Application Note

Recycling of Immobilized Enzymes

Simple and Streamlined Biocatalysis

SPINCHEM®

Authors: Christopher Öberg, Edith Björnberg Kalén and Emil Byström, SpinChem AB

Simple and robust immobilized catalyst recycling using a rotating bed reactor in an advanced synthesis workstation

The use of enzymes as catalysts for organic synthesis is a highly selective and environmentally friendly route. Immobilizing the enzymes on the surface of an insoluble material will increase its thermal and operational stability, and allow for easier washing, manipulation and separation of the enzyme. Even more important, immobilization of the enzyme will allow easier recycling of the catalyst, making the process both resource and cost efficient.

Figure 1. EasyMax™ 102 Advanced glass vessel with SpinChem® rotating bed reactor (RBR) S2.

The SpinChem® rotating bed reactor (RBR) is a device designed for heterogeneous reactions. The solid phase is kept contained as a packed bed within a rotating cylinder, through which the liquid phase is repeatedly percolated. The very efficient mass transfer achieved, together with no filtrating steps or particle grinding, allows for quick, easy and reproducible reactions. In this application note, the esterification of 2-Ethyl-1-hexanol into 2-Ethylhexyl acetate was performed, catalysed by immobilized CalB (Novozyme 435). The enzyme resin was packed into a SpinChem® RBR S2 twice, and each time used for seven consecutive reaction runs in an EasyMax™ 102 Advanced synthesis workstation.

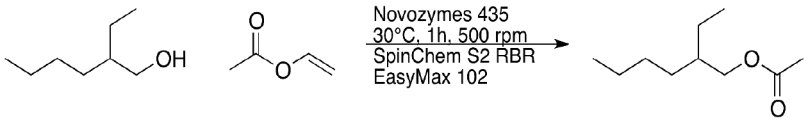

Figure 2. A SpinChem® RBR S2 containing Novozyme 435 (1 g), was rotated at 500 rpm in a preheated (30 °C) substrate solution containing 2-Ethyl-1-hexanol (5.21 g), Vinyl acetate (3.44 mL) and heptane (110 mL). The reaction was repeated for seven cycles, and samples for each run were taken after 0, 10, 20, 30 and 60 min. Analysis of 2-Ethylhexyl acetate product was done suing GC-FID after 1:9 dilution in heptane containing internal standard (tetradecane, 50 mmol). Between cycles, the resin was washed by spinning the RBR in heptane for 1 min.

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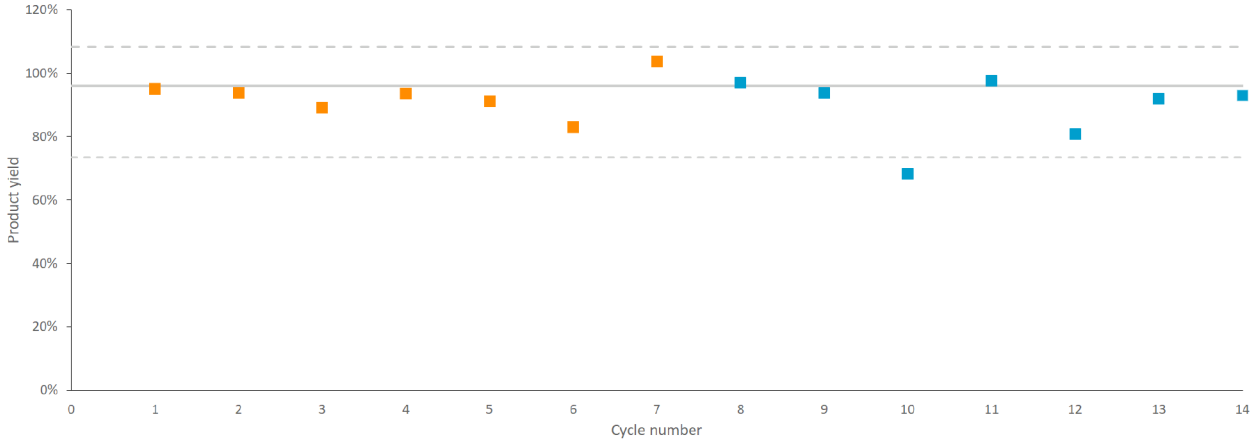

Figure 3. Results from 14 repeated biocatalysis reactions using an RBR in the EasyMax workstation. The immobilized enzyme was changed after 7 cycles. The solid line represents the mean yield of seven cycles run using an anchor stirrer. The dashed lines indicate two standard deviations, in both positive and negative direction from the mean value of the runs perfromed using the RBR.

Results

Product formation was monitored over time the for seven successive catalysis runs using GC-FID. The analysis showed that the Novozyme 435 lipase remained at high enzymatic activity throughout all esterification cycles, indicating that the resin could have been used for several additional runs. The speed and yield of this particular reaction were very similar regardless of using an RBR or an anchor stirrer. The work-up time needed for the anchor stirrer was, however, around 10 times longer than for the RBR, making the RBR experiments significantly more effective.

Conclusions

Recycling of immobilized enzymes catalysing the esterification was successfully performed using a SpinChem® RBR S2 fitted into the EasyMax™ 102 Advanced synthesis workstation. The process proved very time efficient as no filtration steps were needed between cycles, or for the samples extracted for analysis during each run. Washing of the resin between runs was fast, simple and robust, without running the risk of material loss. The easy work-up achieved with the RBR means higher productivity, especially for high reaction rates. This, together with the stable reaction environment of the workstation, ensures a streamlined workflow with high experimental reproducibility, and the possibility of automation.

SpinChem® RBR S2 in an Advanced glass vessel

Quick and robust enzyme recycling:

- Easy sampling and resin recycling
- No filtration and no bead attrition
- Quick and easy work-up

SpinChem® S2 RBR
Efficient rotating bed reactor for 100-500 mL reaction vessels

EasyMax™ 102 Advanced
Robust synthesis workstation for high R&D productivity

Process Analytical Technology
EasyMax integrate seamlessly with real-time in situ probes

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www.spinchem.com
For more information

METTLER TOLEDO AutoChem, Inc.
7075 Samuel Morse Drive
Columbia, MD 21046 USA
Telephone +1 410 910 8500
Fax +1 410 910 8600
Email autochem@mt.com
Internet www.mt.com/autochem

SpinChem AB
Tvistevägen 48C
SE-907 36 Umeå, Sweden
+46 (0)90 19 25 01

info@spinchem.com
www.spinchem.com