Biocatalysis in rotating bed reactors – from screening to production

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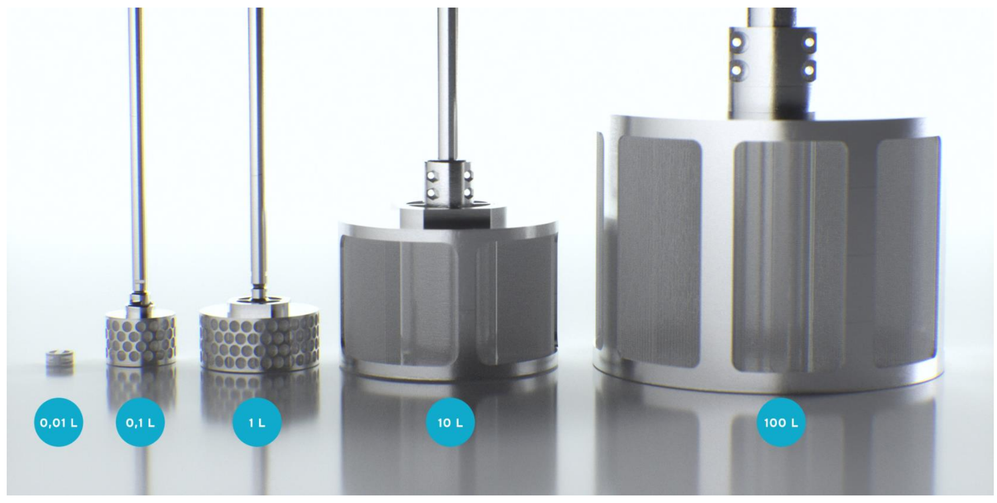
Introduction

Rotating bed reactors (RBR) constitute an exciting alternative for performing biocatalytic reactions with enzymes immobilized onto solid supports. The RBR offers convenient handling of the solid phase by keeping it confined within the reactor. The high convective flows created by the RBR's spinning motion typically translates to fast reactions and high product yields. In the study here presented, we verified the feasibility of the RBR technology for several esterification reactions catalysed by commercially available immobilized lipases from Purolite.



Screening of immobilized enzymes

Rapid screening of six immobilized lipases for an esterification was performed in about 10 mL and 200 mL using a disposable prototype magnetic RBR (MagRBR) and commercial pre-packed cartridges within a SpinChem® RBR S2, respectively (Fig 1). Reactions performed similarly in both cases and handling of the enzyme resins was extremely fast as it required minimum preparations and no filtration during sampling. The screening achieved using the MagRBR was performed with six resins in parallel, but could easily be expanded to further reduce the time.

Fig 1a

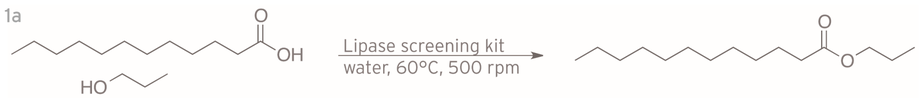


Fig 1b

Fig 1c

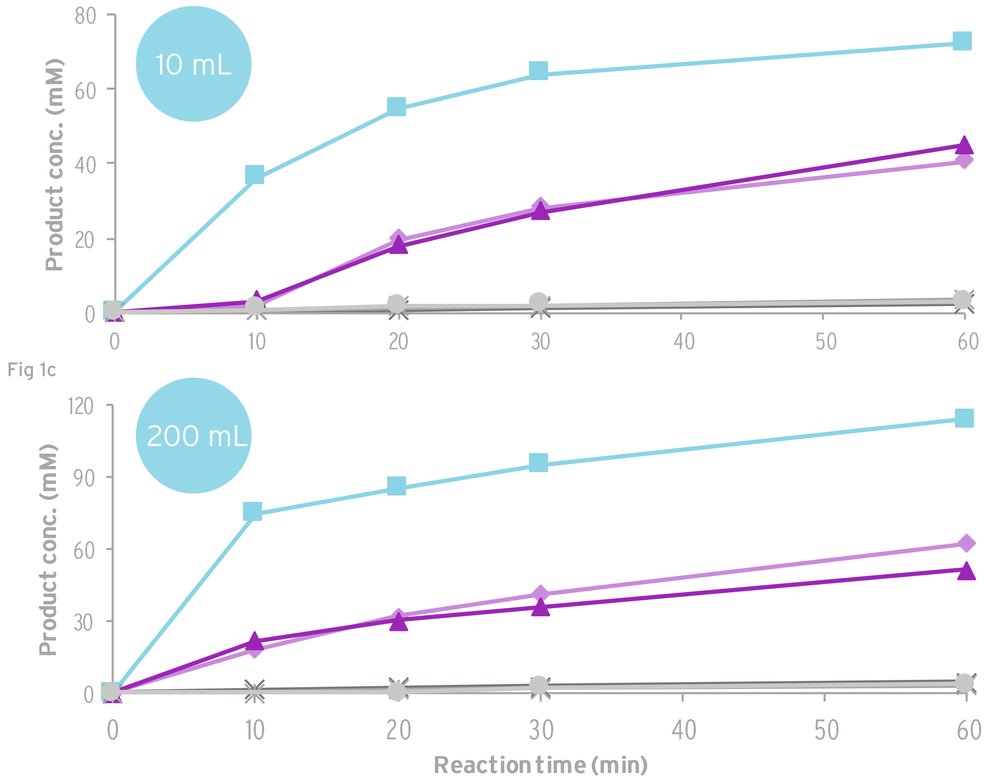


Fig 1. Immobilized enzyme resin screening reaction scheme (top) and plots of results at about 13 mL (middle) and about 130 mL (bottom) liquid volume. Conditions MagRBR: A pre-heated (60 °C, 1 h) mixture of lauric acid (8.01 g, 40 mmol/vial), 1-propanol (2.4 g, 40 mmol/vial) and water (0.32 g, 18 mmol/vial) was distributed into six different plastic vials maintained at 60 °C on a magnetic stirrer with six positions operated at 500 rpm. Each vial contained one prototype MagRBR filled with 0.5 mL of one of six different immobilized enzymes from the Purolite® Lifetech™ lipase screening kit. Conditions S2 RBR: A pre-heated (60 °C, 1 h) mixture of lauric acid (168.2 g, 840 mmol/vessel), 1-propanol (842 mmol, 63 mL/vessel) and water (373 mmol, 6.72 mmol/vessel) was poured into a SpinChem® V2 reaction vessel maintained at 60 °C. Thereafter a SpinChem® S2 RBR fitted with four SpinChem® B2 cartridges containing one of six different immobilized from the Purolite® Lifetech™ lipase screening kit (4x2 mL) was lowered into the solution and rotated at 500 rpm. Analysis: Samples were collected at 0, 10, 20, 30, 60 minutes and diluted with heptane spiked with internal standard (tetradecane), before analysis by GC-FID.

Optimization and scale-up

We studied the influence of rotation speed on conversion for an esterification reaction and concluded that the reaction was mass transfer-limited, showing increased conversion at higher rotation speed (Fig 2). Finally, we performed seven repeated reactions with successful enzyme recycling over a period of one month (Fig 3) to simulate a production environment. Results varied less than two standard deviations and no loss of enzyme activity was observed. The lack of any filtration steps during enzyme recycling, opens up for the possibility of automated semi-continuous processes with improved production economy.

Fig 2a.

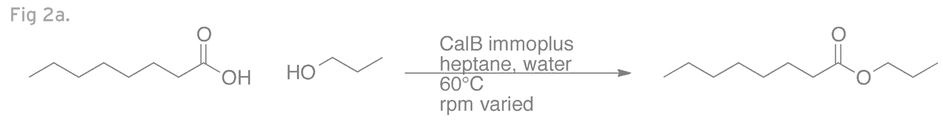


Fig 2b.

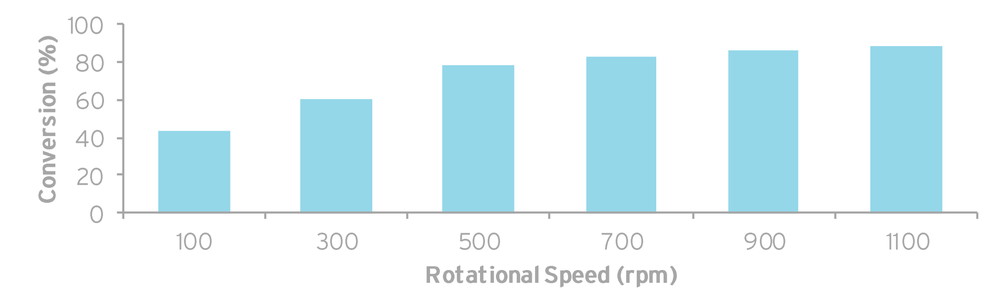


Fig 2. Esterification reaction scheme (top) and conversion diagram (bottom) estimated from remaining starting material after 60 min with the RBR set at different rotational speeds. Conditions: Premixed octanoic acid (6.4 mL, 40 mmol), n-propanol (3.0 mL, 40 mmol), and water (0.32 mL, 18 mmol) dissolved in heptane within a SpinChem® V2 reaction vessel. A SpinChem® S2 RBR charged with Purolite® CalB immo Plus™ lipase (300 mg) was inserted and rotated at various speeds at 30 °C for 30 min. Analysis of amount unreacted octanoic acid by GC-FID.

Fig 3a.

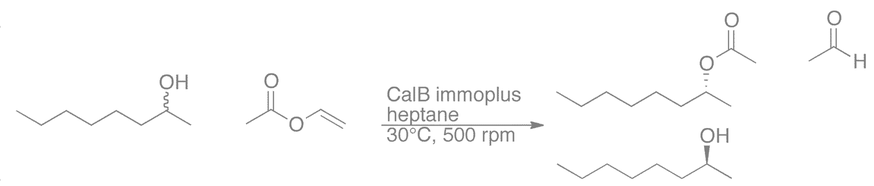


Fig 3b.

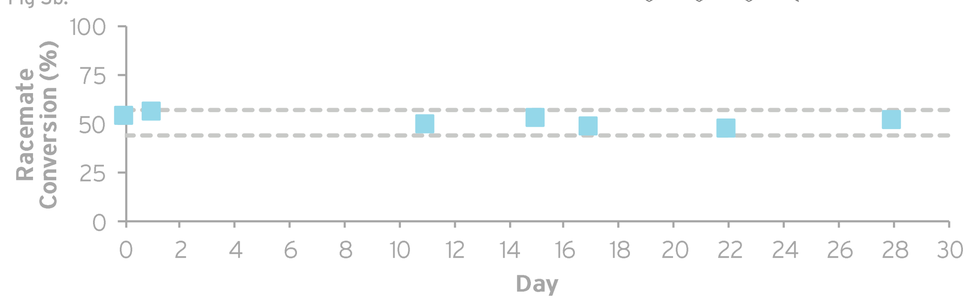
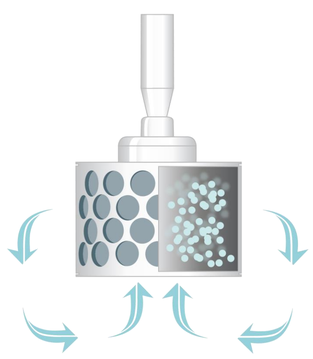


Fig 3. Transesterification reaction scheme (top) and plot (bottom) of racemate conversion after repeated recycling of immobilized enzyme over 28 days. The dotted lines represent two standard deviations of the data. Conditions: Premixed 2-octanol (rac, 4.4 mL, 28 mmol) and vinyl acetate (2.6 mL, 28 mmol), dissolved in heptane (140 mL) within a SpinChem® V2 reaction vessel. A SpinChem® S2 RBR charged with Purolite® CalB immo Plus™ lipase (1.55 g) was inserted and rotated at 500 rpm at 30 °C for 30 min. Washed between batches by spinning the RBR 5 min in heptane followed by storage in heptane at 20 °C. Analysis of amount unreacted 2-octanol by GC-FID.



The SpinChem® RBR creates efficient mass transfer. The RBR aspirates solution from the bottom, percolates it through the bed of solid phase, and finally distributes it towards the vessel wall, thereby creating a continuous flow of solution.

Conclusions

The SpinChem® rotating bed reactor (RBR) design can efficiently facilitate biocatalytic reactions with immobilized enzymes in volumes from a few millilitres up to production scale. The prototype disposable MagRBR enables rapid parallel screening of immobilized enzymes, resulting in fast and convenient laboratory reaction development.

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