Recycling of immobilized enzymes using rotating bed reactor technology

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Introduction

Biocatalytic reactions involving enzymes immobilized to solid supports is a useful method for the synthesis of small chiral molecules. Limited stability or complex post-process clean-ups, however, risk reducing the productivity and scale-up economy. In this poster, a SpinChem® rotating bed reactor (RBR), which enhances mass transfer to solid phases while keeping them protected in a confined compartment, was explored for esterification reactions using immobilized enzymes. After confirming that the reactions likely were mass transfer limited and thus would benefit from the enhanced convection achieved with the RBR, we investigated reaction robustness after many repeated cycles with different starting materials and immobilization supports at several independent laboratories.

Mass transfer limited reaction

Increasing rotational speed of the RBR led to increasing consumption rate of starting material (Fig 1), indicating a mass transfer limited reaction with the immobilized lipase enzyme.

Fig 1.

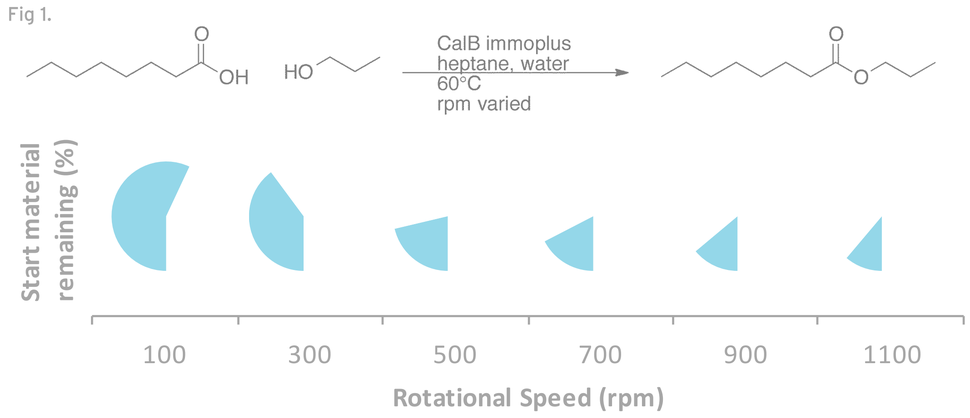


Fig 1. Esterification reaction scheme (top) and pie charts (bottom) of remaining starting material after 60 min at different rotational speed of the RBR. Conditions: Premixed octanoic acid (6.4 mL, 40 mmol), n-propanol (3.0 mL, 40 mmol), and water (0.32 mL, 18 mmol) dissolved in heptane within a SpinChem® V221 reaction vessel. A SpinChem® S221 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.

Successful enzyme recycling

The transesterification reaction with immobilized lipase in an RBR showed reproducible conversion over seven batches during 28 days with recycled enzyme (Fig 2). Similarly, stable esterification synthesis of heptyl acetate and methyl oleate was recorded for 17 and 23 cycles, respectively, using an RBR with immobilized lipase enzymes in different laboratories (Fig 3-4). In all three examples, the recycling step was very quick and convenient. Without any filtration, the RBR was rotated in solvent or air for 1-5 min prior to starting the next cycle. No attrition or turbidity from particle grinding could be observed in any experiment, whereas this was a common problem in stirred tank reactors under similar conditions (data not shown). It was estimated from Fig 4 that the enzyme half-life would allow more than 200 cycles, processing more than 50 million catalytic turnovers, thus enabling production of 50 kg per g catalyst.

Fig 2.

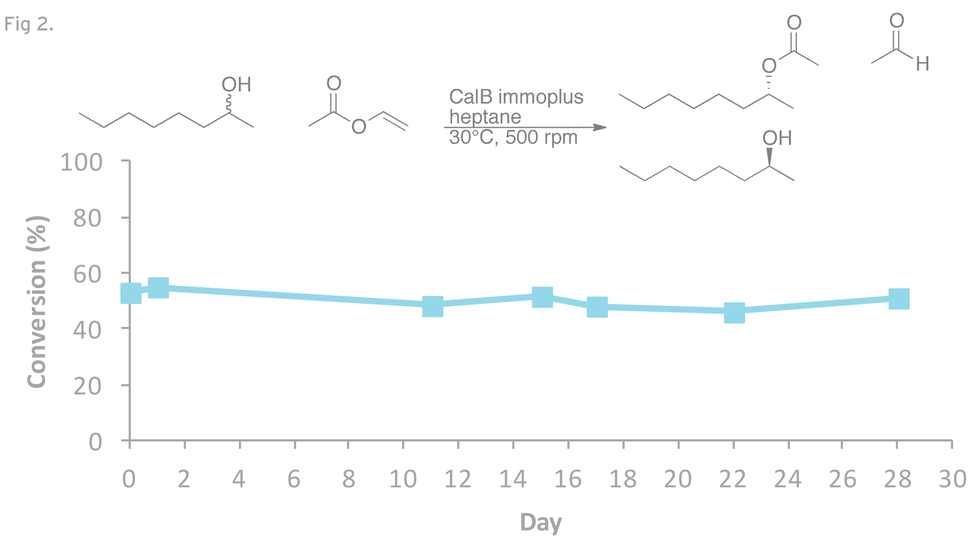


Fig 2. Transesterification reaction scheme (top) and plot (bottom) of remaining starting material after repeated recycling of immobilized enzyme in transesterification reaction over 28 days. Conditions: Premixed 2-octanol (rac, 4.4 mL, 28 mmol) and vinyl acetate (2.6 mL, 28 mmol), dissolved in heptane (140 mL) within a SpinChem® V221 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 un-reacted 2-octanol by GC-FID.

Fig 3.

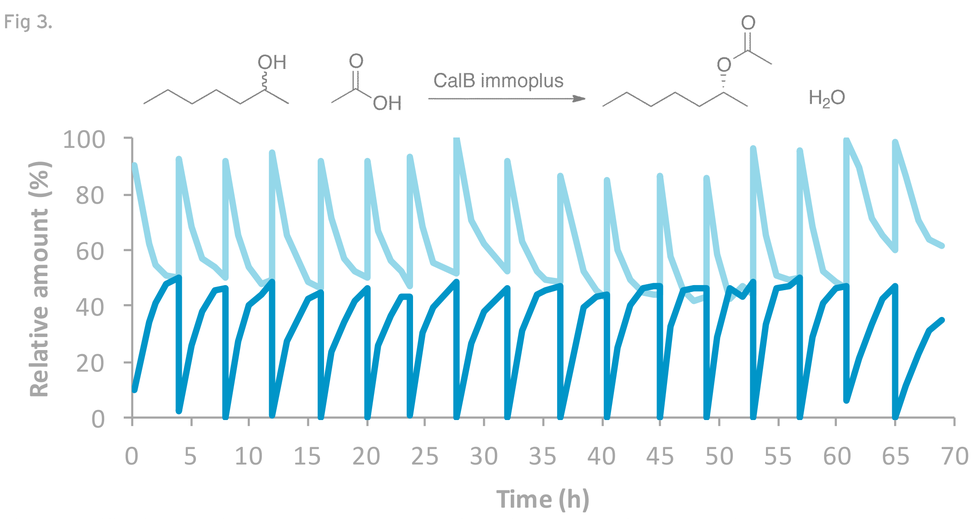


Fig 3. Esterification reaction scheme (top) and plot of relative amount of substrate (light blue) and product (dark blue) during 4 h batch cycles with the same immobilized enzyme. Conditions: Premixed acetic acid (1.201 mL, 20 mmol) and 2-heptanol (rac, 2.324 mL, 16 mmol) dissolved in 100 mL heptane within a SpinChem® V211 reaction vessel. A SpinChem® S221 RBR charged with Purolite® CalB immo Plus™ lipase (1.1 g, 10000 PLU) was inserted and rotated at 100 rpm at 30 °C for 4 h. Washed between cycles by spinning the RBR in 100 ml heptane. Storing at 4 °C at end of day. Analysis of amount of substrate and product by GC-FID.

Fig 4.

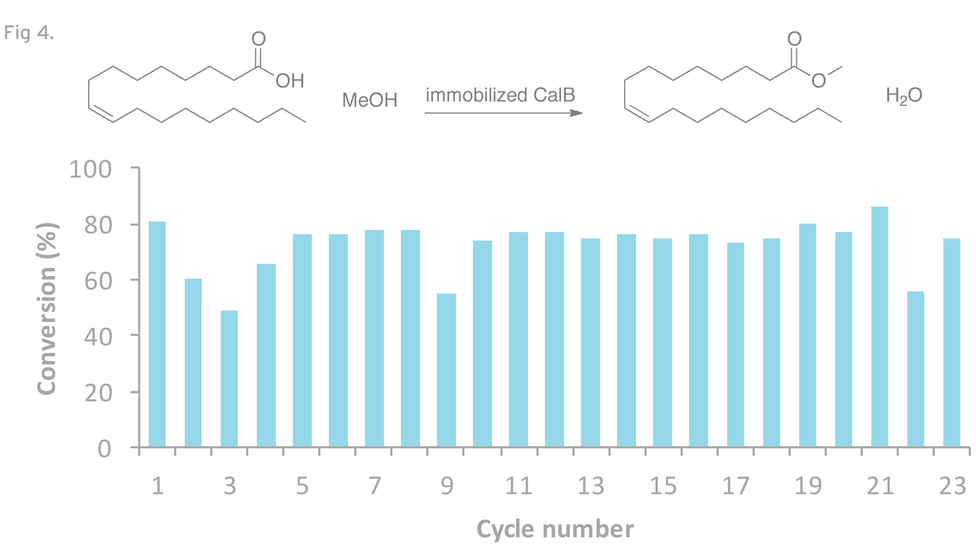
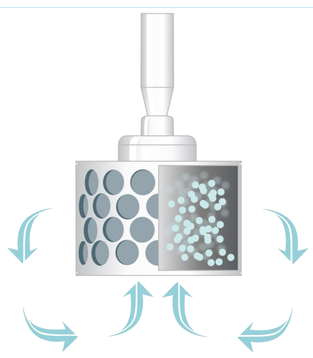


Fig 4. Esterification reaction scheme (top) and plot (bottom) of conversion after recycling of immobilized enzyme. Conditions: Oleic acid (750 mmol, tech.) and methanol (1.5 mol) within a SpinChem® V211 reaction vessel. A SpinChem® S211 RBR charged with ChiralVision's proprietary adsorbed CalB lipase (2 wt%) rotated at 500 rpm at 40 °C. Dry spinning of RBR 1 min in air at 1000 rpm after each cycle. Analysis of amount unreacted oleic acid by GC-FID. The lower conversions at cycle 3, 9 and 22 was due to insufficient heating time after weekend storage at 4 °C. Conversion after 4 h was 90-95%.



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 significantly enhance the recycling of immobilized enzymes. The high catalyst stability and simple handling during recycling opens for the possibility of automated semi-continuous processes with greatly improved production economy.

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