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Efficient Biocatalysis with Immobilized Enzymes or Encapsulated Whole Cell Microorganism by Using the SpinChem Reactor System

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Nowadays, biocatalysis is an established method for the enzymatic synthesis of chiral building blocks for organic compounds and pharmaceuticals, compounds for the flavor and fragrance industry, the production of bulk chemicals, and the modification of lipids for the food industry.[1]

Biocatalysis has become highly competitive with classical (asymmetric) chemical routes that use transition-metal catalysts, especially in combination with new methods for enzyme discovery and protein engineering,[2] as recently shown for the synthesis of the drug Sitagliptin.[3] The cost-effective application of enzymes, in particular for the synthesis of cheap products, requires immobilization of the biocatalyst (or the encapsulation of whole cells) to enhance their long-term stability[4,5] and facilitate their reuse. At the same time, immobilization of the biocatalyst should enable the use of established reactor setups, such as fixed-bed reactors (FBRs), instead of simple stirred-tank reactors (STRs, Figure 1).[1b,6] FBRs are used, for instance, for the large-scale production of chiral amines[7] or emollient esters for the cosmetic sector[8] by using lipase catalysts. However, several disadvantages are encountered with FBRs, which depend on, for example, the length, diameter, and particle size in the reactor, the flow rate, the pressure drop within the column, and reactant and pH gradients, as well as inactivation profiles after extended use. In contrast, the more operationally simple STR encounters mechanical challenges for the carrier, which results in abrasion of the biocatalyst material and severe damage of encapsulated whole cells beside the fact that the recycling of the immobilized biocatalyst is rather laborious.

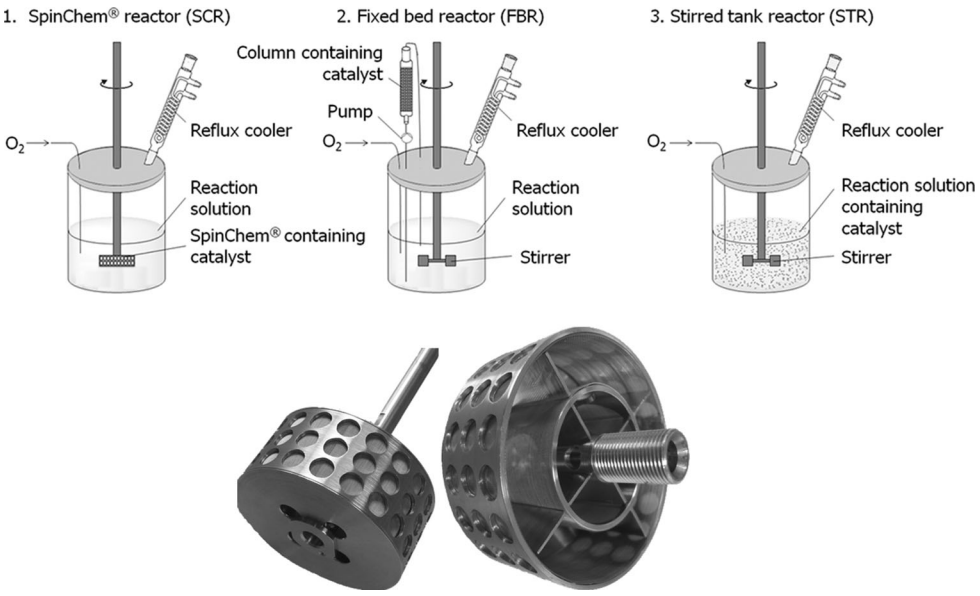

Figure 1. Top: Schematic representation of the three reactor setups that were investigated. Bottom: Photograph of the SpinChem device (reflux cooler and oxygen supply only for BVMO reaction).

Herein, we have investigated the use of an alternative setup for the application of immobilized enzymes and encapsulated whole cells. This SpinChem reactor (SCR; SpinChem is a registered trademark by Nordic ChemQuest AB, Umeå, Sweden) enables the simultaneous stirring and efficient percolation of a liquid through packed particle beds, which is implemented by a hollow stirring device that allows the solid reaction chamber to be located inside the stirring element itself. The SCR can be seen as an evolution of the standard basket reactor.[9,10] The basket reactor, first published by Carberry in 1964, is a setup in which four baskets rotate inside a well for gas/solid reactions. This concept was later developed as the "annular spinning basket reactor" by Mahoney et al. in 1978. However, in the SpinChem reactor, the solid phase (such as an immobilized enzyme) is present in the stirring element itself in up to four separate compartments, which provides greater mixing and flexibility compared to the basket reactors.

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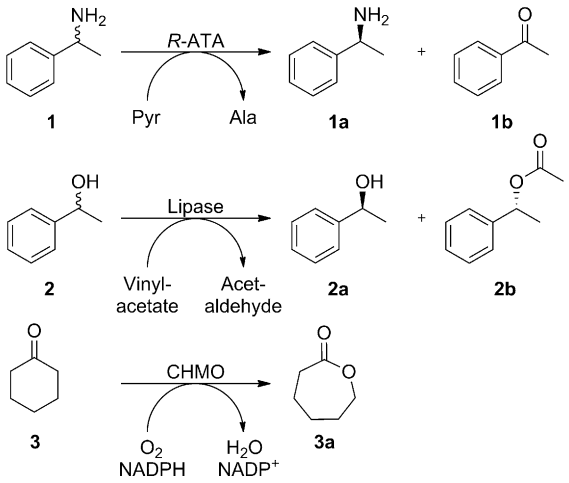
In this way, a variety of heterogeneous operations (catalysis, solid-phase reactions, scavenging, etc.) can be performed in an efficient and convenient fashion, because the material is contained in the overhead stirring device and is not subject to mechanical wear or filtration problems. By rotating the SCR, the liquid inside is "thrown out" through a centrifugal effect and the new liquid will be drawn into the SCR from both the bottom and the top (Figure 1). The main advantages of the SpinChem system are easier downstream processing and simple recycling of the biocatalyst, because the compartment that contains the immobilized enzyme can be easily separated from the bulk reaction solution.

To verify the properties of the SCR compared to established reactor systems for biocatalysis, we have investigated 1) the kinetic resolution of (*R*,*S*)-1-phenylethylamine by using an immobilized (*R*)-transaminase from *Gibberella zeae* (GibZea)[11] and 2) the kinetic resolution of (*R*,*S*)-1-phenylethanol by using an immobilized *Candida antarctica* lipase B (CAL-B,[12] Novozyme 435, N435) in *n*-hexane (Scheme 1).



Scheme 1. Biocatalytic reactions that were studied by using the different reactor systems. *R*-ATA = (*R*)-amine transaminase, CHMO = cyclohexanone monooxygenase.

Furthermore, 3) calcium-alginate-encapsulated *Escherichia coli* whole cells that harbor the cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871[13] were used for the production of ε-caprolactone from cyclohexanone (Scheme 1). Stability has been a particularly challenging issue for O_2-consuming enzymes, which still has to be addressed. Furthermore, in FBRs, the O_2 supply is a difficult issue and, thus, an alternative reactor system is sought.

The reactions in the SCR and the STR were performed with a volume of 0.5 L in a New Brunswick BioFlo 110 Fermentor/Bioreactor (total volume: 0.9 L). In the FBR reactions a reservoir with a volume of 0.5 L was used. In all three setups, we used identical amounts of enzyme (based on units of activity; for details, see the Supporting Information). For the lipase and transaminase reactions, we operated at high substrate concentrations of 122.17 g L^{-1} (1 M) and 16.12 g L^{-1} (0.133 M), respectively.

Because Baeyer–Villiger monooxygenases work best at lower substrate concentrations, only 1.96 g L^{-1} (0.02 M) cyclohexanone was used for the CHMO-catalyzed reaction. To ensure an optimal mass transfer, we first determined the optimal stirring speed for the SCR, which was found to be 500 rpm for all three reactions that were studied (the range 100–1000 rpm was investigated; see the Supporting Information, Table S6).

In the transaminase-catalyzed kinetic resolution (Scheme 1, Figure 2, and Table 1), the SCR and the STR gave the same conversions after 6 h, whereas the FBR gave a 1.2-fold-lower conversion. For the lipase-catalyzed kinetic resolution (Scheme 1, Figure 3, and Table 1), almost-identical conversions (close to 50%) were determined after only 4 h, even at a substrate concentration of 1 M. The production of ε-caprolactone catalyzed by the CHMO also showed the same conversions (35%) after 24 h in both the SCR and the STR (Scheme 1, Figure 4, and Table 1). In contrast, for the FBR, a significant nine-fold-lower conversion was obtained. This dramatic slowdown could be explained by the decreased oxygen supply in the column. Thus, SCR and STR enable similar conversions for CHMO-, lipase-, and transaminase-catalyzed reactions, thus indicating that, for these reactors, mass transfer is not a limiting issue.

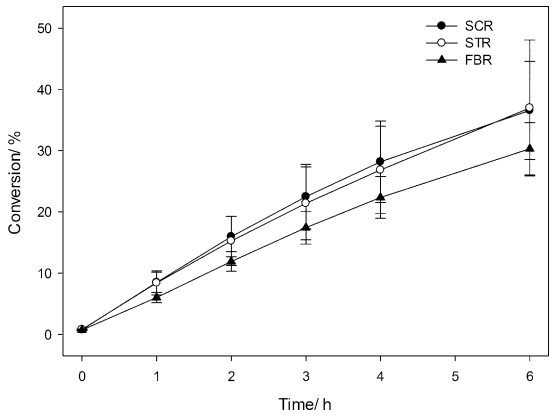


Figure 2. Kinetic resolution of (*R*,*S*)-1-phenylethylamine to afford (*S*)-1-phenylethylamine by using the immobilized GibZea (*R*)-transaminase.

Table 1. Conversions that were achieved with three different reactor systems.

Enzyme	Conversion [%]		
	SCR	STR	FBR
transaminase[a]	37 ± 8.0	37 ± 11	30 ± 4.3
lipase[b]	45 ± 1.0	46 ± 1.0	n.d.
CHMO[c]	36 ± 6.1	35 ± 6.0	4 ± 0.2

[a] After 6 h; [b] after 4 h; [c] after 24 h; n.d. = not determined.

Next, reuse and downstream processing were studied in the SCR and the STR under identical conditions. In the case of the SCR, this study was simply performed by taking the stirrer out of the reactor and washing it three times in small beakers

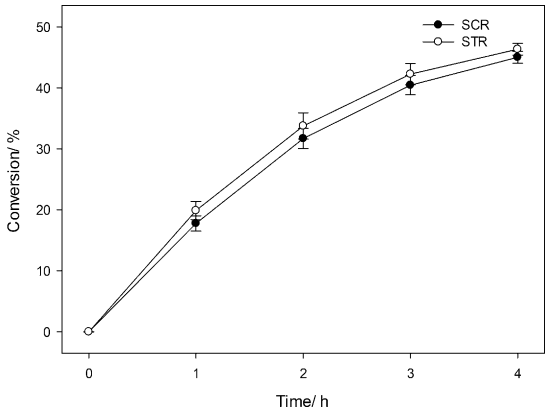


Figure 3. Transesterification of (*R*,*S*)-1-phenylethanol into the corresponding (*R*)-acetate by using immobilized lipase CAL-B in the SCR and the STR.

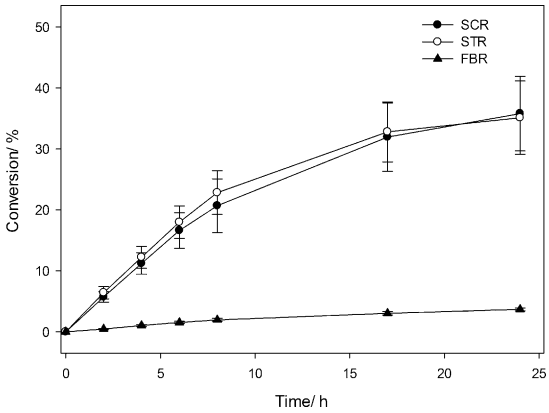


Figure 4. Formation of ε-caprolactone by using alginate-encapsulated resting *E. coli* cells that harbored a CHMO.

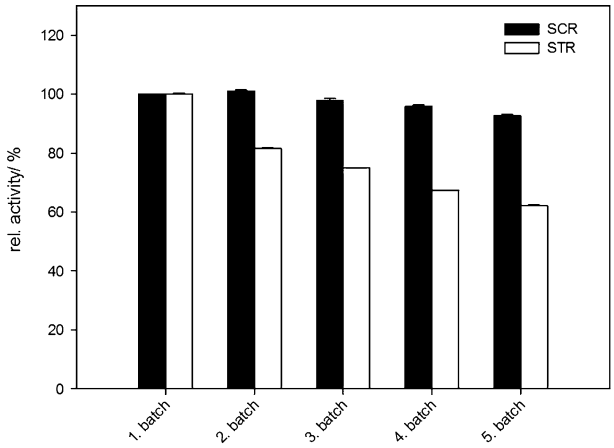


Figure 5. Recycling study with immobilized GibZea (*R*)-transaminase (2 h per batch) in the SCR and the STR; 100% relative activity refers to 19% conversion of (*R*)-1-phenylethylamine in the first batch.

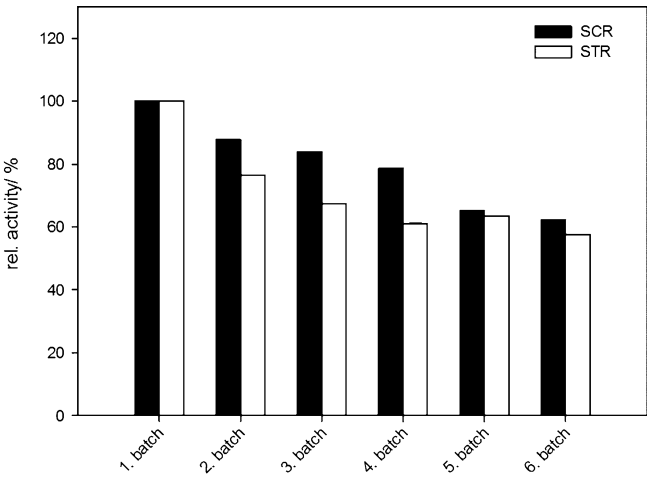


Figure 6. Recycling study with immobilized lipase CAL-B (2 h per batch) in the SCR and the STR; 100% relative activity refers to 33% (SCR) and 39% conversions (STR) of (*R*,*S*)-1-phenylethanol in the first batch.

under stirring for 30 s. Then, the biocatalysts that were present in the SpinChem compartment were ready to use in the next cycle. In the STR, the reaction solution was first filtered, followed by washing the immobilized catalyst before the next cycle was started. These recycling studies revealed that, for the transaminase reaction, the SpinChem system was superior: 93% relative activity was recovered in the SCR compared to only 62% in the STR (Figure 5).

This result suggested that the immobilized transaminase was better protected from mechanical forces in the SpinChem device. In the lipase-catalyzed kinetic resolution, the SCR was slightly superior until the fourth cycles; after the sixth cycle, both systems gave similar conversions (Figure 6).

Notably, CAL-B is a highly robust lipase and, hence, losses in activity are difficult to observe because the immobilized biocatalyst is typically stable for several months under process conditions. Furthermore, this initial decrease in activity could be caused by accumulation of the reaction compounds on the carrier or in the enzyme environment. In the oxidation reactions that were catalyzed by CHMO whole cells, the SCR was clearly superior and showed 41% residual activity after six cycles (versus 14% relative activity in the STR). Furthermore, between the fourth and fifth cycles, the encapsulated cells were stored overnight at 4 °C (Figure 7). The cells in the SCR showed no significant loss of activity, whereas, in contrast, only half of the relative activity was determined for the cells in the STR. In addition, for successful recycling of the alginate capsules, the presence of 10 mM $CaCl_2$ in the reaction and washing solution was necessary. Without this additive, the beads completely dissolved after the second cycle, owing to removal of the Ca^{2+} ions from the calcium-alginate complex (data not shown).

In conclusion, we have developed an alternative reactor concept for the use of immobilized enzymes or whole cells in biocatalysis. The SCR was shown to be equivalent to—or even superior to—conventional setups. In particular, recycling experiments in consecutive batch reactions are facilitated and the activity loss was reduced in the SCR. The SCR does not require special laboratory equipment because, in essence, only the stir-

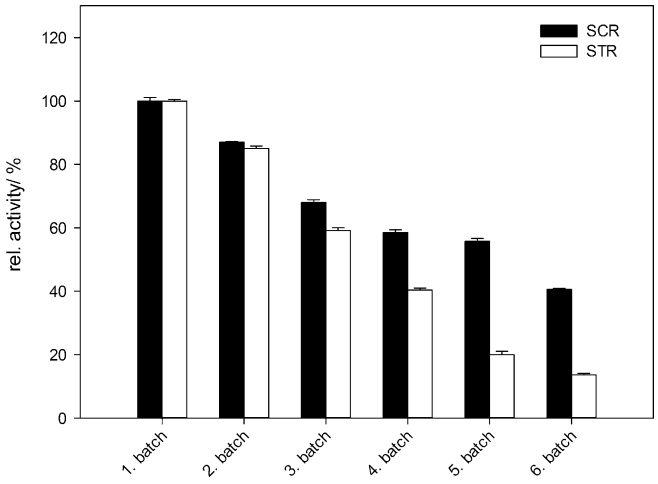


Figure 7. Recycling study with encapsulated resting *E. coli* cells that expressed the CHMO in the SCR and the STR (2 h per batch); 100% relative activity refers to 7.5% conversion of cyclohexanone in the first batch.

rer needs to be modified with the Spin-Chem compartment chamber.

Acknowledgements

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Keywords: biocatalysis · enzymes · immobilization · kinetic resolution · microreactors

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