Efficient synthesis of chiral lactones by encapsulated cells in a rotating bed reactor

Encapsulating whole cells for biocatalytic reactions avoids recycling of cofactors but may result in lower enzyme activity and the need for more complicated equipment. In this application note we demonstrate how a SpinChem® rotating bed reactor (RBR) retains the benefits of cell encapsulation while avoiding poor oxygen transport and time-consuming downstream processing. The result was an efficient path to asymmetric synthesis of ε-caprolactone through Baeyer-Villiger oxidation by cyclohexanone monooxygenase. The SpinChem® RBR was easy to set up and outperformed traditional reaction systems for this process throughout several batches. The processing time to recover and recycle the encapsulated cells after each batch was reduced by a factor of 10 to 25, depending on conditions.

Keywords: Biotransformation, Organic molecules, Alginate, Gas-distribution

Biocatalytic reactions have become a competitive route to production of many small chiral molecules. To facilitate handling and enhance activity, the enzymes employed are often immobilized on solid supports. For complicated reactions with multiple steps and for reactions that require regeneration of cofactors such as NADPH, encapsulation of whole cells can be a more viable approach. These strategies may, however, show limited productivity due to reduced transport of substrates or dissolved oxygen to the cells, and require complex procedures for downstream processing.

For such challenging situations the SpinChem® rotating bed reactor (RBR) can provide an attractive solution as it ensures extremely efficient mass transfer of both dissolved molecules and gases to solid materials packed into beds. The bed format also simplifies recovery of the particles and stabilizes activity of the encapsulated cells since they are protected from mechanical wear.

In this application note we investigated the effects of different reactor systems on the production of ε-caprolactone (Fig 1) by the enzyme cyclohexanone monooxygenase (CHMO) within encapsulated cells. A setup with SpinChem® RBR (Fig 2) was compared to a traditional stirred tank reactor (STR) and a fixed bed reactor (FBR).

Fig 1.

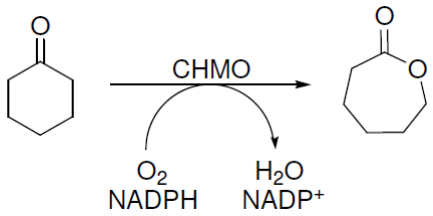

Fig 2.

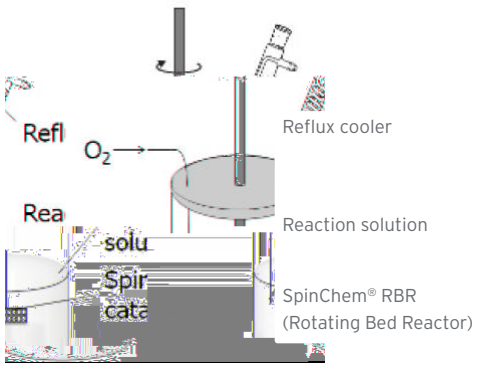

Fig 1. Synthesis scheme for production of ε-caprolactone from cyclohexanone by cyclohexanone monooxygenase (CHMO).

Fig 2. Reaction vessel setup including the SpinChem® RBR (Rotating Bed Reactor) operated by an overhead stirrer at 500 rpm within an open vessel New Brunswick BioFlo 110 Fermenter (0.9 L) with 0.25 L/min O_2 under reflux at 25°C.

Changing from the STR to the RBR was a simple procedure and required only that the stirrer was replaced by the SpinChem® RBR device inside the reaction vessel. Conversely, the FBR had an external bed of particles through which a separate pump circulated liquid at a constant rate. Comparing the three types of reactors (Fig 3) revealed that the conversion was similar using the SpinChem® RBR and the traditional STR, but about ten times lower with the FBR. The most likely explanation for this was poor oxygen transport to the FBR's external bed.

Fig 3.

Fig 3. Formation of ε-caprolactone by CHMO enzyme inside calcium-alginate encapsulated *E. coli* cells (28 g wet) using either SpinChem® RBR, STR or FBR reactor setups. Conditions: cyclohexanone (20 mM) and D-glucose monohydrate (2.5 g/L) in 0.5 L Tris-HCl buffer (20 mM, pH 7.5, 1% NaCl). Vessel setup cf. Fig 2. FBR flow 3 mL/min.

The ability of the SpinChem® RBR to preserve activity of the encapsulated cells was investigated by running consecutive synthesis batches and recycling the cell particles between each run. The results (Fig 4a) revealed that the cells within the SpinChem® RBR were indeed protected. After six batches the activity with the SpinChem® RBR was three times higher compared to the traditional STR setup.

The time savings when recycling particles with the SpinChem® RBR was even more striking (Fig 4b). The only cleaning needed for the RBR was to spin it three times in a solvent for 30 seconds, summing up to a total recycling time of less than 5 minutes. In contrast, the STR setup required filtration and workup of the dispersed particles for 1-2 hours.

Fig 4a.

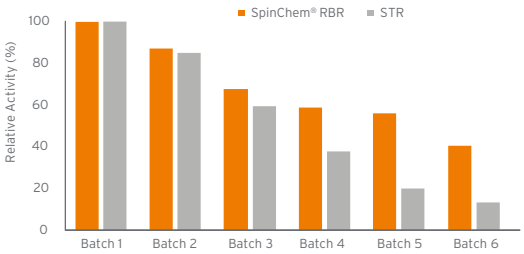

Fig 4b.

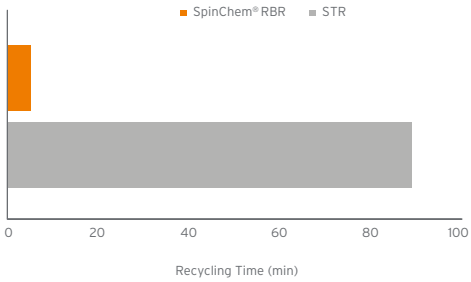

Fig 4. Remaining activity (a) of calcium-alginate encapsulated *E. coli* cells with CHMO after recycling to consecutive batches. Total time for recycling (b). Two hours reaction time for each batch. Conditions cf. Fig 3 plus 20 mM $CaCl_2$.

Conclusions:

- It was simple to convert a traditional STR setup to a SpinChem® RBR without affecting the intrinsic reaction rates negatively
- The collection of particles in a bed within the SpinChem® RBR protected the encapsulated cells and better preserved activity throughout several batches
- Avoiding filtration to recover the encapsulated cells reduced the recycling time by a factor of 10 to 25, reducing it to less than 5 minutes

References:

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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 needs 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® RBR S311 (1311-001)

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