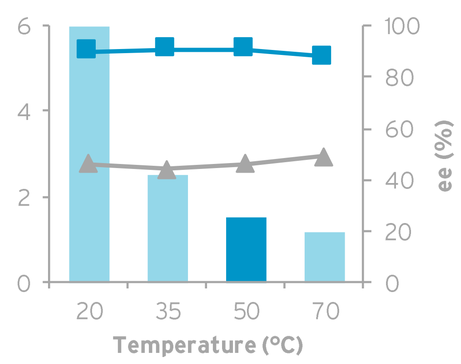


Fig 2. Plot of reaction time (blue bars, left axis) to reach conversion (grey line, right axis) and the achieved enantiomeric excess (blue line, right axis) during univariate laboratory scale reaction optimization. Final optimized conditions in each experiment is highlighted by a dark blue bar. Conditions as outlined in Fig 1 with modifications according to figure text. The studied immobilized enzymes were Purolite Lifetech CalB Immo 5872 (P58), 5587 (P55), 8285 (P82), Immozyme IMMCALB-T2-150XL (IT2), Purolite ImmoPlus (PIP), and Novozymes 435 (435).

Enzyme recycling

The reusability of the immobilized enzyme was evaluated through recycling experiments. After each cycle, the reaction mixture was simply drained out and the RBR was rinsed with reaction solvent prior to the next cycle experiment. It was found (Fig 3) that the enzyme was very stable and could be reused for 10 consecutive recycling runs with preserved reaction rate and enantioselectivity.

Fig 3

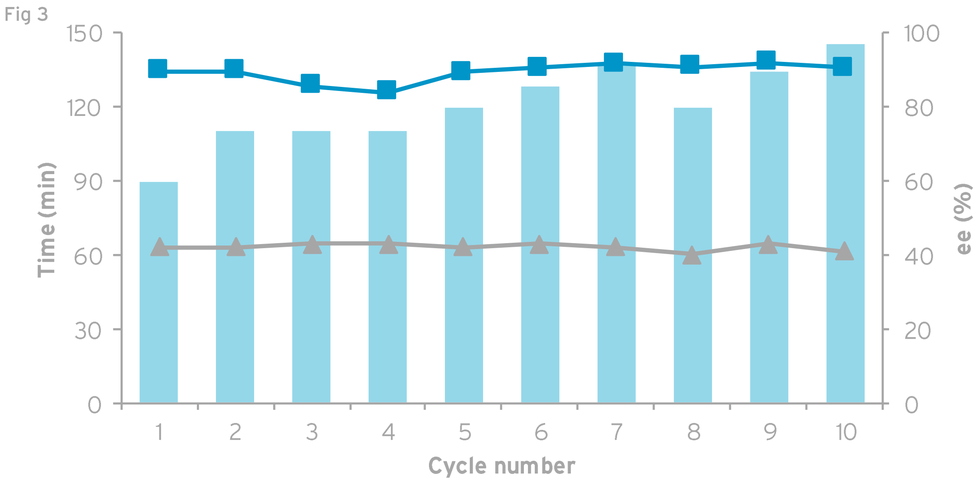


Fig 3. Plot of reaction time (blue bars, left axis) to reach the desired conversion (grey line, right axis) and the achieved enantiomeric excess (blue line, right axis). Recycling reactions were performed as specified in Fig 1, but at 50 °C and with inline monitoring of reaction progress and conversion using a FT-IR probe in the reaction vessel. The reaction vessel and RBR containing the immobilized enzyme was rinsed with 50 mL iPrOAc under vigorous agitation between each cycle.

Kilogram scaleup

To investigate the suitability of the RBR technology for manufacturing, the volume of the enzyme catalysed synthesis was increased from laboratory development to kilogram production scale. This scaleup was very successful and the reaction reached 46% conversion within 90 minutes (Fig 4). After an aqueous acidic workup and recrystallization, the desired product was obtained with a 39% overall yield with 99% ee.

Fig 4

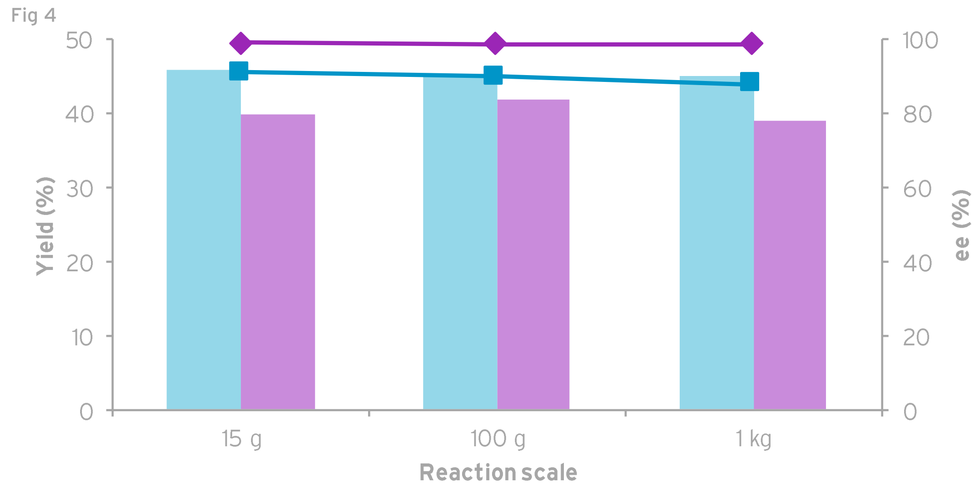
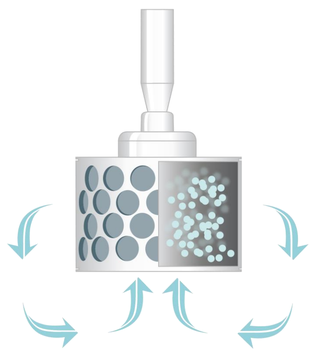


Fig 4. Plot of conversion (blue bars, left axis) and reaction yield after workup (purple bars, left axis) and the achieved enantiomeric excess of crude product (blue line, right axis) and after crystallization workup (purple line, right axis) at different reaction scales. The 15 g scale experiment was performed as specified in Fig 3 during 90 minutes and the scales of 100 g and 1 kg were performed for the same amount of time at matching conditions. Specifically, the 1 kg scale was using 100 g enzyme resin in a SpinChem® RBR S4 prototype submerged in 10 L iPrOAc containing 1 kg (5.18 mol) of amine 1, finally resulting in a total of 460 g (99% w/w, 2.02 mol, 39.1% yield, ee >98.8%) of compound 2 after workup and recrystallization.



The SpinChem® RBR creates efficient mass transfer and simplifies solid catalyst recovery. The RBR creates a circulating flow through the rotating catalyst bed, giving the reactants numerous chances to come in close contact with the catalyst sites.

Conclusions

The SpinChem® rotating bed reactor (RBR) design was successfully applied for enzymatically mediated synthesis of pharmaceutical building blocks. The RBR technology enabled easy enzyme recycling for more than 10 cycles with preserved yield, enantioselectivity and reaction rate. Reaction scaleup was feasible and provided similar good results in the small-scale development laboratory as in kilogram scale production.

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