Screening of immobilized lipases using magnetic rotating bed reactors

The SpinChem® magnetic rotating bed reactor (MagRBR) was proven to be a time and labour efficient tool in the screening of biocatalysts. In this application note, six different immobilized lipases were screened in parallel for the esterification of lauric acid into propyl laurate using the pre-packed MagRBR Lipase screening kit. The process proved fast and simple, as efficient sampling and monitoring of the process was achieved without filtration steps, by keeping the immobilized catalyst confined inside the MagRBR.

Keywords: Immobilized enzymes, Rapid Screening, Synthesis, Easy handling, Fast reaction

The use of enzymes to catalyse the synthesis of organic compounds is an efficient and widely applied science due to the high selectivity and specificity of these biocatalysts. A frequently employed group of biocatalysts is lipases. These enzymes are used to catalyse important steps within the processing of food, production of biofuels as well as in the synthesis of fine chemicals, cosmetics and pharmaceuticals. Reactions mediated by lipases include the hydrolysis of lipids into fatty acids, and the synthesis of esters and amides.

A beneficial approach when working with biocatalysis is to immobilize the enzyme of interest on an insoluble carrier material. This will allow for easier handling of the enzyme, as well as an increase its thermal and operational stability. This means faster and simpler screening and recycling of suitable enzymes.

The SpinChem® magnetic rotating bed reactor (MagRBR) is a tool for screening of biocatalysts and biocatalytic reactions. The MagRBR consists of a hollow, magnetic cylinder, pre-packed with immobilized enzyme that is held in place by filters (Figure 1). This tool is designed to preserve resin integrity and eliminate the need for filtration of the reaction solution. As the MagRBR is spun in substrate solution by means of magnetic coupling, the solution is repeatedly pushed through the packed bed within by the centrifugal forces created by the rotary movement. This allows soluble substrate to do multiple passages through the bed, leading to fast and convenient reaction screenings.

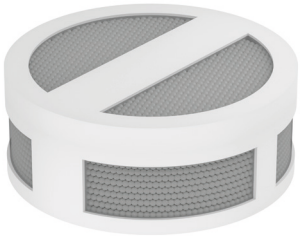

Fig 1. SpinChem® magnetic rotating bed reactor (MagRBR), packed with resin and used for screening applications of biocatalysts and biocatalytic reactions.

In this application note the SpinChem® MagRBR Lipase screening kit was used to screen six different lipases immobilized on ECR enzyme carrier resin (Purolite® Lifetech™) for the esterification of lauric acid into propyl laurate. The six MagRBRs included in the kit, each packed with 0.5 mL of one of the immobilized lipases, were spun in parallel on a six-position magnetic plate at 500 rpm for 1 h in preheated substrate solution, containing equimolar amounts of lauric acid and 1-propanol.

Three of the screened lipases successfully catalysed the reaction (Figure 2). The most efficient enzyme to mediate the conversion was found to be recombinant Lipase B from *Candida antarctica* (CalB) immobilized by adsorption on a DVP/methacrylate bead (CalB immo Plus™, Purolite® Life Sciences).

Using the SpinChem® MagRBR Lipase screening kit for parallel evaluation of catalyst candidates proved to

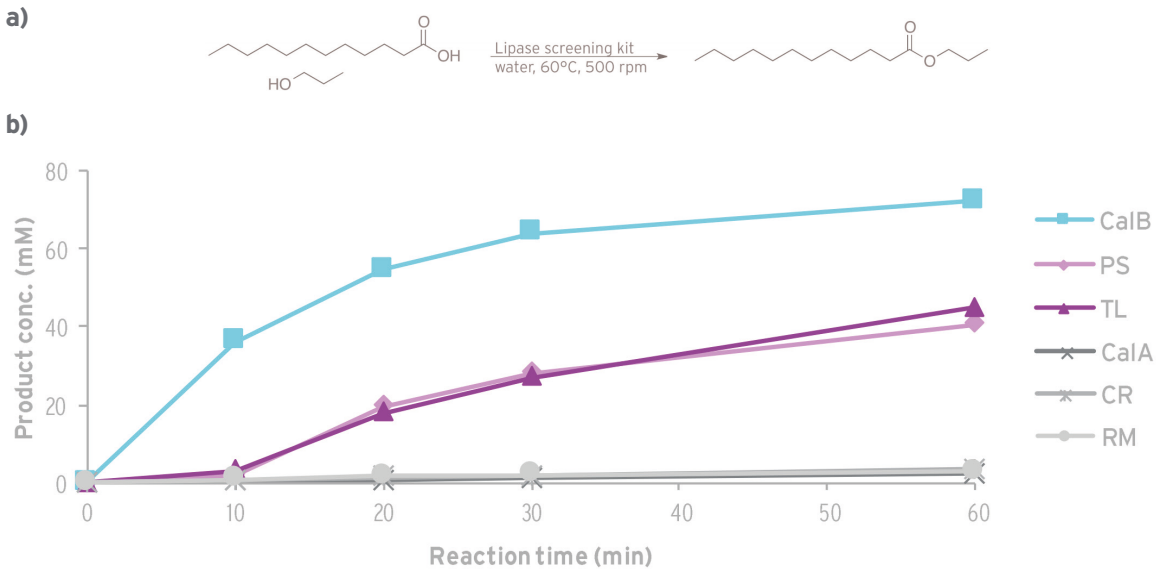

Fig 2. a) Reaction scheme. Six MagRBRs, each packed with 0.5 mL of one of the immobilized enzymes, were spun in parallel on a six-position magnetic plate at 500 rpm for 1 h in preheated (60°C) substrate solution containing lauric acid (8.01 g, 40 mmol/vial), 1-propanol (2.4 g, 40 mmol/vial), and water (0.32 g, 18 mmol/vial). Samples were collected at 0, 10, 20, 30 and 60 min. Analysis of the propyl laurate product was done using GC-FID after 1:50 dilution in heptane containing internal standards (tetradecane). b) Formation of propyl laurate over time for esterification reactions catalysed by immobilized lipases CalB (lipase B from *Candida antarctica*), CalA (lipase A from *Candida antarctica*), TL (lipase from *Thermomyces lanuginosa*), RM (lipase from *Rhizomucor miehei*), CR (lipase from *Candida rugosa*) and PS (lipase from *Pseudomonas cepacia*).

be a fast and simple approach to finding the enzyme best suited for a particular reaction. The reactions were monitored by withdrawing samples directly from the reaction tubes with a pipette, as filtration of the solution was not required before analysis. The MagRBR screenings could easily be expanded to more than six reactions in parallel, thus further increasing the efficiency.

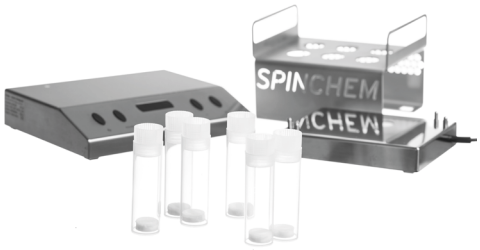

Fig 3. SpinChem® MagRBR starter kit, including six MagRBRs pre-packed with resin, reaction tubes, magnetic stirrer, stirrer control unit and tube rack.

Conclusions:

- The SpinChem® MagRBR Lipase screening kit enables rapid parallel screening of immobilized lipases, resulting in fast and convenient laboratory reaction development.
- The lack of filtration steps allows for easy sampling and monitoring of the process.

The SpinChem® rotating bed reactor (RBR) is revolutionizing mass transfer in heterogeneous reactions where solid phases are used for catalysis, enzymatic reactions, adsorption, scavenging and other processes. The convenience of a protected bed within an RBR significantly reduce the need for post-reaction work-up. The SpinChem® RBR concept is fully scalable from laboratory to production, thus providing both more efficient reaction development and improved production economy.

Products: SpinChem® MagRBR Lipase screening kit (5101-220), SpinChem® MagRBR starter kit (9100-001)

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